Biochem. J. (1975) **151**, 699–706
Printed in Great Britain

Purification of the Thy-1 Molecule from Rat Brain

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(*Received* 20 *May* 1975)

The present paper describes the characterization of the Thy-1 molecule from rat brain. The molecule was recognized by its antigens, which could be solubilized from brain membrane with deoxycholate. In the solubilized form the Thy-1 antigens were associated with a homogeneous component with the following hydrodynamic properties: $s_{20,w}$ = 2.2S, $\bar{v}$ = 0.72ml/g and Stokes radius = 3.0nm. The mol.wt. of the deoxycholate–antigen complex was estimated to be 27000; these values are not significantly different from those obtained thymocyte Thy-1. Brain Thy-1 was further purified by affinity chromatography with lentil lectin coupled to Sepharose 4B, and more than 80% of the antigen was bound. The material eluted with methyl α-D-glucopyranoside was then filtered on a column of Sephadex G-200, and only one glycoprotein was found in the antigenically active fraction. On sodium dodecyl sulphate–polyacrylamide-gel electrophoresis the glycoprotein was very similar to the Thy-1 from thymocytes that binds to lentil lectin. Its apparent mol.wt. on 12.5% acrylamide gels was 24000, and it electrophoresed as a symmetrical band. Brain Thy-1 was antigenically indistinguishable from thymocyte Thy-1 when analysed with rabbit antisera raised against brain or thymocyte Thy-1.

Antigens of the Thy-1 (θ) molecule are found in large amounts on thymocytes and in the brain of adult mice and rats. In neonatal animals of the same species the antigens are present in low amounts in-brain, and they increase in specific activity from birth to reach adult values at about 3 weeks of age (Reif & Allen, 1964, 1966; Douglas, 1972; Acton *et al.*, 1974; Morris & Williams, 1975). This increase correlates with developmental events such as the formation of synaptic junctions, the adoption of behavioural patterns and the onset of bioelectric activity (Aghajanian & Bloom, 1967; Tilney, 1933; Deza & Eidelberg, 1967), and precedes the main period of myelination (Davison & Peters, 1970). Other cell-surface antigens show a similar increase in specific activity (Schachner & Hämmerling, 1974; Schachner, 1974).

The Thy-1 antigen is found in all regions of mouse and rat brain in various amounts (Reif & Allen, 1964; Moore *et al.*, 1971; Grabar *et al.*, 1974), but there may be considerably less on peripheral nerves (R. T. Acton, J. Addis, G. F. Carl & W. F. Bridgers, unpublished work). In cultures of foetal mouse brain, Thy-1 is present on neuronal-like cells and on an indistinct class of cells, but not on glial-like cells (Mirsky & Thompson, 1975). The expression of Thy-1 antigen on neuronal cell lines is variable, with small amounts present on one mouse neuroblastoma of peripheral sympathetic origin (Schachner, 1973), but apparently not on others (Joseph & Oldstone, 1974). Thy-1.1 is found on some rat neuronal cell lines (R. T. Acton, J. Addis, G. F. Carl & W. F. Bridgers, unpublished work). Although the data are confused, a differential localization of Thy-1 within nervous tissue may eventually be found.

The molecular nature of brain Thy-1 has not been established, although Esselman & Miller (1974) suggest that the Thy-1 antigens are on gangliosides in the mouse. In rat thymocytes there is no evidence for Thy-1 antigen on gangliosides, and a glycoprotein of apparent mol.wt. 25000 has been characterized as the Thy-1 molecule (Letarte-Muirhead *et al.*, 1975). It is important to establish whether the antigen is in the same form in thymocytes and brain since antigens can exist on different molecular types, as is clearly established by the existence of ABO blood-group antigen on glycolipid or glycoprotein molecules (Hakomari & Kobata, 1974).

Thus in the present paper we describe the purification of the Thy-1 molecule from rat brain, and its characterization in crude extracts and purified form. The antigen could be solubilized from crude brain membrane with deoxycholate, but not with non-ionic detergents (Letarte-Muirhead *et al.*, 1974), and was purified by affinity chromatography with lentil lectin, followed by gel filtration on Sephadex G-200.

Materials and Methods

Unless otherwise stated all procedures were at 0–4°C and all methods were as described in Letarte-Muirhead *et al.* (1975).

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Antibodies

To prepare rabbit anti-(rat brain Thy-1) sera, two rabbits which had been primed with brain glycoprotein obtained after lentil lectin affinity chromatography were further immunized with pure Thy-1 5 months later. The priming involved two intramuscular injections of 1 mg of glycoprotein in complete Freund's adjuvant, followed by subcutaneous injection of 1 mg in incomplete Freund's adjuvant. The boosting with pure Thy-1 involved three injections of 40 μg of Thy-1 given intravenously at weekly intervals. Animals were bled weekly after this.

Crude membrane preparation from brain and solubilization in deoxycholate

Whole brains were removed from rats of 6–12 weeks of age and frozen in liquid N_2 for storage at −70°C. This procedure had no effect on Thy-1.1 activity compared with fresh material. Brain homogenate was prepared by using a Potter–Elvehjem homogenizer with rat brains suspended in 10 vol. of 0.32 M-sucrose. The large debris was removed by centrifugation for 16000 *g*-min and the pellet washed once. The pooled supernatants were centrifuged for 2000000 *g*-min and the membrane pellet obtained was resuspended in 0.05 M-NaCl–0.01 M-Tris–HCl, pH 7.4, to give 12 mg of protein/ml. An equal volume of 4% (w/v) Lubrol-PX in the same buffer was added and the mixture stirred on ice for 60 min. The extract was centrifuged at 6000000 *g*-min and the pellet obtained was resuspended in 0.02% (w/v) NaN_3–0.01 M-Tris–HCl, pH 8.0, to give 12 mg of protein/ml. An equal volume of 4% (w/v) deoxycholate in the same buffer was then added, and the mixture was frozen and stored overnight at −20°C. On thawing it was centrifuged at 6000000 *g*-min and the pellet extracted again with 2% (w/v) deoxycholate in the above buffer. The supernatants were pooled to give the solubilized Thy-1 fraction.

Affinity chromatography with lentil lectin coupled to Sepharose 4B

A column (35 ml) of lentil lectin–Sepharose 4B (10 mg of lectin/ml of swollen beads) was used in a typical preparation of Thy-1 from 63 g of rat brain. The column was pre-washed with 1 M-methyl α-D-glucopyranoside in 1% (w/v) deoxycholate buffer, then with 0.5% (w/v) deoxycholate buffer. The solubilized brain membrane fraction was then passed through the column at 15 ml/h, and the column was washed with 3–4 column-volumes of 0.5% (w/v) deoxycholate buffer. Bound material was then eluted with 1 M-methyl α-D-glucopyranoside in 1% (w/v) deoxycholate buffer at 5 ml/h. Protein was estimated from the E_{280}^{1cm} readings, and Thy-1.1 antigenic activities were assayed.

Sucrose-gradient centrifugation

This was carried out in H_2O and ^2H_2O as described by Morris *et al.* (1975) except that 1% (w/v) deoxycholate was used in the gradients and ^{125}I-labelled myoglobin was used as an extra marker. Deoxycholate extract was sedimented on the gradients and for analysis in ^2H_2O the sample was dialysed against deoxycholate buffer in ^2H_2O before application.

Gel filtration in deoxycholate

To measure Stokes radius, and in preparative procedures, Pharmacia K-26 and K-50 upward-flowing columns containing Sephadex G-200 were used (Letarte-Muirhead *et al.*, 1974, 1975).

Concentration of fractions

Fractions were concentrated by ultrafiltration with a Bio Fibre 80 beaker (Bio-Rad Laboratories, Bromley, Kent, U.K.) dialysing simultaneously with 0.01 M-Tris–HCl, pH 8.0, where it was desirable to decrease the deoxycholate concentration (e.g. in concentrating the fractions after Sephadex G-200 chromatography in 0.5% deoxycholate). Further concentration was effected with an Amicon Diaflo ultrafiltration apparatus by using a PM 10 membrane which had been equilibrated with deoxycholate.

Analytical methods

Protein was determined by Hartree's (1972) modification of the method of Lowry *et al.* (1951) with bovine serum albumin as standard. Samples in deoxycholate were estimated either by comparing them with standards in the same concentration of deoxycholate or by trichloroacetic acid precipitation of the sample and the removal of deoxycholate with acetone.

Phospholipid was determined from the phosphorus present in a chloroform–methanol (2:1, v/v) extract. Phosphorus was determined by a modification of the method of Bartlett (1959) and 1 μg of phosphorus was taken to be equivalent to 25 μg of phospholipid.

Results

Estimation of the amount of Thy-1 in brain and the degree of purification required

It can be estimated that there is approx. 2.8 mg of Thy-1 per 1×10^{11} thymocytes (Letarte-Muirhead *et al.*, 1975). If the inhibition of the Thy-1.1 assay by brain homogenate is compared with that produced by thymocytes, an estimate of the amount of Thy-1 in brain can also be made. Assuming the same molecular weight as for thymocyte Thy-1 (see below),

Table 1. *Purification of brain Thy-1*

A unit of activity is defined as the amount of antigen needed to give 50% inhibition of the appropriate binding assay. Specific activity is the number of units of activity per mg of protein and is related to that of brain homogenate.

			Thy-1.1 antigen			Thy-1 xenoantigens		
Fraction	Protein (mg)	Phospholipid (mg)	$10^{-5}\times$activity (units)	Relative sp. activity	Yield (%)	$10^{-5}\times$activity (units)	Relative sp. activity	Yield (%)
Brain homogenate	7300	2900	18.7	1.0	100	81.5	1.0	100
Crude membrane pellet	2880	2000	13.0	1.8	70	65.5	2.0	80
Lubrol-PX pellet	1600	1200	8.0	2.0	43	63.0	3.5	77
Deoxycholate extract	1440	799	13.0	3.5	70	34.0	2.1	42
Eluted from lentil lectin	74	10	4.5	24	24	28.0	34	34
After Sephadex G-200 chromatography	2.0	<0.1	2.2	440	12	16.0	720	20

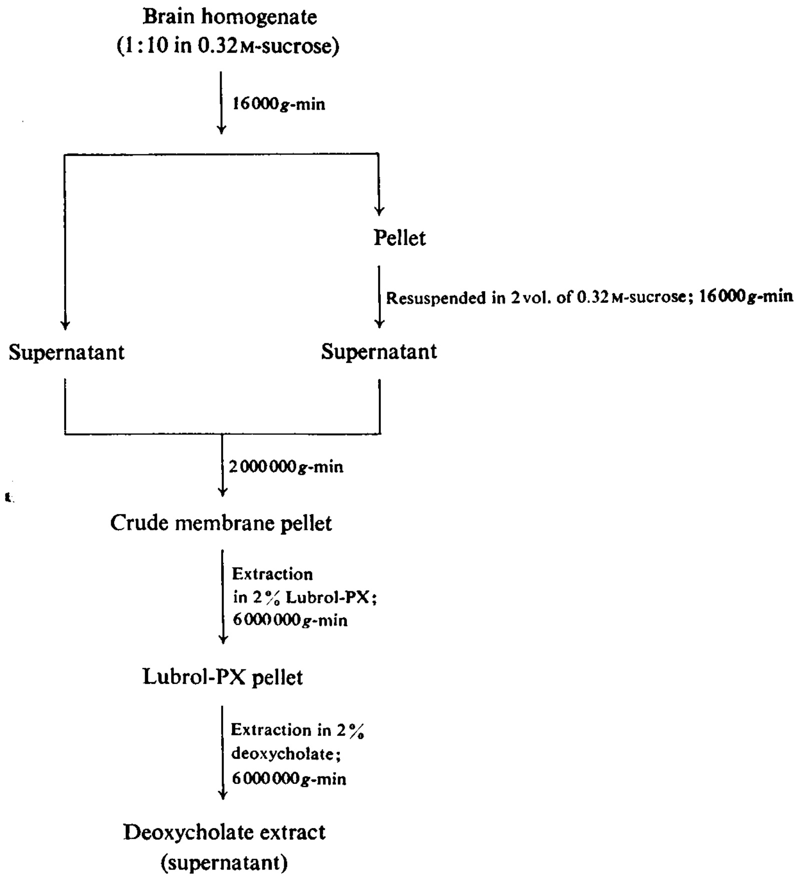


Fig. 1. *Scheme for the solubilization of brain Thy-1*

this amount would be 95 μg of Thy-1 antigen per g wet wt. of brain, or 145 μg per average rat brain. Thus on a per rat basis there is more Thy-1 in brain than thymus, which contains about 40 μg per animal (1.5×10^9 thymocytes).

In a typical preparation of brain Thy-1 (see below) 63 g wet wt. of brain was used, and this contained about 6.0 mg of Thy-1 compared with 7300 mg of protein (Table 1). Thus a purification of about 1200-fold should result in pure Thy-1.

Solubilization of Thy-1 antigens

The scheme for the solubilization of brain Thy-1 is shown in Fig. 1 and details of yields of antigen activity and of protein are listed in Table 1. The first step was the preparation of a crude membrane fraction from which solubilization in detergent was attempted. Non-ionic detergents did not inactivate Thy-1.1 antigen, but nor did they give effective solubilization (Letarte-Muirhead *et al.*, 1974). Deoxycholate did not solubilize crude membrane very well, but, if membrane was first extracted with 2% Lubrol-PX, effective solubilization of Thy-1.1 antigen was possible from the Lubrol-PX pellet. Moreover, solubilization was improved when membrane in deoxycholate was frozen–thawed before centrifugation. With the procedures outlined in the Materials and Methods section and in Fig. 1, about 50% of the Thy-1 antigens (Table 1) from brain homogenate were obtained in a 6×10^6g-min deoxycholate supernatant.

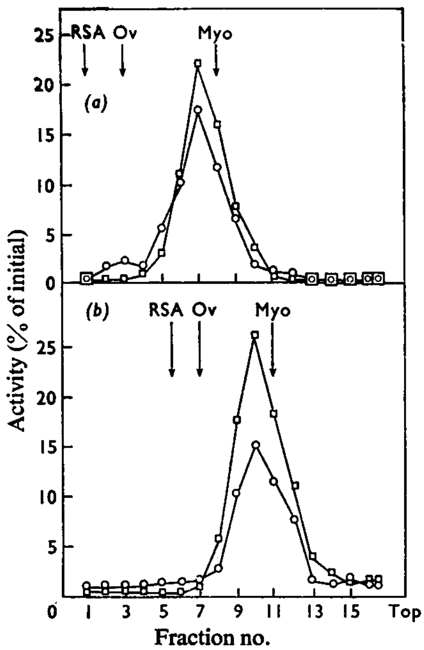


Fig. 2. *Sucrose-gradient centrifugation of solubilized Thy-1 in deoxycholate*

(*a*) shows the distribution of Thy-1.1 (○) and Thy-1 xenoantigenic (□) activities, after centrifugation for 40h at 44000rev./min ($r_{av.}$ 8.35cm), of deoxycholate extract of brain membrane on a 5–20% (w/v) sucrose gradient in 1% (w/v) deoxycholate buffer in H_2O. Activity is expressed as the percentage of total antigenic activity in the original extract. The positions of marker proteins sedimenting on the same gradient are shown: RSA, rat serum albumin; Ov, ovalbumin; Myo, myoglobin. (*b*) shows antigenic activities and marker positions after centrifugation as above but with gradients and extract prepared in ^2H_2O.

Sedimentation of brain Thy-1 on sucrose density gradients

To determine the nature of the solubilized antigen the deoxycholate extract was subjected to zone sedimentation on sucrose gradients in H_2O and ^2H_2O; such an analysis at an early stage in the purification may indicate whether multiple forms of the antigen exist.

The results are shown in Fig. 2 and the position of sedimentation of Thy-1.1 and Thy-1 xenoantigens is plotted in comparison with the positions of marker proteins. There is a high recovery of antigenic activity and both antigens move in an identical way as homogeneous components. The $s_{20,w}$ and $\bar{v}$ values were calculated as described by Meunier *et al.* (1972) and were 2.2S and 0.72ml/g respectively.

The results shown in Fig. 2 suggested that the antigens were solubilized in deoxycholate and gave no evidence for multiple forms of Thy-1 antigens in rat brain.

Affinity chromatography on lentil-lectin columns

To further purify Thy-1 from the deoxycholate extract, affinity chromatography on lentil lectin–Sepharose 4B columns was carried out. The results are shown in Fig. 3, and the major part of the protein,

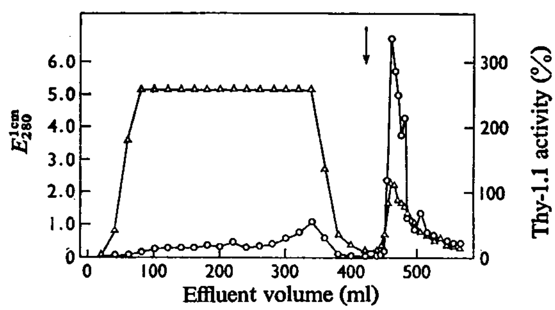
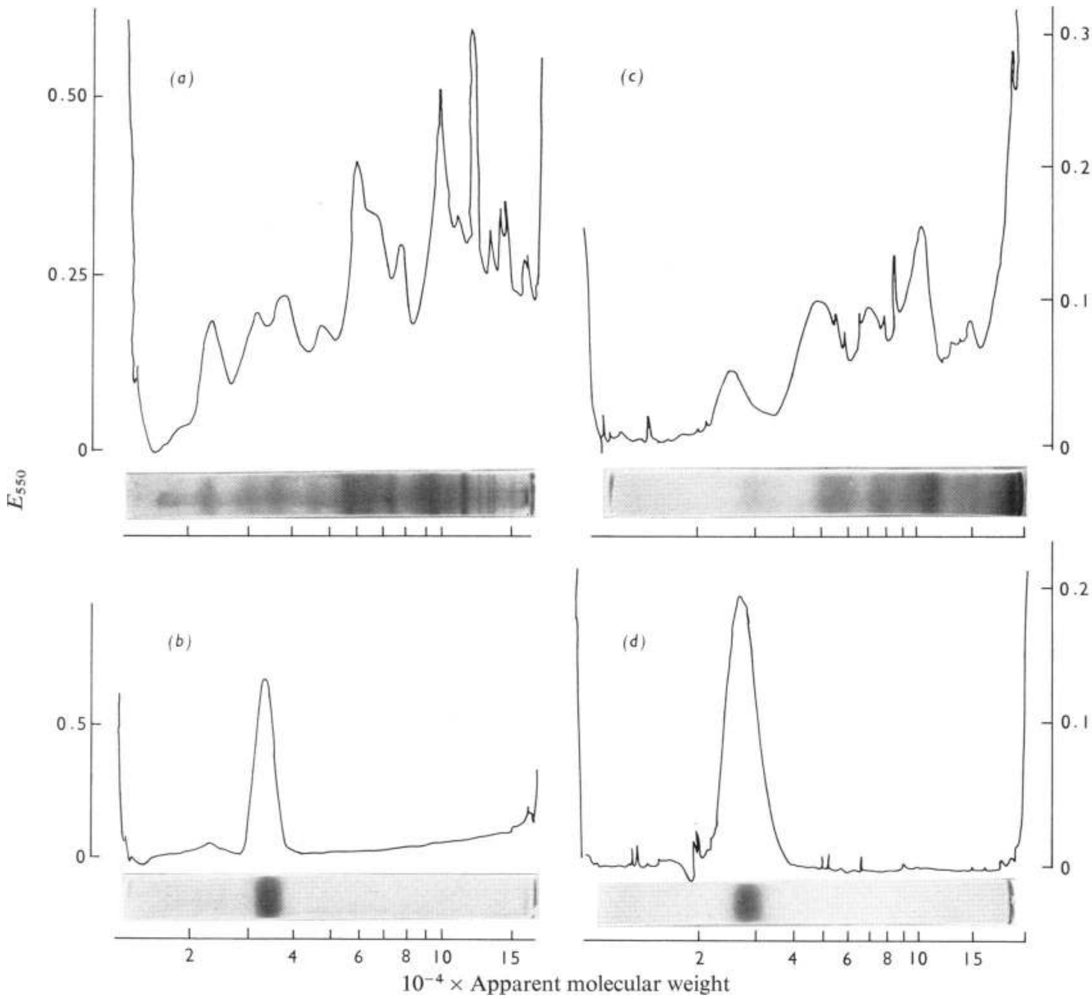


Fig. 3. *Affinity chromatography with lentil lectin–Sepharose 4B*

325ml of Thy-1 extract in deoxycholate was loaded on to a lentil lectin–Sepharose 4B affinity column (volume 35ml) and bound material was eluted with 1M-methyl α-D-glucopyranoside in 1% (w/v) deoxycholate buffer. Elution commenced at the point indicated by the arrow. Thy-1.1 antigenic activity was assayed (○) and expressed as a percentage of that in the starting extract (3600units of Thy-1.1 antigenic activity/ml). Protein is indicated by E^{1cm}_{280} (△); the background E^{1cm}_{280} values for 2% (w/v) deoxycholate and 1M-methyl α-D-glucopyranoside in 1% (w/v) deoxycholate buffer were 0.23 and 0.21 respectively.



EXPLANATION OF PLATE 1

Sodium dodecyl sulphate–polyacrylamide-gel electrophoresis of fractions during purification

The following samples were loaded on to 5.6% acrylamide gels in sodium dodecyl sulphate: 45 μg (*a*) and 220 μg (*c*) of material eluted from lentil lectin; 9 μg (*b*) and 22 μg (*d*) of the Thy-1 fraction after rechromatography on Sephadex G-200. Gels were stained for protein (*a* and *b*) or carbohydrate (*c* and *d*) and scanned at 550 nm and 560 nm respectively.

as indicated by E_{280}^{1cm} values, was not retarded, whereas the Thy-1.1 activity was bound, and was eluted with methyl α-D-glucopyranoside. In this experiment the affinity column was approaching saturation, but under non-saturating conditions (e.g. in the first fractions through the column) more than 80% of the Thy-1.1 activity was bound. This is in contrast with Thy-1 from thymocytes; in that case there is a clear distinction between the Thy-1 which binds to lentil lectin and that which is unretarded, the two forms being present in approximately equal amounts (Letarte-Muirhead *et al.*, 1975).

The yields obtained with the Thy-1 xenoantigen assay indicate that approx. 80% of the Thy-1 loaded on to the affinity column is eluted with methyl α-D-glucopyranoside. In terms of Thy-1.1 antigen activity there appeared to be a loss of Thy-1, but this may be more apparent than real, since the Thy-1.1 assay is less reproducible than the Thy-1 xenoantigen assay, and backgrounds are more affected by deoxycholate. The overall yield after the lentil lectin step was in reasonable agreement for the two assays. The profile in Fig. 3 clearly shows that this a particularly good purification step, since the major part of the Thy-1 activity is eluted with only 5% of the protein and less than 2% of the phospholipids loaded on to the affinity column (Table 1).

Gel filtration on Sephadex G-200 in deoxycholate

To further purify Thy-1, the material eluted from the lentil lectin column was chromatographed on Sephadex G-200. This was done on small columns (2.6cm×90cm) to measure Stokes radius, and on large columns (5.0cm×90cm) in preparative procedures. In Fig. 4 the result of column measurement of Stokes radius is shown, and Thy-1.1 antigen emerges at the same elution volume as ovalbumin and has a Stokes radius of 3.0nm (Tanford *et al.*, 1974). The antigen is separated from the bulk of the protein and the Sephadex G-200 column provides a very good purification step (Table 1). On some occasions gel filtration was repeated to achieve complete purification (see later).

Analysis of the purification by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis

From the results given in Table 1, Thy-1 should be a major component in the antigenically active fraction after gel filtration, and may also be detectable in the post-lentil-lectin fraction. The samples from these stages were analysed by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis and stained for carbohydrate and protein. The results are shown in Plate 1, and after gel filtration only one major glycoprotein band remains (Plate 1*b* and 1*d*), and this band can be detected in the material eluted from

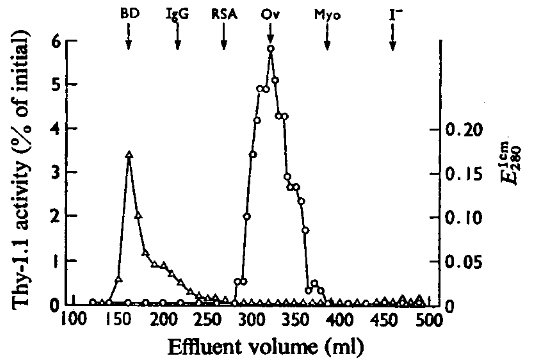


Fig. 4. *Chromatography of eluate from lentil lectin on Sephadex G-200 in deoxycholate*

5ml of eluate from lentil lectin was chromatographed on a column (2.6cm×90cm) of Sephadex G-200 in 0.5% (w/v) deoxycholate buffer. Fractions (5ml) were assayed for Thy-1.1 activity (○), which is expressed as the percentage of the activity of the loaded extract, and the E_{280}^{1cm} (△) measured. The elution position of protein markers run immediately before and after the fractionation are shown: IgG, bovine IgG (immunoglobulin G); RSA, rat serum albumin; Ov, ovalbumin; Myo, myoglobin. The void and total volumes are indicated by Blue Dextran (BD) and $Na^{125}I$ (I^-).

lentil lectin (Plate 1*a* and 1*c*). After one passage of this material through Sephadex G-200, a minor glycoprotein was also detected (not shown), but this could be eliminated by a second step of gel filtration (Plate 1*b* and 1*d*).

To be sure that the antigenicity was associated with the major glycoprotein, a gel was run in 0.1% sodium dodecyl sulphate, and antigenic activities were assayed from the gel in exactly the same way as described for thymocyte Thy-1 (Letarte-Muirhead *et al.*, 1975). Both Thy-1.1 and Thy-1 xenoantigens ran with the glycoprotein band.

Reduction and alkylation of Thy-1 had no effect on its mobility; thus the molecule consists of a single polypeptide chain.

The homogeneity and apparent molecular weight were also established after sodium dodecyl sulphate–polyacrylamide-gel electrophoresis and it can be seen from Plate 1(*b*) and 1(*d*) that brain Thy-1 electrophoreses as a symmetrical band. The apparent molecular weight by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis varied with acrylamide concentration in the same manner as did thymocyte Thy-1, and the mean±S.E.M. was 31300±1000 on 5.6% acrylamide gels (seven determinations) and 24100±150 (four determinations) on 12.5% gels.

Immunogenicity of brain Thy-1

To show further that the glycoprotein purified by this procedure was identical with the Thy-1 molecule, antisera were raised in two rabbits against purified brain Thy-1 (see the Materials and Methods section) and analysed for binding to thymocytes. From previous studies (Morris & Williams, 1975) such sera should have antibodies against a component absent from liver, but found in large amounts on thymocytes and brain and in much smaller amounts on spleen and lymph-node cells. This pattern was also obtained for rabbit anti-(thymocyte Thy-1) (Letarte-Muirhead *et al.*, 1975), and, when the anti-(brain Thy-1) was analysed in the same way, the same result was obtained for both sera.

The concentration of antibody was estimated as described by Morris & Williams (1975), and the bleeds of highest activity from one rabbit contained 0.3 mg/ml of anti(Thy-1), whereas the other contained 0.1 mg/ml.

The antisera were further analysed for their specificities against Thy-1 by absorption with A/Thy-1.1 and A/Jax congenic mouse brain and Wistar-rat brain. The results are shown for the more active serum in Fig. 5, and it can be seen that the absorption was 26, 30 and 100% for A/Jax and A/Thy-1.1 mouse and Wistar-rat brain respectively. Thus 70% of the antiserum was against the rat-specific Thy-1 xenoantigen, 26% against the rat–mouse cross-reacting xenoantigen and only 4% against Thy-1.1. This serum was used for antibody affinity columns to purify thymocyte Thy-1 (Letarte-Muirhead *et al.*, 1975).

In serum of the second rabbit, the antibody specificities as described above were 45, 37 and 18% respectively.

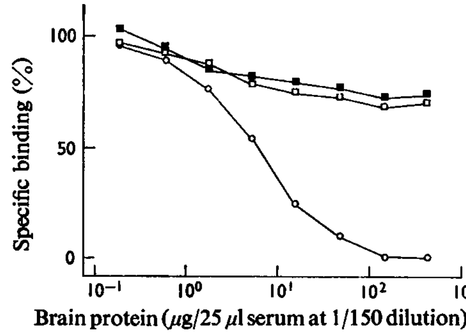


Fig. 5. *Analysis of the specificities of rabbit anti(brain Thy-1) serum*

Antiserum at a dilution of 1/150 was preincubated with brain homogenate from Wistar rat (○), A/Thy-1.1 mouse (□), or A/Jax mouse (■) and after centrifugation was analysed for residual binding by using indirect binding assays as 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).

Antigenicity of rat brain Thy-1

Antigenicity of brain Thy-1 and thymocyte Thy-1 was compared by studying inhibition of the binding of rabbit anti-(brain Thy-1), or rabbit anti-(thymocyte Thy-1_{L+}) sera (Letarte-Muirhead *et al.*, 1975) to thymocyte target cells. This analysis should reveal if thymocyte Thy-1 displays antigens not present on brain Thy-1. The results are shown in Fig. 6 and it is clear that the brain Thy-1 and thymocyte Thy-1 molecules cannot be distinguished in terms of their antigenicity. The two assay systems differed in their sensitivity to inhibition and this was probably due to differences in affinity rather than to antigenic specificity. The rabbit anti-(thymocyte Thy-1) serum was an early bleed after limited immunization, whereas the anti-(brain Thy-1) serum was obtained after prolonged immunization.

To summarize the similarities between brain Thy-1 and thymocyte Thy-1_{L+}, the amount of antigen required for 50% inhibition of the Thy-1.1 and Thy-1 xenoantigen assays is given in Table 2. The specific activities for the antigens are similar in all cases, and this is consistent with estimates of

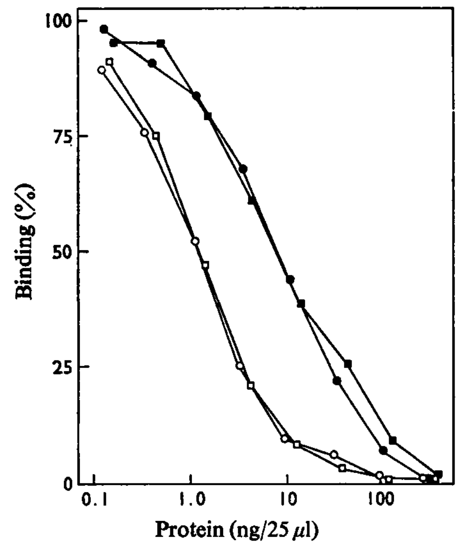


Fig. 6. *Inhibition of rat Thy-1 xenoantigen assays by purified Thy-1 from brain and thymocytes*

Rabbit anti-(brain Thy-1) serum (25 μl) at a dilution of 1/1500 was incubated with 25 μl dilutions of Thy-1 purified by lentil lectin affinity chromatography from brain (○) and thymocytes (□). Similarly rabbit anti-(thymocyte Thy-1) at 1/400 was incubated with Thy-1 from brain (●) and thymocytes (■). The binding of residual antibodies to excess of target cells (1 × 10^6 rat thymocytes per assay) was revealed with trace amounts of ^{125}I-labelled horse anti-(rabbit IgG) as described by Morris & Williams (1975) and expressed as a percentage of the binding of unabsorbed antiserum.

Table 2. *Amount of antigen preparation needed for 50% inhibition of standard binding assays*

Values are given as the means±S.E.M. with the numbers of determinations given in parentheses. Two assays for Thy-1 xenoantigens are used: (*a*), with rabbit anti-(brain Thy-1), and (*b*), with rabbit anti(thymocyte Thy-1).

	Amount of fraction (ng) needed for 50% inhibition of assays for		
		Thy-1 xenoantigens	
Thy-1 fraction	Thy-1.1	(*a*)	(*b*)
Thy-1 (brain)	8.5±1.1 (4)	1.4±0.1 (5)	5.4±2.0 (3)
Thy-1_{L+} (thymocytes)	10.5±2.3 (3)	1.9±0.3 (4)	8.0±3.3 (3)

purity for thymocyte Thy-1 and brain Thy-1 obtained by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis.

Discussion

Purification of Thy-1 from brain

The large quantities of Thy-1 in rat brain and the availability of the tissue make it a good source for the purification of quantities of antigen sufficient for chemical characterization. With thymocytes preparing membrane is a good initial purification step, but with the brain, already mostly membrane, this gives little purification. However, a relatively simple procedure using two detergents has been successful in purifying Thy-1 to homogeneity.

Hydrodynamic properties of Thy-1 in deoxycholate

Sucrose-gradient centrifugation and gel filtration in deoxycholate were used to determine some hydrodynamic parameters of Thy-1 by methods described in Letarte-Muirhead *et al.* (1974). These apply to antigen plus bound detergent and the molecular size is likely to be an overestimate as parameters are determined by comparison with marker proteins which bind much less deoxycholate than membrane proteins (Helenius & Simons, 1972). The following values were obtained: $s_{20,w}$, 2.2S; $\bar{v}$, 0.72ml/g; Stokes radius, 3.0nm; and from these a frictional coefficient of 1.52 and mol.wt. of 27000 were calculated. These values are not significantly different from those for Thy-1 from thymocytes (Letarte-Muirhead *et al.*, 1974).

Chemical nature of Thy-1

The Thy-1 antigen has been purified from brain and shown to be a single symmetrical glycoprotein band, by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis, of apparent mol.wt. 24100. This value is consistent with that of 27000 obtained above from the hydrodynamic properties for the deoxycholate–Thy-1 complex. The glycoprotein appears to constitute the main structure carrying Thy-1 antigens in brain, and no evidence for antigenically active gangliosides as reported by Esselman & Miller (1974) for mouse brain Thy-1 has been obtained.

The apparent mol.wt. of 24100 for brain Thy-1 determined by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis was consistently slightly lower than for thymocyte Thy-1 that binds to lentil lectin (Thy-1_{L+}, mol.wt. 25300), and considerably lower than the thymocyte Thy-1 that does not bind (Thy-1_{L-}, mol.wt. 27200). Thus the brain Thy-1 is more similar to thymocyte Thy-1_{L+}, and a brain equivalent of Thy-1_{L-} is yet to be found, although it could exist in the 10–20% of brain Thy-1 that did not bind to lentil lectin. In terms of antigenicity and immunogenicity, brain Thy-1 could not be distinguished from thymocyte Thy-1_{L+} or Thy-1_{L-}, and the differences between these three forms of Thy-1 must be established by chemical analysis.

Importance of Thy-1 in rat brain

The Thy-1 glycoprotein may play an important role in brain function, and the increase in the amount of antigen during development is perhaps significant. The clear determination of the localization of Thy-1 in the brain, yet to be achieved, may also suggest possible functions. The studies on distribution of Thy-1 antigens referred to in the introduction indicate that preferential localization may be found, but so far this has been mainly limited to studies by absorption analysis using fractions of brain. Localization by immunological techniques on histological sections has been attempted (Moore *et al.*, 1971; Grabar *et al.*, 1974), but convincing results are yet to be obtained, probably because specific antisera of high concentration have not been available.

As an alternative to examining antigen, the distribution of Thy-1 glycoprotein could be determined. A number of workers have studied glycoprotein of brain synaptosomal membrane, and bands produced on sodium sulphate–polyacrylamide-gel electrophoresis that could indicate Thy-1 were only sometimes found (Banker *et al.*, 1972; Morgan *et al.*, 1973; Zanetta *et al.*, 1975; Waehneldt *et al.*, 1971; Gurd *et al.*, 1974). Given the characterization of Thy-1 glycoprotein in the present paper, more definitive experiments should now be possible.

We are grateful to Marilyn Simpkins for expert technical assistance, to Mr. F. Caddick for photographs of gels, and also to Professor R. R. Porter for advice and encouragement. M. L.-M. was a Centennial Fellow of

The Medical Research Council of Canada and A. N. B. was supported by a Medical Research Council Research Studentship.

References

Acton, R. T., Morris, R. J. & Williams, A. F. (1974) *Eur. J. Immunol.* **4**, 598–602
Aghajanian, G. K. & Bloom, F. E. (1967) *Brain Res.* **6**, 716–727
Banker, G., Crain, B. & Cotman, C. W. (1972) *Brain Res.* **42**, 508–513
Bartlett, G. R. (1959) *J. Biol. Chem.* **234**, 466–468
Davison, A. N. & Peters, A. (eds.) (1970) *Myelination*, Charles C. Thomas, Springfield
Deza, L. & Eidelberg, E. (1967) *Exp. Neurol.* **17**, 425–438
Douglas, T. C. (1972) *J. Exp. Med.* **136**, 1054–1062
Esselman, W. J. & Miller, H. C. (1974) *J. Exp. Med.* **139**, 445–450
Grabar, P., Loisillier, F. & Burtin, P. (1974) *C. R. Hebd. Séances Acad. Sci. Ser. D* **279**, 1225–1228
Gurd, J. W., Jones, L. R., Mahler, H. R. & Moore, W. J. (1974) *J. Neurochem.* **22**, 281–290
Hakomari, S. & Kobata, A. (1974) in *The Antigens* (Sela, M., ed.), vol. 2, pp. 79–140, Academic Press, London and New York
Hartree, E. F. (1972) *Anal. Biochem.* **48**, 422–427
Helenius, A. & Simons, K. (1972) *J. Biol. Chem.* **247**, 3656–3661
Joseph, B. S. & Oldstone, M. B. A. (1974) *Brain Res.* **80**, 421–434
Letarte-Muirhead, M., Acton, R. T. & Williams, A. F. (1974) *Biochem. J.* **143**, 51–61
Letarte-Muirhead, M., Barclay, A. N. & Williams, A. F. (1975) *Biochem. J.* **151**, 685–697
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
Meunier, J.-C., Olsen, R. W. & Changeux, J. P. (1972) *FEBS Lett.* **24**, 63–68
Mirsky, R. & Thompson, E. J. (1975) *Cell* **4**, 95–101
Moore, M. J., Dikkes, P., Reif, A. E., Romanul, F. C. A. & Sidman, R. L. (1971) *Brain Res.* **28**, 283–293
Morgan, I. G., Zanetta, J. P., Breckenridge, W. C., Vincendon, G. & Gombos, G. (1973) *Brain Res.* **62**, 405–411
Morris, R. J. & Williams, A. F. (1975) *Eur. J. Immunol.* **5**, 274–281
Morris, R. J., Letarte-Muirhead, M. & Williams, A. F. (1975) *Eur. J. Immunol.* **5**, 282–285
Reif, A. E. & Allen, J. M. V. (1964) *J. Exp. Med.* **120**, 413–433
Reif, A. E. & Allen, J. M. V. (1966) *Nature (London)* **209**, 523
Schachner, M. (1973) *Nature (London) New Biol.* **243**, 117–119
Schachner, M. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1795–1799
Schachner, M. & Hämmerling, U. (1974) *Brain Res.* **73**, 362–371
Tanford, C., Nozaki, Y., Reynolds, J. A. & Makino, S. (1974) *Biochemistry* **13**, 2369–2376
Tilney, F. (1933) *Bull. Neurol. Inst. N.Y.* **3**, 252–358
Waehneldt, T. V., Morgan, I. G. & Gombos, G. (1971) *Brain Res.* **34**, 403–406
Zanetta, J. P., Morgan, I. G. & Gombos, G. (1975) *Brain Res.* **83**, 337–348