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Purification of the Thy-1 Molecule, a Major Cell-Surface Glycoprotein of Rat Thymocytes

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The Thy-1 molecule, which was identified by its antigenic activities, has been purified from rat thymocytes. The purification involved preparation of crude membranes and solubilization in deoxycholate, followed by gel filtration and affinity chromatography on antibody or lectin columns. In all cases the purified molecule was a glycoprotein that did not form higher polymers and was not associated with other polypeptide chains. The Thy-1 glycoprotein could be found in two forms, one binding to lentil lectin, the other not. Both forms had the same detectable antigens and were of a similar but not identical size. After sodium dodecyl sulphate–polyacrylamide-gel electrophoresis the apparent molecular weight of Thy-1 binding to lentil lectin was 25000, whereas that not binding to the lectin was 27000, with heterogeneity towards forms of apparently higher molecular weight.

A study of cell-surface antigens provides one method of identifying tissue-specific membrane molecules (Bennett *et al.*, 1972; Morris & Williams, 1975). Antigens which are restricted to particular differentiated cell types may be carried by molecules with functions related to the specific role of the cells which display them. The Thy-1 (θ) alloantigens are of this type, being found in large amounts in brain and thymocytes of rodents (Reif & Allen, 1964, 1966; Douglas, 1972; Acton *et al.*, 1974). In the mouse, the Thy-1 molecule occurs in two allelic forms resulting in two alloantigens Thy-1.1 (θ-AKR) and Thy-1.2 (θ-C_3H) (Reif & Allen, 1964; Snell & Cherry, 1972). In the rat, Thy-1.1 antigen, identified with mouse alloantiserum, is present in the thymus and brain of all strains which have been studied, but the Thy-1.2 antigen has not been found (Douglas, 1972; Acton *et al.*, 1974). It was also established that the rat Thy-1 molecule carries two other antigenic determinants which can be recognized by xenogeneic antiserum, in this case a rabbit anti-(rat brain) serum (Morris & Williams, 1975; Morris *et al.*, 1975). One antigen is found on rat but not on mouse tissues and is referred to as the 'rat-specific Thy-1 xenoantigen', whereas the other is common to rat and mouse and is called the 'rat–mouse cross-reacting xenoantigen'. Quantitative studies using antisera against Thy-1.1 antigen and Thy-1 xenoantigens have shown that there are about 600000 antigenic sites per rat thymocyte, and this suggests that the Thy-1 molecule is a major membrane component (Acton *et al.*, 1974; Morris & Williams, 1975).

To elucidate the function of the Thy-1 molecule an understanding of its molecular structure is required. In preliminary investigations (Letarte-Muirhead *et al.*, 1974; Morris *et al.*, 1975) it was established that rat Thy-1 antigens are not inactivated by detergents, and that the Thy-1 molecule can be solubilized in deoxycholate. In this detergent Thy-1 was found to have a sedimentation coefficient of 2.4S, a Stokes radius of 3.0nm and a partial specific volume of 0.70ml/g. The mol.wt. of the Thy-1–detergent complex was calculated to be 28000, and it seemed likely that Thy-1 was a protein, carbohydrate or glycoprotein, rather than a glycolipid as suggested by others (Esselman & Miller, 1974; Vitetta *et al.*, 1973).

The present paper reports the continuation of these studies, which resulted in the purification of the Thy-1 molecule from deoxycholate extracts of rat thymocyte membrane. Purification was achieved by two independent methods, one involving gel filtration and affinity chromatography with lectins (Hayman & Crumpton, 1972), the other involving antibody affinity chromatography against the brain Thy-1 molecule (Barclay *et al.*, 1975). The results show that rat Thy-1 is a glycoprotein with an apparent mol.wt. of about 25000 and consists of a single polypeptide

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chain. Evidence for possible heterogeneity in the carbohydrate part of the molecule was obtained.

Materials and Methods

Materials

Animals. These were as described by Acton *et al.* (1974) except for Wistar rats which were obtained from the M.R.C. Radiobiology Unit, Harwell, Oxon, U.K.

Buffers. Deoxycholate buffer used for gel filtration and affinity chromatography contained 0.5% deoxycholate, 0.02% NaN_3, and 0.01 M-Tris–HCl, pH 8.0. The Tris was neutralized at 20°C, and at 4°C the pH was 8.4.

Chemicals. Avidin, fetuin, deoxycholate and Tween-40 were from Sigma Chemical Co., London S.W.6, U.K.; acrylamide, bisacrylamide and sodium dodecyl sulphate were from BDH Chemicals Ltd., Poole, Dorset, U.K.; Schiff reagent was from Difco Labs., Surrey, U.K.; Coomassie Blue was from Michrome, Edward Gurr Ltd., London S.W.14, U.K.; concanavalin A was from Pharmacia Fine Chemicals, S-751 04 Uppsala 1, Sweden.

Antibodies. The following were used. (1) Balb/c mouse anti-(Wistar rat brain) serum, specific for Thy-1.1 alloantigen on AKR mouse thymocytes (Acton *et al.*, 1974). (2) Rabbit anti-(rat brain Thy-1) serum, specific for Thy-1 on rat thymocytes with 70% of antibody against Thy-1 rat-specific xenoantigen, 26% against Thy-1 rat–mouse cross-reacting xenoantigen, and 4% against Thy-1.1 (Barclay *et al.*, 1975); the strength of the antiserum was estimated to be approx. 0.3 mg of anti-(Thy-1) antibody/ml. (3) Rabbit anti-(thymocyte Thy-1) serum, raised against Thy-1 purified by affinity chromatography with lentil lectin as described in the Results section. Two rabbits were immunized three times at 8-day intervals with 30 μg of Thy-1 per injection. The first two doses were in complete Freund's adjuvant and given intramuscularly, the third was in incomplete Freund's adjuvant and given subcutaneously. The animals were bled 8 days after the third injection and analysed as described in the Results section. (4) Purified horse IgG* anti-(rabbit IgG) antibody labelled with ^{125}I (20 μCi/μg) (Morris & Williams, 1975). (5) Purified rabbit $F(ab')_2$ anti-(mouse IgG) antibody labelled with ^{125}I (20 μCi/μg) and prepared as described for anti-(mouse Fab fragment) antibody (Acton *et al.*, 1974). (6) Rabbit anti-(lentil lectin) antibody which was raised by immunization with lentil lectin in the same way as for antiserum (3) above, by using 600 μg of lectin per dose.

* Abbreviations: IgG, immunoglobulin G; Thy-1_{L+}, Thy-1 binding to lentil lectin; Thy-1_{L-}, Thy-1 not binding to lentil lectin; Thy-1_{total}, Thy-1 containing a mixture of Thy-1_{L+} and Thy-1_{L-} purified by antibody affinity chromatography.

Methods

Unless otherwise stated all procedures were carried out at 0–4°C.

Indirect binding assays and units of antigenic activity. Antigen activity was measured by inhibition of indirect radioactive-binding assays; the different types of assay possible are as described by Morris & Williams (1975). To assay antigen in detergent extracts, trace amounts of second antibody were used, whereas in the quantitative analysis of anti-(thymocyte Thy-1) antibody saturating amounts of ^{125}I-labelled horse anti-(rabbit IgG) were added. In all cases the standard assay contained 25 μl of antiserum, 25 μl of inhibitor and 50 μl of target cells. In the second step 100 μl of ^{125}I-labelled anti-(IgG) was added.

In the Thy-1.1 assay (Letarte-Muirhead *et al.*, 1974), Balb/c anti-(Wistar rat brain) serum was added at 1:50 (v/v) (final dilution 1:200) and ^{125}I-labelled anti-(mouse IgG) was used in the second step rather than ^{125}I-labelled anti-(mouse Fab fragment).

In the Thy-1 xenoantigen assay (Morris *et al.*, 1975) an antiserum against purified brain Thy-1 (see under 'Antibodies' above) was used rather than one against whole brain homogenate. The target cells were rat thymocytes, and the antiserum did not need absorption as only 4% of the antibody was against Thy-1.1 antigen. Distinction was not made between rat-specific and rat–mouse cross-reacting xenoantigen, and the serum was added at 1:1500 (v/v) (final dilution 1:6000).

To compare antigenic activities at different stages of the purification a unit of activity was defined as the amount of antigen needed to give 50% inhibition of the appropriate standard binding assay. Thus 1 ml of extract of which 25 μl gave 50% inhibition at a dilution of 1:100 would contain 4000 units of antigenic activity.

Preparation and solubilization of thymocyte membranes. Thymus glands were obtained from 70–100 rats (approx. 10^{11} thymocytes) and membrane was prepared by the Tween-40 method (Morris *et al.*, 1975) with volumes increased in proportion with increased cell numbers. The membrane was resuspended in 25 ml of 0.01 M-Tris–HCl, pH 8.0, plus 0.02% (w/v) NaN_3, to which was added 25 ml of 4% (w/v) deoxycholate in the same buffer. After 60 min at 0°C the extract was centrifuged for 6750000 *g*-min, leaving solubilized antigen in the supernatant.

Gel filtration in deoxycholate. Upward-flowing columns of Sephadex G-200 were eluted with deoxycholate buffer. For gel filtration of solubilized membrane, 50 ml of the supernatant was loaded on to a Pharmacia K-50 (5 cm × 90 cm) column and 20 ml fractions were collected. For final purification of Thy-1 obtained by antibody affinity chromatography, a Pharmacia K-26 (2.6 cm × 90 cm) column was used, and 5 ml fractions were collected.

Affinity chromatography with columns of Sepharose 4B coupled with lentil lectin and concanavalin A. *Lens culinaris* phytohaemagglutinin (referred to as lentil lectin) was prepared from lentils by the method of Howard *et al.* (1971) as modified by Hayman & Crumpton (1972). The lentil lectin was pure as judged by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis and was coupled to activated Sepharose 4B (Cuatrecasas, 1970) at a ratio of between 3 and 14mg of lectin to 1ml of packed swollen beads. The Sepharose 4B–lectin was always used in amounts such that saturation did not occur during absorption. In large-scale preparations, as in Table 1, 2.5ml of beads with 14mg of lentil lectin/ml was sufficient.

Before use, the lentil lectin–Sepharose 4B column was washed with deoxycholate buffer, then with 0.5M-methyl α-D-glucopyranoside in deoxycholate buffer, and finally with deoxycholate buffer. The sample was then applied and the column washed with deoxycholate buffer until the E_{280}^{1cm} reading was zero. Bound material was then eluted with 0.5M-methyl α-D-glucopyranoside in deoxycholate buffer and the eluate assayed for protein and antigenic activity.

Affinity chromatography with concanavalin A–Sepharose 4B (10mg/ml of beads) was carried out in the same way.

Affinity chromatography with antibody–Sepharose 4B columns. γ-Globulin [obtained by precipitation with 16% (w/v) Na_2SO_4] from rabbit anti(brain Thy-1) and anti(lentil lectin) antisera was coupled to Sepharose 4B at 20–35mg of protein/ml of beads as described by Cuatrecasas (1970) for anti-insulin antibody. Before use, anti-(Thy-1) columns were pre-eluted with 3vol. of 0.05M-diethylamine–HCl buffer, pH11.5 (Read *et al.*, 1974), and washed with deoxycholate buffer. This eluted a small amount of IgG (detected by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis) and thereafter IgG was not detectable in any fractions.

For affinity chromatography, columns of volume 9–15ml were set up with a 5ml column of normal rabbit IgG–Sepharose 4B preceding the antibody column (to absorb aggregates and decrease non-specific binding). Deoxycholate extract of thymocyte membrane was passed through the column at approx. 0.3ml/min and washed through with deoxycholate buffer until no further E_{280}-absorbing material emerged. Then the upper column was disconnected and the antibody column eluted at 0.7ml/min with 2 column-volumes of 0.05M-diethylamine–HCl, pH11.5, plus 0.5% deoxycholate, followed by deoxycholate buffer again. The eluted fractions were immediately brought to pH8.3 with solid glycine and then dialysed overnight against deoxycholate buffer. Antigenic activities and protein contents were then determined. Columns were used three times without detectable loss of antigenic activity.

Sodium dodecyl sulphate–polyacrylamide-gel electrophoresis. This was carried out as described by Fairbanks *et al.* (1971), by using 5.6% (w/v) acrylamide gels (containing 1% sodium dodecyl sulphate) and 0.2% sodium dodecyl sulphate in the electrophoresis buffer as the standard system. When 5, 7.5, 10 and 12.5% (w/v) gels were used, the different concentrations were achieved by adding more of the acrylamide–bisacrylamide mixture. Samples were concentrated for electrophoresis by precipitation with 20% (w/v) trichloroacetic acid for 60min at 0°C followed by washing twice with acetone (0°C), which removed both deoxycholate and trichloroacetic acid. Some variation in the effectiveness of precipitation of deoxycholate extracts was observed; thus in all cases where purity was being estimated gels were also run with material concentrated by ultrafiltration (see below). Sodium dodecyl sulphate (1% or more to give a sodium dodecyl sulphate/protein ratio of >7:1) and 4M-urea, plus either 2mM-iodoacetamide or 1% dithiothreitol, was added to the precipitates, which were solubilized by heating at 100°C for 3min; reduced samples were then alkylated with iodoacetamide. Gels were stained for protein with Coomassie Blue (Weber & Osborn, 1969) and de-stained electrophoretically with a Canalco de-stainer. For carbohydrate stain the periodic acid–Schiff procedure of Glossmann & Neville (1971) was used except that 25% (v/v) propan-2-ol plus 10% (v/v) acetic acid was used to fix the gels. The relative mobility of the bands was determined by comparison with the mobility of the Bromophenol Blue marker and the apparent mol.wt. estimated by using standard curves constructed with marker proteins (Weber & Osborn, 1969). The marker proteins used (with mol.wts. in parentheses) were: H chain (52000) and L chain (23500) of rabbit IgG; bovine serum albumin (68000); hen ovalbumin (43000); whale myoglobin (17000). Densitometry was carried out on a Gilford 2410-S gel scanner at 550nm for Coomassie Blue, and 560nm for the periodic acid–Schiff stain.

To assay Thy-1.1 and Thy-1 rat xenogeneic antigen activities after sodium dodecyl sulphate–polyacrylamide-gel electrophoresis, the concentration of sodium dodecyl sulphate in the gel and buffer was decreased to 0.1% (w/v) and the sample was loaded in 0.1% (w/v) sodium dodecyl sulphate–0.01M-Tris, pH8.0. ^{125}I-labelled hen ovalbumin and ^{125}I-labelled whale myoglobin iodinated as for ^{125}I-labelled antibodies (Jensenius & Williams, 1974) were loaded in trace amounts as internal markers. After electrophoresis the gels to be assayed were cut into 3mm slices which were crushed in 0.5ml of 0.05% (w/v) sodium dodecyl sulphate in 10mM-Tris, pH8.0, counted for radioactivity in a Nuclear

Enterprises γ-radiation counter to determine marker positions, and left overnight at 4°C. The material eluted from every slice after centrifugation was tested for antigenic activity by inhibition of the indirect binding assays. In these assays, the concentration of sodium dodecyl sulphate during absorption was 0.025% (w/v), and this decreased the antigenic activity of pure Thy-1 treated in an identical way, except for the electrophoresis step, to 10% of its activity in deoxycholate buffer. Thus to estimate the recovery of antigen activity from the gel a standard curve of inhibition by Thy-1 in the presence of sodium dodecyl sulphate was used.

Concentration of fractions throughout purification. Fractions were concentrated for analysis or further procedures by ultrafiltration in an Amicon apparatus by using a PM10 filter.

Determination of protein. Protein was determined by the method of Lowry *et al.* (1951); bovine serum albumin (concentration determined by using $E_{280}^{1cm} = 6.74$ for a 1% solution) was used as standard. When assays were carried out in the presence of deoxycholate the standards used to construct the curve contained the same amount of deoxycholate as the unknowns.

Results

Procedures used in the purification of the Thy-1 molecule

A summary of the procedures used in purification of the Thy-1 molecule is shown in Fig. 1. In all cases the purification of Thy-1 was assessed by measurement of its antigenic activities (Thy-1.1 and Thy-1 xenoantigens) by inhibition assays (see under 'Methods'). The first step always involved preparation of crude thymocyte membrane followed by solubilization in deoxycholate. After this, a combination of affinity chromatography (using antibody or lectin columns) and gel filtration resulted in purification of the Thy-1 molecule. The steps summarized in Fig. 1 are discussed in detail below.

Estimation of the degree of purification required

In a typical preparation of Thy-1, approx. 1×10^{11} thymocytes were used. Given that there are approx. 6×10^5 antigenic sites per thymocyte for Thy-1.1 (Acton *et al.*, 1974) or Thy-1 xenoantigens (Morris & Williams, 1975) it can be calculated that 1×10^{11} cells have about 100nmol of Thy-1. Taking a mol.wt. of 28000 this amounts to 2.8mg of antigen. This can be compared with a total protein content of 1810mg per 1×10^{11} thymocytes (Table 1). Thus it can be estimated that purification of the order of 650-fold is required to obtain pure antigen on the basis of protein content.

Preparation of crude membrane with Tween-40

Morris *et al.* (1975) showed that if thymocytes are suspended in 2% (w/v) Tween-40 at 0°C most of the Thy-1.1 antigen is released as a membrane fragment which sediments at $4.5\times10^6 g$-min but not at $1\times10^5 g$-min. The advantage of this method is that it can easily be increased in scale for use with large batches of cells. Table 1 shows that approx. 50% of the Thy-1.1 antigen activity is recovered in the membrane fraction at a ninefold purification. In Plate 1 the gel profiles for membrane material analysed by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis and stained with Coomassie Blue for protein and with periodic acid–Schiff reagent for carbohydrate are shown. Plate 1(*a*) shows that a large number of protein bands are present, but that the periodic acid–Schiff-staining profile is simpler (Plate 1*e*). The main bands, which are likely to be glycoproteins, are labelled from 1 to 5 and have apparent mol.wts. of 32000, 60000, 90000, 110000 and 150000 respectively (in 5.6% acrylamide gels). Band 2 is broad, and band 4 appears as a doublet in gels when small amounts of material are electrophoresed. In Plate 1(*e*) there is another band of periodic acid–Schiff-staining material

Table 1. *Purification of Thy-1.1 antigen by affinity chromatography on lentil lectin–Sepharose 4B*

Fraction	$10^{-4}\times$Total Thy-1.1 activity (units/fraction)	Protein (mg/fraction)	Relative specific activity	Yield of antigenic activity (%)
Cells (1.3×10^{11})	84	2360	1	100
90000*g*-min pellet	15	1428	0.3	18
4500000*g*-min supernatant	1.5	714	0.06	2
4500000*g*-min pellet (=membrane)	42.3	128	9	50
Membrane in deoxycholate	42.3	128	9	50
6800000*g*-min pellet	2.0	18	3	2
6800000*g*-min supernatant (=soluble fraction)	27.6	108	7	33
After Sephadex G-200 chromatography	14.9	14	30	18
Sepharose 4B–lectin unbound fraction	8.3	9	26	10
Sepharose 4B–lectin bound and eluted fraction	8.3	0.9	260	10

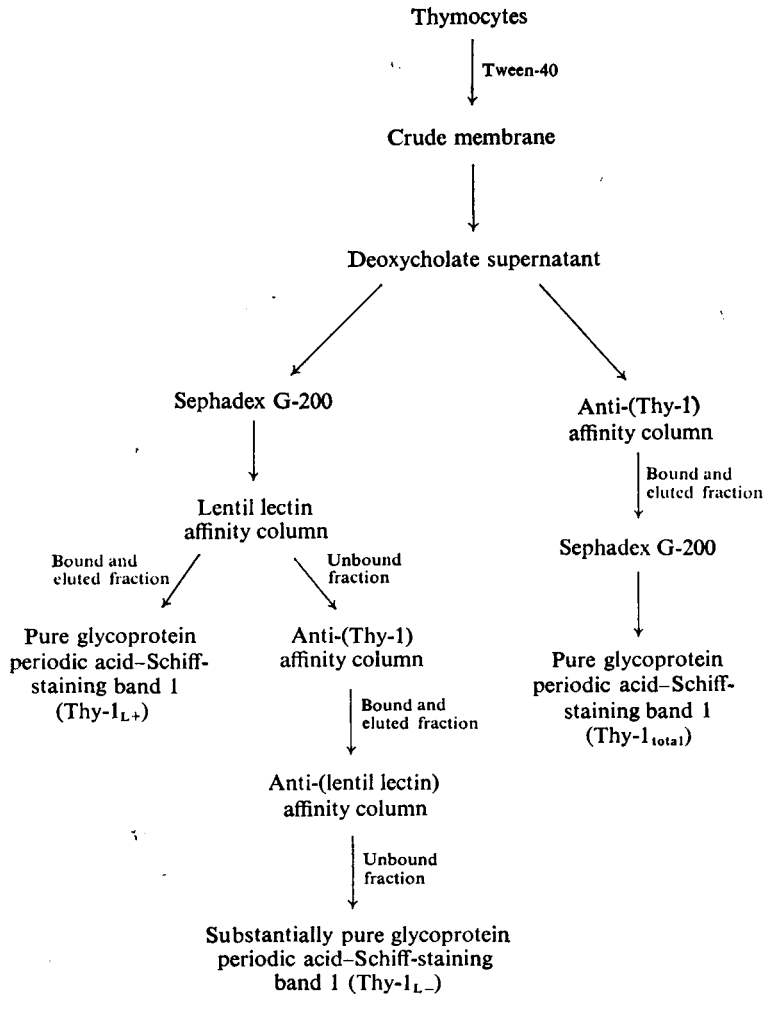


Fig. 1. *Flow sheet for the purification of the Thy-1 molecule*

running slightly ahead of the Bromophenol Blue marker and this is probably glycolipid (Glossman & Neville, 1971).

The periodic acid–Schiff staining pattern is similar to that obtained by Ladoulis *et al.* (1974) for rat thymocyte plasma membrane prepared by conventional methods.

Solubilization in deoxycholate

Thy-1.1 antigen is effectively solubilized by deoxycholate (Letarte-Muirhead *et al.*, 1974), and in the present preparations the same method was used except that the ratio of membrane protein/deoxycholate was increased to 1:8 (w/w), which was the maximum ratio compatible with solubilization. At this ratio, membrane from 1.3×10^{11} cells was solubilized in 50ml of 2% (w/v) deoxycholate, and 65% of the Thy-1.1 activity of the crude membrane was found in the 6.8×10^{6} *g*-min supernatant (Table 1). Sodium dodecyl sulphate–polyacrylamide-gel electrophoresis of material from the solubilized extract gave gels which did not differ markedly from those carried out with membrane in either protein (compare Plate 3*a* with Plate 1*a*) or carbohydrate (compare Plate 3*e* with Plate 1*e*) bands. The only difference was a decreased glycolipid band; this material may be lost during trichloroacetic acid precipitation and acetone extraction in the presence of deoxycholate.

Gel filtration

The deoxycholate extract of membrane (50ml) was applied to a large column (1800ml; 5cm×90cm) of Sephadex G-200 and eluted with deoxycholate buffer. The elution profile was as previously described for chromatography on a smaller column (Morris *et al.*, 1975), and most of the Thy-1.1 activity was eluted at the elution volume of ovalbumin. The fractions containing Thy-1.1 activity were pooled and contained 13 and 55% respectively of the protein and antigenic activity applied to the column (Table 1); a 4.3-fold purification of antigen resulted. The pooled fraction contained a number of polypeptides as detected by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis (Plate 1*b*), but only one periodic acid–Schiff-staining component (Plate 1*f*). As might be expected this was the low-molecular-weight band 1 seen in the whole-membrane profile (Plate 1*e*).

Affinity chromatography with lentil lectin and concanavalin A columns

In preliminary experiments a deoxycholate extract of crude thymocyte membrane was passed through a lentil lectin–Sepharose 4B column which was then washed with buffer and eluted with methyl α-D-glucopyranoside. The fractions were assayed for Thy-1.1 antigen and, as Fig. 2(*a*) shows, a substantial proportion of the antigen was not retarded by the column, whereas the rest bound to the column and was eluted by methyl α-D-glucopyranoside. Re-running of the unretarded (Fig. 2*b*) and eluted fractions (Fig. 2*c*) confirmed that they were distinct and henceforth they are referred to as 'Thy-1.1_{L-}' and 'Thy-1_{L+}'.

In large-scale preparations the pooled Thy-1.1-containing fractions from a Sephadex G-200 column were passed through a lentil lectin column in conditions where saturation was not reached, and the unretarded and eluted fractions were recovered and analysed. The unretarded fraction had a decreased specific activity for Thy-1.1 antigen (per mg of protein), and contained similar protein bands after sodium dodecyl sulphate–polyacrylamide-gel electrophoresis as the fraction applied to the column (Table 1 and Plate 1*c*). This fraction also retained part of periodic acid–Schiff-staining band 1 (Plate 1*g*) seen in the starting G-200 fraction, but this was clearly and reproducibly decreased in amount. The eluted fraction was purified for Thy-1.1 antigen ninefold compared with the Sephadex G-200 fraction, and the overall purification was 260-fold compared with the starting cells (Table 1). This approaches the estimated requirement of a 650-fold purification and suggested that Thy-1_{L+} should be a major component of this fraction. In fact when sodium dodecyl sulphate–polyacrylamide-gel electrophoresis was carried out, only one major band was seen after staining for protein (Plate 1*d*) or carbohydrate (Plate 1*h*). There was also one minor protein band present, but this was almost certainly lentil lectin eluted from the column, since it was obtained in washings of the column before and after application of the post-G-200 material.

To verify that the Thy-1.1 antigenic activity coincided with the major band in Plate 1(*d*) and 1(*h*), material eluted from gel slices after sodium dodecyl sulphate–polyacrylamide-gel electrophoresis was assayed for antigenic activity. Sodium dodecyl

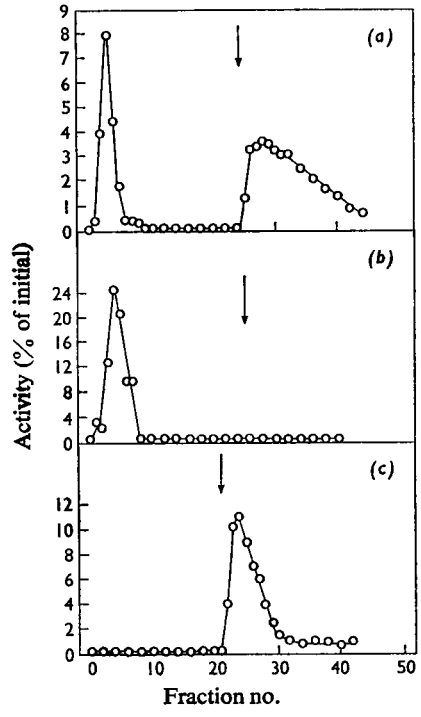
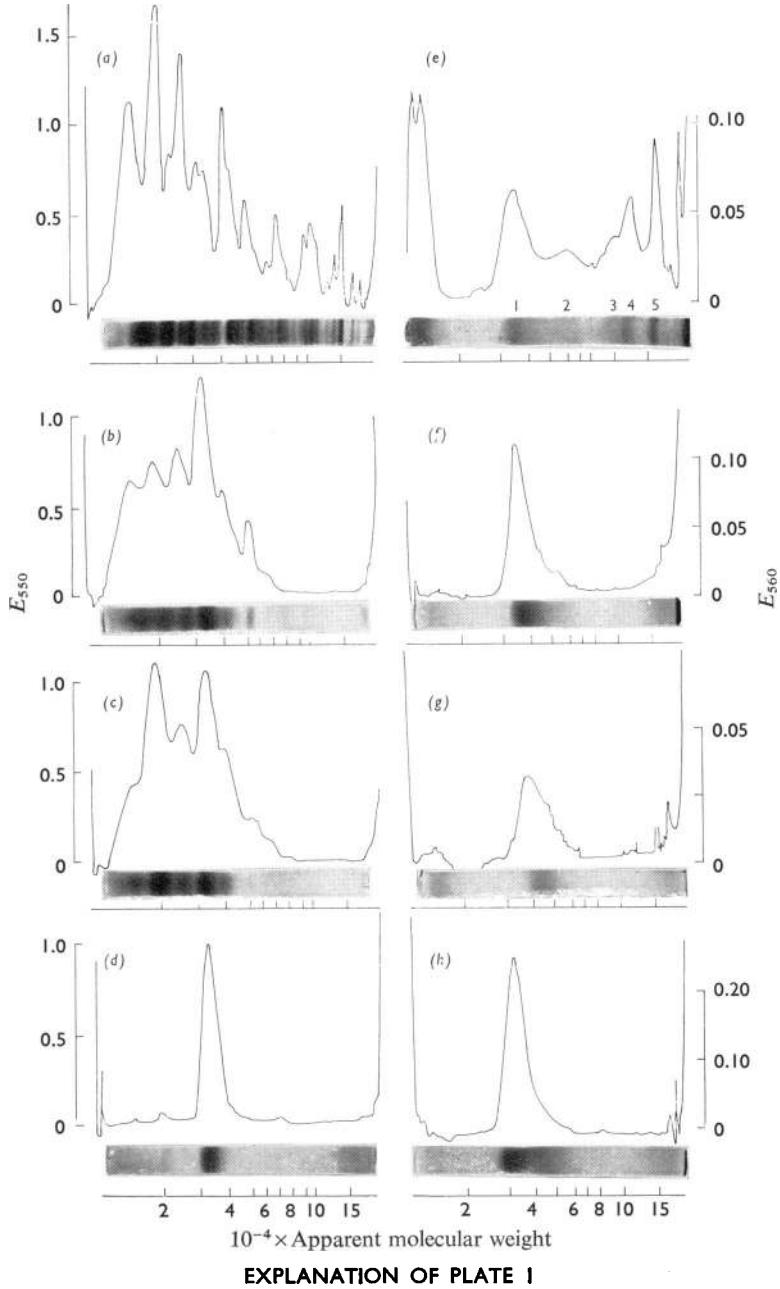


Fig. 2. *Affinity chromatography of Thy-1.1 on Sepharose 4B–lentil lectin*

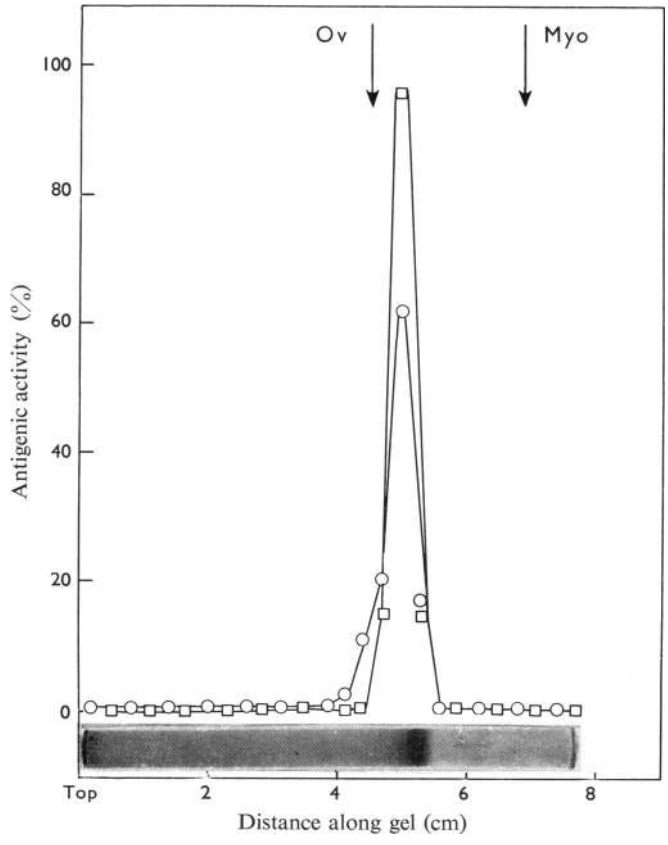
(*a*) 2ml of a 2% deoxycholate extract from thymocyte membranes prepared from 1.3×10^9 cells was applied to a 2.0ml Sepharose 4B–lentil lectin column (3mg of lectin/ml of beads) which was then washed with deoxycholate buffer (44ml) and eluted with 0.5M-methyl α-D-glucopyranoside in deoxycholate buffer; an arrow marks the beginning of elution. The collected fractions (2ml) were assayed for Thy-1.1 activity, which is expressed as the percentage of the activity applied to the column. (*b*) shows chromatography as in (*a*) except that the applied fraction was the material which did not bind to lentil lectin (fractions 2–5 from *a*). (*c*) shows chromatography as (*a*) except that the applied fraction was the material which had bound to lentil lectin (fractions 26–36 from *a*).



EXPLANATION OF PLATE 1

Purification of Thy-1_{L+} *analysed by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis*

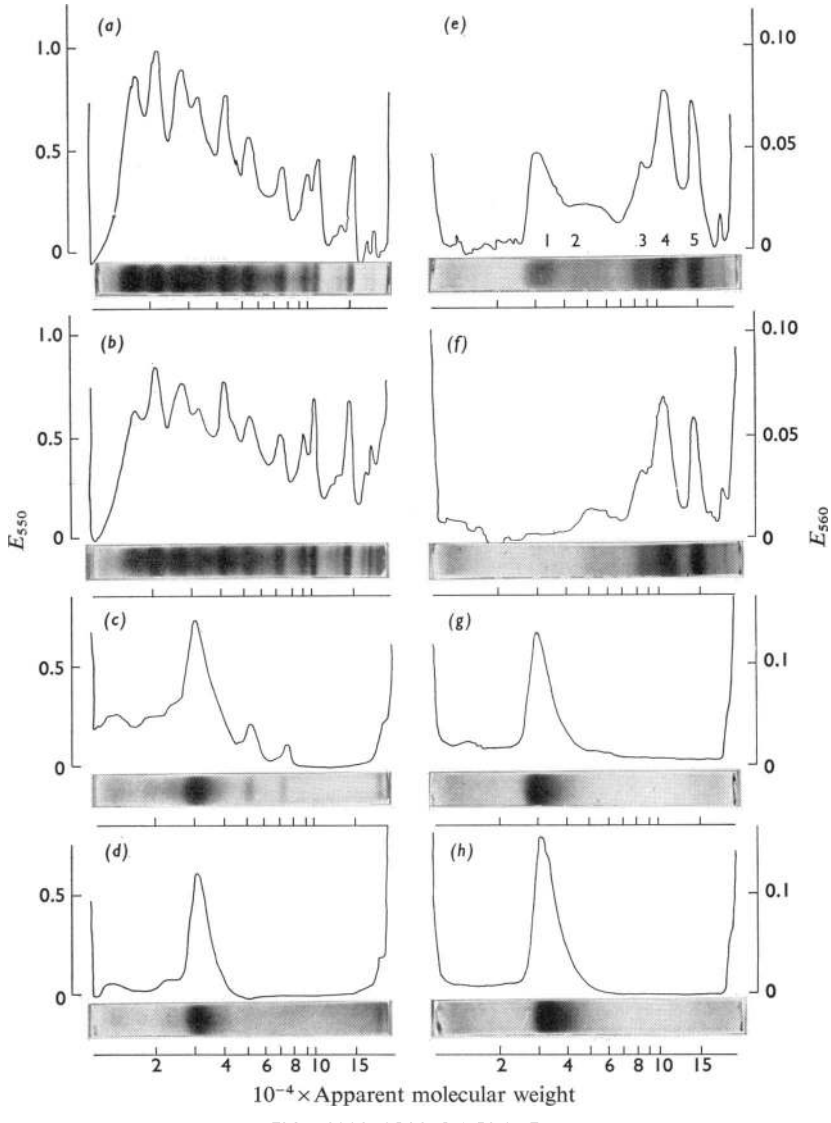
Fractions after reduction and alkylation were electrophoresed on 5.6% gels and stained with Coomassie Blue (*a*, *b*, *c*, *d*) or periodic acid–Schiff reagent (*e*, *f*, *g*, *h*). The following fractions are shown: Tween-40 membrane, 60μg (*a*) and 340μg (*e*); Thy-1.1 active fraction from Sephadex G-200, 50μg (*b*) and 150μg (*f*); lentil lectin unretarded fraction, 50μg (*c*) and 180μg (*g*); lentil lectin absorbed and specifically eluted fraction, 13μg (*d*) and 30μg (*h*).



EXPLANATION OF PLATE 2

*Assay of Thy-*1.1 *and Thy-*1 *xenoantigenic activity after sodium dodecyl sulphate–polyacrylamide-gel electrophoresis*

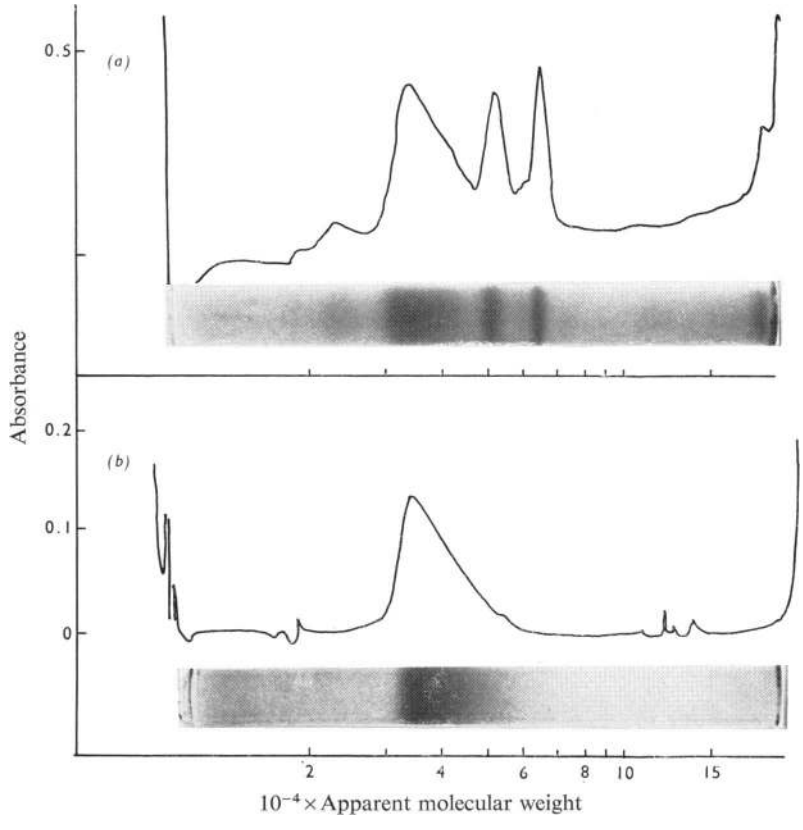
2.5μg of Thy-1 purified by the lentil lectin column was electrophoresed on a 5.6% acrylamide gel containing 0.1% sodium dodecyl sulphate with ^{125}I-labelled whale myoglobin (Myo) and ^{125}I-labelled hen ovalbumin (Ov) as markers. After electrophoresis the gel was sliced and assayed for Thy-1.1 activity (○) and Thy-1 xenoantigen activity (□) as described under 'Methods'. Also shown is the photograph of a gel on which 7μg of Thy-1 was electrophoresed under the same conditions and stained with Coomassie Blue.



EXPLANATION OF PLATE 3

Purification of Thy-1_{total} *analysed by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis*

Fractions, after reduction and alkylation, were electrophoresed on 5.6% gels and stained with Coomassie Blue (*a*, *b*, *c*, *d*) or periodic acid–Schiff reagent (*e*, *f*, *g*, *h*). The following fractions are shown: deoxycholate extract of Tween-40 membrane, 80μg (*a*) and 350μg (*e*); deoxycholate extract depleted of Thy-1 antigens, 80μg (*b*) and 350μg (*f*); fraction eluted from antibody column by pH11.5 buffer, 20μg (*c*) and (*g*); eluted fraction after Sephadex G-200 chromatography, 20μg (*d*) and (*h*).



EXPLANATION OF PLATE 4

Thy-1_{L-} analysed by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis

23μg of the Thy-1_{L-} fraction obtained by using the antibody column (Table 3) was electrophoresed after reduction and alkylation on 5.6% gels and stained with Coomassie Blue (*a*) or periodic acid–Schiff reagent (*b*).

sulphate caused a tenfold decrease in the Thy-1.1 antigenic activity compared with that in deoxycholate buffer. However, the inhibition curve was not altered in shape and analysis of the gels was possible. Plate 2 shows that Thy-1.1 antigenic activity was in the same position on the gel as the major protein band visible if a gel run in the same conditions was stained with Coomassie Blue. In addition to assaying Thy-1.1 activity in this experiment, Thy-1 xenoantigen activity was also measured. As shown below, the glycoprotein purified with lentil lectin has strong Thy-1 xenoantigenic activity as well as Thy-1.1 activity, and this activity coincided with Thy-1.1 on the polyacrylamide gels (Plate 2).

Thus it appeared that the glycoprotein bound to, and eluted from, lentil lectin–Sepharose 4B was pure Thy-1_{L+}, and that the residual glycoprotein found in the unretarded fraction (Plate 1*g*) was Thy-1_{L-}. If this were so, it was possible that another agglutinin might bind the Thy-1_{L-}. Concanavalin A was tried for, although it binds to the same sugar residues as lentil lectin, it has a binding constant for methyl α-D-mannoside and methyl α-D-glucopyranoside 50 times higher than that of lentil lectin (Stein *et al.*, 1971). Thus the Sephadex G-200 fraction containing Thy-1.1 activity was passed twice through a lentil lectin column and then chromatographed on a concanavalin A–Sepharose B absorbent. Approx. 50% of the activity was bound and could be eluted with methyl α-D-glucopyranoside. This supported the view that Thy-1_{L-} was also a glycoprotein.

Affinity chromatography with anti-(brain Thy-1) antibody columns

To further substantiate the above results another affinity method for purification was tried. Antibody against purified Thy-1 from rat brain was coupled to Sepharose 4B and used to absorb antigen from a deoxycholate extract of thymocyte membrane. Throughout the purification both Thy-1.1 and Thy-1 xenoantigens were assayed.

Details of the affinity chromatography are described in Fig. 3 and Table 2. When deoxycholate extract was passed through the antibody affinity column (Fig. 3*a*), antigen was depleted from a large volume, and, when the column was saturated, both Thy-1.1 and Thy-1 xenoantigens appeared simultaneously in the eluate.

Sodium dodecyl sulphate–polyacrylamide-gel electrophoresis was carried out on the starting extract and on the antigen-depleted effluent (at 60ml in Fig. 3) and the results are shown in Plate 3(*a*) and 3(*b*) for protein and Plate 3(*e*) and 3(*f*) for carbohydrate stains. No clear removal of any protein band can be associated with absence of antigen, but the removal of periodic acid–Schiff-staining band 1 occurred in the antigen-depleted fractions. After

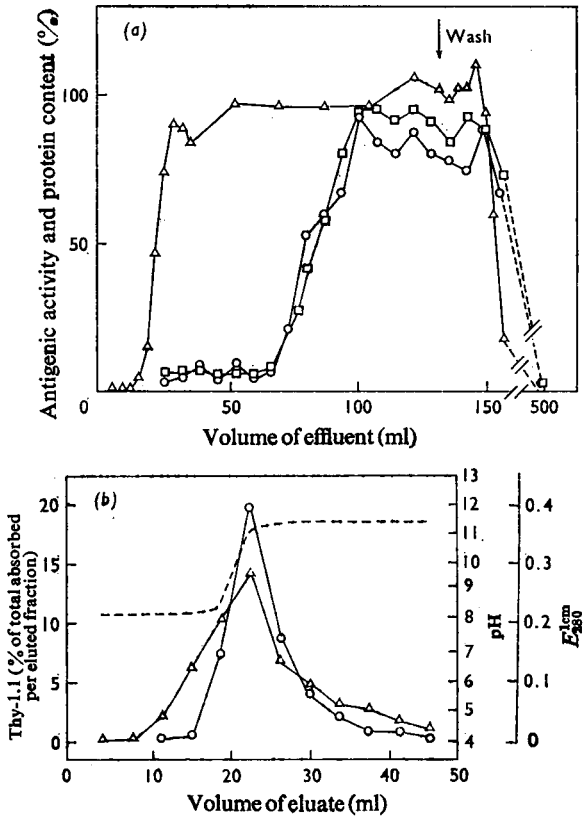


Fig. 3. *Affinity chromatography for Thy-1 with an antibody–Sepharose 4B column*

Membrane from 8×10^{10} thymocytes was solubilized in 160ml of 1% deoxycholate, and 140ml of the extract was passed through a 15ml column of Sepharose 4B to which was coupled rabbit anti-(rat brain Thy-1) antibody. As shown in (*a*), the effluent was assayed for protein (△), Thy-1.1 antigen (○) and Thy-1 xenoantigen activity (□) and the results are plotted as a percentage of the antigenic activity or protein content of the starting extract (100% = 450μg/ml of protein, 1.8×10^5units/ml of Thy-1.1 antigen, and 7.6×10^3units/ml of Thy-1 xenoantigen). After washing, the column was eluted with pH11.5 buffer as in (*b*), where the pH (——), E^{1cm}_{280} (△) and Thy-1.1 antigen activity (○) of the eluted fractions are shown. Antigen activity is plotted as percentage of the total absorbed antigen recovered per eluted fraction, and each point represents one fraction.

the column became saturated with antigen, the periodic acid–Schiff-staining band 1 reappeared (not shown but identical with those in Plate 3*a* and 3*e*).

Elution of the column with 0.05M-diethylamine buffer, pH11.5, is shown in Fig. 3*b*. It can be seen that 44% of the absorbed Thy-1.1 antigenic activity was

Table 2. *Purification of Thy-1_{total} with antibody columns*

Affinity chromatography with antibody columns was as described in Fig. 3 and data shown below are pooled from the experiment in Fig. 3 plus one other.

Fraction	Protein (mg)	Thy-1.1 antigen $10^{-4}\times$ units	Thy-1.1 antigen Relative sp. activity	Thy-1.1 antigen Yield (%)	Thy-1 xenoantigen $10^{-4}\times$ units	Thy-1 xenoantigen Relative sp. activity	Thy-1 xenoantigen Yield (%)
Membrane extract from which antigen was depleted	43	17.3	1	100	74.3	1	100
Fraction eluted by pH11.5 buffer	2.1	8.8	10.5	51	40	11	54
Fraction after Sephadex G-200 column chromatography	0.7	5.3	19	31	25	22	35

recovered in a peak associated with material having absorbance at 280nm. This peak also contained 54% of the absorbed Thy-1 xenoantigen activity (Table 2). Analysis of the eluted material by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis showed one main protein band with an apparent mol.wt. of 32000, plus a number of less-prominent bands (Plate 3*c*). Only one periodic acid–Schiff-staining band was found and this corresponded to the major protein band (Plate 3*h*). For further purification the material eluted from the affinity column was chromatographed on Sephadex G-200 and the Thy-1.1 antigenic activity was eluted at a Stokes radius of 3.0nm as before. Analysis of this fraction by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis showed only the major protein band (Plate 3*d*) and periodic acid–Schiff-staining band 1 (Plate 3*h*). (This preparation is henceforth referred to as 'Thy-1_{total}'.)

Parameters of the purification by antibody affinity chromatography are summarized in Table 2 for Thy-1.1 and Thy-1 xenoantigen. It is noteworthy that yields and purification are calculated with respect to deoxycholate extract and thus yield from starting cells would be about half of that shown, and the purification factor about ten times greater (compare with Table 1).

Thus the results of purification by an antibody column were consistent with those obtained by lectin affinity chromatography and supported the view that the part of periodic acid–Schiff-staining band 1 not retarded by lentil lectin was Thy-1_{L-}, since all of this band was removed by the antibody column. To further establish this, the antibody column was used to purify Thy-1_{L-} from a Sephadex G-200 fraction which had been passed twice through a lentil lectin column. Thy-1.1 and Thy-1 xenoantigenic activities were depleted from the passed fraction, as found for whole extract in Fig. 3(*a*), and analysis by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis showed complete removal of periodic acid–Schiff-staining band 1 found in the starting material shown in Plate 1(*g*).

When the column was eluted with pH11.5 buffer, 41% of the Thy-1.1 antigen was recovered and the eluted fraction was similar after electrophoresis to that eluted from whole extract (Plate 3*c*), with the exception of one extra major polypeptide band. This band had the same mobility as lentil lectin and it was considered likely that it was lectin which had eluted from previous columns and then bound to carbohydrate on the antibody affinity column. This view was confirmed when the band was removed without loss of antigenic activity by an anti-(lentil lectin) affinity column (see under 'Methods').

Details of the recovery of antigenic activity and of the purification are given in Table 3, and the final purification approached that for Thy-1_{L+} (Table 1). In Plates 4(*a*) and 4(*b*) are shown gels stained for protein and carbohydrate after electrophoresis of the eluted fraction after removal of contaminating lectin. The fraction contained one carbohydrate band running in approximately the same position as periodic acid–Schiff-staining band 1, but the band was broader, indicating apparent heterogeneity (see below). The protein pattern showed one major band (50–60% of the stain by densitometry), which was in the same position as the carbohydrate band, and two prominent contaminant proteins of higher molecular weight. Analysis of antigenic activity after electrophoresis as in Plate 2 showed association with the glycoprotein, but not with the contaminant bands.

Antigenicity of the various preparations

To obtain a direct comparison of the specific activities of the various Thy-1 preparations, inhibition assays were done at the same time for Thy-1.1 antigen and for the Thy-1 xenoantigens. The results are shown in Table 4 as the protein (in ng) of each fraction required for 50% inhibition of the respective binding assays. This provided a valid comparison as the inhibition curves for the three preparations were roughly parallel in each assay.

Table 3. *Purification of Thy-1_{L-} by an antibody column*

Fraction	Protein (mg)	Thy-1.1 antigen $10^{-4}\times$ units	Thy-1.1 antigen Relative sp. activity	Thy-1.1 antigen Yield (%)	Thy-1 xenoantigen $10^{-5}\times$ units	Thy-1 xenoantigen Relative sp. activity	Thy-1 xenoantigen Yield (%)
Extract after Sephadex G-200 chromatography and passage through a lentil lectin column	10.5	8.0	1	100	2.8	1	100
Fraction eluted by pH11.5 buffer and after passage through an anti-(lentil lectin) column	0.51	2.7	7	34	1.1	8	39

Table 4. *Amount of antigen preparation needed for 50% inhibition of standard binding assays*

Values shown are means of the individual determinations given in parentheses.

Thy-1 fraction	Amount of fraction (ng) needed for 50% inhibition of assay for: Thy-1.1	Thy-1 xenoantigens
Thy-1_{L+}	7.8 (6, 7, 10.3)	1.9 (1.7, 1.8, 2.1)
Thy-1_{total}	12.6 (11, 11.6, 15.2)	2.8 (2.1, 3.2, 3.1)
Thy-1_{L-}	19 (17, 21)	4.5 (4.3, 4.8)

In all cases less protein was needed for the inhibition of the Thy-1 xenoantigen assays than for Thy-1.1. This may be due to the higher affinity of the hyperimmune rabbit antiserum and is in agreement with previous observations (Morris *et al.*, 1975). The three separate preparations of antigen all carried the same antigenic determinants, since all gave total inhibition of each assay. In terms of specific activity all preparations were of the same order, except that Thy-1_{L-} had a lower activity, as would be expected as it is less pure when analysed by sodium dodecyl sulphate – polyacrylamide-gel electrophoresis. It is also possible that there was some loss of antigenic activity in the preparations purified by antibody columns, since Thy-1_{total} appears as pure as Thy-1_{L+}, yet had a lower specific activity.

Further characteristics of the purified preparation determined by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis

Thus far sodium dodecyl sulphate–polyacrylamide-gel electrophoresis has been used as a criterion of purity of reduced and alkylated antigen preparations; in addition to this, electrophoresis was carried out to further characterize the antigens.

The effect of reduction was first examined, and unreduced antigen ran with the same mobility as reduced and alkylated material in 5.6% gels.

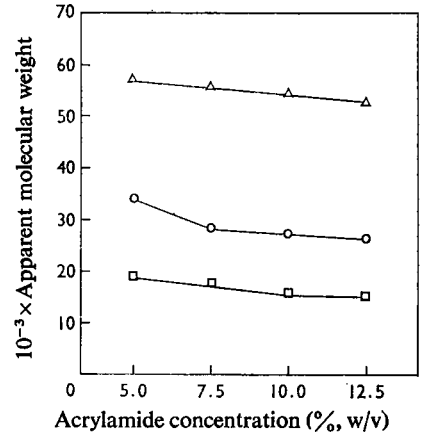


Fig. 4. *Apparent molecular weight of Thy-1_{L+} on gels of different acrylamide concentrations*

The molecular weight of Thy-1_{L+} (○), fetuin (△) and avidin (□) was estimated after sodium dodecyl sulphate–polyacrylamide-gel electrophoresis by using gels at four different acrylamide concentrations as shown on the abscissa.

Secondly, the effect on electrophoresis of the concentration of acrylamide in the gel was determined and apparent molecular weights were measured. It is known that reduced and alkylated polypeptides electrophorese in sodium dodecyl sulphate with a mobility proportional to log(mol.wt.) (Weber & Osborn, 1969), whereas many glycoproteins behave anomalously (Glossmann & Neville, 1971; Segrest *et al.*, 1971). Usually the glycoproteins appear larger than predicted (compared with protein markers), particularly when run on gels with a low percentage of acrylamide. In Fig. 4 the apparent molecular weight is plotted against acrylamide concentration and the result compared with that obtained by using two other glycoproteins, fetuin and avidin. The apparent

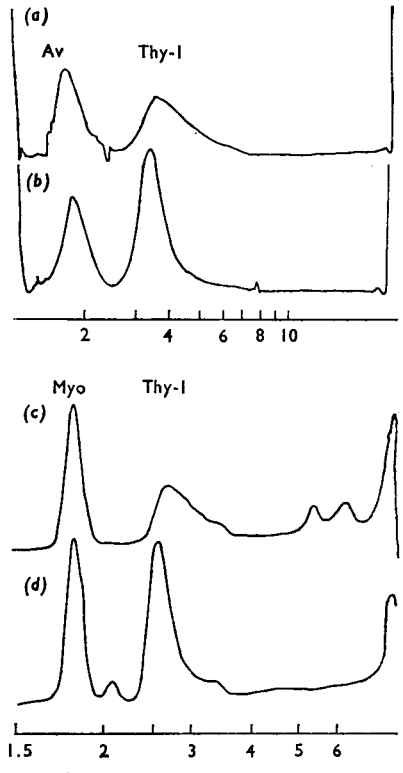


Fig. 5. *Differences between Thy-1 preparations after sodium dodecyl sulphate–polyacrylamide-gel electrophoresis*

(*a*) Thy-1_{L-} (23 μg) and (*b*) Thy-1_{L+} (17 μg) were electrophoresed with 50 μg of avidin on 5.6% gels and stained with periodic acid–Schiff reagent. (*c*) Thy-1_{L-} (23 μg) and (*d*) Thy-1_{L+} (17 μg) were co-electrophoresed with 5 μg of myoglobin in 12.5% gels and stained with Coomassie Blue. All samples were reduced and alkylated.

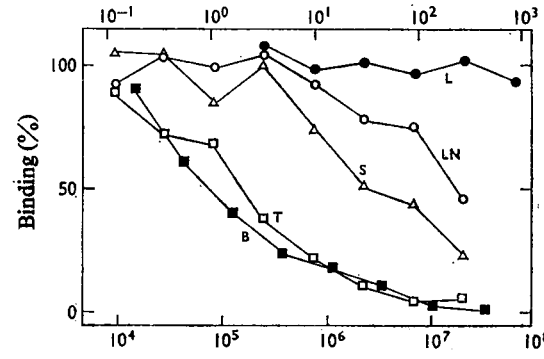


Fig. 6. *Inhibition of the binding of rabbit anti-Thy-1_{L+} antibodies to rat thymocytes*

Antiserum at a dilution of 1/50 was pre-absorbed with the amounts of tissue shown on the abscissa and after centrifugation was assayed for residual binding by using indirect binding assays described by Morris & Williams (1975). Excess of rat thymocyte target cells was used, and the second antibody [^{125}I-labelled horse anti-(rabbit IgG)] was added in saturating amounts (20 μg/ml). Inhibition by liver homogenate (●), lymph-node cells (○), spleen cells (△), thymocytes (□) and brain homogenate (■) is shown. One abscissa (defining inhibiting cell numbers) applies to □, △ and ○, and the other (defining protein) applies to ■, ● and □.

mol.wt. of the Thy-1_{L+} fell from 34000 to 26000 in going from 5.0 to 12.5% gels. The more correct value is likely to be that determined from the 12.5% gels since both avidin and fetuin approach their correct mol.wts. of 15600 and 50500 respectively on the 12.5% gel (Glossmann & Neville, 1971). The apparent mol.wt. was determined a number of times on 12.5% gels and a mean±S.E.M. of 25300±240 (four determinations) was obtained for Thy-1_{L+}. In contrast, the apparent mol.wt. of Thy-1_{L-} was 27200±390 (five determinations). This difference was also found on 5.6% gels, where the values were 31600±400 (five determinations) and 34800±380 (seven determinations) for Thy-1_{L+} and Thy-1_{L-} respectively.

Finally, the apparent heterogeneity, particularly of Thy-1_{L-}, was analysed by using sodium dodecyl sulphate–polyacrylamide-gel electrophoresis. Thy-1_{L+} and Thy-1_{L-} were electrophoresed along with markers to act as internal standards, and the gels were stained for protein or carbohydrate. Typical results of gel scans are shown in Fig. 5, where (*a*) shows electrophoresis on 5.6% gels of Thy-1_{L-} and (*b*) that for Thy-1_{L+}, both stained with periodic acid–Schiff reagent; the marker is avidin. Figs. 5(*c*) and 5(*d*) also show Thy-1_{L-} and Thy-1_{L+} respectively, but electrophoresis was on 12.5% gels which were stained for protein, and the marker was myoglobin. For both carbohydrate and protein stain, and on 5.6 and 12.5% gels, Thy-1_{L-} was more heterogeneous in the direction of high molecular weight, but not of lower molecular weight as compared with Thy-1_{L+}. In Fig. 5 the small differences in apparent molecular weight between Thy-1_{L-} and Thy-1_{L+} can be seen when gels (*a*) and (*b*), and (*c*) and (*d*), are compared. [As Thy-1_{L-} is heterogeneous towards higher-molecular-weight forms, readings of mobility on gels were taken to the leading peak (Plate 4 and Fig. 5), which is most comparable with

the more symmetrical peak of Thy-1_{L+} (Plate 1*d* and Fig. 5).]

Immunogenicity of purified Thy-1

To further check that the purified glycoprotein was identical with the Thy-1 molecule as defined by its antigenicity, the antisera raised by immunization of two rabbits with Thy-1_{L+} was analysed (see under 'Antibodies' for the immunization schedule). From previous observations (Morris & Williams, 1975) rabbit antisera against Thy-1 would be expected to contain antibodies against a component absent from liver, but found in large amounts on brain and thymocytes and in very much smaller amounts on spleen and lymph-node cells. Both antisera against the pure preparation showed this pattern in absorption analysis which is illustrated for one in Fig. 6.

The antibody could be further analysed for specificity against Thy-1.1 antigen, Thy-1 rat-specific xenoantigen and Thy-1 rat–mouse cross-reacting xenoantigen. This was done as described previously (Morris & Williams, 1975) by comparing absorption of binding by rat brain with that by A/JAX (Thy-1.2) and A/Thy-1.1 mouse brain. The two antisera were 45% and 60% specific for rat xenoantigen respectively, and in both cases the rest of the antibody was against the rat and mouse cross-reacting xenoantigen. The absence of significant antibody against Thy-1.1 was not unusual in that analysis of anti-(rat brain) sera binding to thymocytes showed that the appearance of anti-(Thy-1.1) antibodies was an inconsistent event (Morris & Williams, 1975).

Discussion

Procedures for the purification of Thy-1.1

With the procedures summarized in Fig. 1 the Thy-1 glycoprotein can be obtained in two forms and in large enough amounts for chemical analysis. The method using lentil lectin is simple and effective, the only problem being that the lectin appears to be continuously eluted in small amounts from the columns. Lentil lectin exists as a dimer of non-covalently bound subunits (Howard *et al.*, 1971), and if one monomer was coupled to the Sepharose 4B the other could dissociate and be eluted. Lectin can be simply removed from a preparation with an antibody affinity column (see under 'Methods'), and this is possibly the best method for removing minor contamination because even inactivated lectin would probably retain its antigenicity.

Antibody columns should also be very useful in further studies on the Thy-1 molecule. Thy-1_{L-} can be obtained, and also preparations of Thy-1_{total} can be made. Considerable improvement of the method as reported here may be possible with the use of more concentrated antibody, which would increase the specific binding relative to non-specific absorption. Obviously a one-step affinity-chromatography procedure resulting in pure antigen would be desirable.

Properties of the rat Thy-1 *molecule*

The purifications described above have established that the rat Thy-1 molecule is a glycoprotein and confirm the previous observations (Morris *et al.*, 1975) that it carries xenoantigenic specificities as well as the Thy-1.1 antigen. Heterogeneity in the carbohydrate portion of the glycoprotein was suggested by the finding that only about half of the antigen would bind to lentil lectin columns. Estimates of molecular size by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis gave apparent mol.wt. values of 25300 and 27200 for the main bands of Thy-1_{L+} and Thy-1_{L-} respectively. Both values are in reasonable agreement with an estimate of 28000 for the mol.wt. of Thy-1.1 antigen in deoxycholate, based on determination of hydrodynamic properties (Letarte-Muirhead *et al.*, 1974). As this latter estimate includes deoxycholate bound to the antigen, and as the technique of sodium dodecyl sulphate–polyacrylamide-gel electrophoresis is not reliable for estimates of the molecular weights of glycoproteins, all these values may be overestimates of the true molecular weight. With the pure preparations of Thy-1 an accurate estimate of molecular weight should be possible by using the methods of Tanford *et al.* (1974).

Further heterogeneity seemed to exist in Thy-1_{L-}, since this preparation did not run as a sharp band on sodium dodecyl sulphate–polyacrylamide-gel electrophoresis. The heterogeneity was towards apparently higher-molecular-weight forms, and this, together with the larger size of the main band, is consistent with the existence of extra carbohydrate on this form of Thy-1. It is possible that addition of carbohydrate obscures lentil-lectin-binding sites, but this can only be established by chemical analysis.

The rat Thy-1 glycoprotein has a single polypeptide chain as judged by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis of reduced and unreduced antigen. Histocompatibility antigens are associated with a shorter polypeptide-chain-$\beta 2$ microglobulin, which occurs in large amounts in the membranes of most tissues (Tanigaki & Pressman, 1974). No evidence for this was obtained in association with Thy-1. Further, the Thy-1 molecule shows no tendency to form polymers, as appears to be the case with the major erythrocyte glycoprotein (Slutzky & Ji, 1974; Tuech & Morrison, 1974).

The above properties of the rat Thy-1 molecule are at variance with suggestions of some workers as to the molecular nature of mouse Thy-1. Atwell *et al.*

(1973) have suggested that Thy-1.2 antigen is a protein of mol.wt. 60000, and other groups of workers (Vitetta *et al.*, 1973; Esselman & Miller, 1974) have suggested that the antigens are on a glycolipid molecule. In contrast with this, Sauser *et al.* (1974) have identified mouse Thy-1.2 as a molecule of size more similar to that of rat Thy-1. Moreover, Trowbridge *et al.* (1975) found that a molecule similar to rat Thy-1 could be precipitated from detergent extracts of iodinated mouse thymocytes with antiserum to rat brain Thy-1, which cross-reacts with mouse Thy-1 antigen (Barclay *et al.*, 1975). Conventional alloantisera would not precipitate the Thy-1 molecule, possibly owing to low affinity, and this may explain why Atwell *et al.* (1973) and Vitetta *et al.* (1973) obtained different results.

In preliminary experiments we have found that Thy-1.1 is obtained in good yield from membrane prepared by the Tween-40 method from AKR mouse thymocytes. After solubilization in deoxycholate the mouse antigen activity was found to have a Stokes radius of 3.0nm by gel filtration on Sephadex G-200 in deoxycholate. Moreover mouse thymocyte membrane gave a similar pattern of bands staining with periodic acid–Schiff reagent after sodium dodecyl sulphate–polyacrylamide-gel electrophoresis to that with rat membrane. In particular, a prominent band, equivalent to rat periodic acid–Schiff-staining band 1, was seen. Thus it seems possible that the mouse Thy-1 molecule will eventually be found to be very similar to its equivalent in the rat.

Quantitative importance of the Thy-1 glycoprotein in thymocyte membranes

The Thy-1 molecule is likely to constitute a significant proportion of the surface area of mouse and rat thymocytes. The number of Thy-1.2 antigenic sites per cell in the mouse was estimated to be >400000 (Hämmerling & Eggers, 1970), and Thy-1.1 in the mouse and rat, and Thy-1 xenoantigens in the rat, were found at about 600000 sites/cell (Acton *et al.*, 1974; Morris & Williams, 1975). Also, from sodium dodecyl sulphate–polyacrylamide-gel electrophoresis of thymocyte membranes, it is clear that Thy-1 is quantitatively a major glycoprotein. The results for membrane purified by the Tween-40 method are supported by those of Ladoulis *et al.* (1974) on membrane prepared by conventional techniques. They identified a major glycoprotein of apparent mol.wt. 27000 on rat thymocyte, but not spleen lymphocyte membrane. This glycoprotein was almost certainly the Thy-1 molecule, and its absence from spleen is consistent with the presence of only very small amounts of Thy-1 antigens in the spleen and lymph-node cells of the rat (Acton *et al.*, 1974; Morris & Williams, 1975).

Given that the Thy-1 glycoprotein can be clearly identified on gels after sodium dodecyl sulphate–polyacrylamide-gel electrophoresis by staining for carbohydrate, the presence of a similar molecule might be evident in gels of thymocyte membrane from other species. As stated above, this is so for mouse thymocytes, but the literature suggests that this does not hold for rabbit or calf thymocyte membranes (Schmidt-Ullrich *et al.*, 1974, 1975). Also, in our laboratory, no periodic acid–Schiff-staining band equivalent to rat Thy-1 could be identified in membrane prepared by the Tween-40 method from human thymocytes (Dr. J. Fabre, unpublished work). It would be surprising if a functionally equivalent glycoprotein were not present in other species, and it may be that sodium dodecyl sulphate–polyacrylamide-gel electrophoresis gives misleading results for glycoproteins in comparison between species. In support of this is the finding that for erythrocyte membranes of different species there are marked differences in the major glycoprotein bands, whereas the protein band pattern is relatively constant (Hamaguchi & Cleve, 1972).

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