Biochem. J. (1976) **155**, 25–30
Printed in Great Britain

Physicochemical Characterization of Proteolytic Cleavage Fragments of Bovine Colostral Immunoglobulin G_1 (IgG_1)

By WEI D. FANG* and T. K. S. MUKKUR†‡
†*Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, Sask. S7N 0W0, Canada, and* **Department of Biology, University of Windsor, Windsor, Ont. N9B 3P4, Canada*

(*Received* 1 *August* 1975)

Normal bovine colostral immunoglobulin G_1 was subjected to enzymic digestion (pepsin, papain and trypsin) and the resulting fragments separated by a combination of molecular-sieve and phosphocellulose chromatography. Fragments $F(ab')_2$ derived from peptic digestion, fragment Fab from papain digestion and fragment Fab(t) from tryptic digestion showed complete antigenic identity with each other. Although fragment $F(ab')_2$ (peptic digestion) had a sedimentation coefficient ($s_{20,w}$) of 5.3S, those for fragments Fab' (peptic digestion), Fab (papain digestion) and Fab(t) (tryptic digestion) were found to be 3.9S, 3.7S and 3.7S respectively. The mol.wts. calculated for the various fragments from the sedimentation equilibrium data were: $F(ab')_2$, 104000 ± 200; Fab', 51900 ± 340; Fab, 50900 ± 230; Fab(t) 50900 ± 300. Fragment Fc' (peptic digestion) had an $s_{20,w}$ of 3.2S and a mol.wt. of 42900 ± 650; fragment Fc (papain digestion) had an $s_{20,w}$ of 3.7S and a mol.wt. of 50800 ± 300; fragment Fc(t) had an $s_{20,w}$ of 3.7S and a mol.wt. of 50800 ± 450.

Different classes of immunoglobulins known to be present in bovine colostrum are IgM§, IgA, IgG_1 and IgG_2 (Butler, 1969; Duncan *et al.*, 1972; Mach *et al.*, 1968; Porter, 1971; Tewari & Mukkur, 1975). However, unlike colostrum from other mammals, IgA forms only a minor constituent of bovine milk (Mach *et al.*, 1969). Colostral IgM and IgG_1, on the other hand, are present in a much larger quantity as compared with IgG_2 (Butler, 1969; Mukkur & Froese, 1971; Tewari & Mukkur, 1975). The fact that colostral IgG_1 was shown to possess a higher molecular weight than IgG_2 (Duncan *et al.*, 1972; Tewari & Mukkur, 1975) led to a postulation that an immunoglobulin molecule such as IgG_1 with a molecular weight greater than that found conventionally for mammalian IgG might act as a functional substitute for secretory IgA in milk, especially since the latter is present in very small quantities (Mach *et al.*, 1969). Secretory IgA has been found to be relatively resistant to the action of certain proteolytic enzymes such as papain, pepsin, trypsin and chymotrypsin (Ghetie & Mota, 1973; Tomasi & Bienenstock, 1968), the resistance being due to the presence of the secretory component (Ghetie & Mota, 1973). The present investigation was therefore performed to determine the susceptibility of normal bovine colostral IgG_1 to the action of various proteolytic enzymes including pepsin, papain and trypsin and to isolate and characterize the cleavage products.

‡ To whom reprint requests should be sent.

§ Abbreviations: IgM, immunoglobulin M; IgA, immunoglobulin A; IgG_1, immunoglobulin G_1; IgG_2, immunoglobulin G_2.

Materials and Methods

Isolation of bovine colostral IgG_1

Bovine colostral IgG_1 was isolated as described by Tewari & Mukkur (1975). Briefly, bovine colostral whey was prepared from colostrum as described previously (Mukkur & Froese, 1971). The immunoglobulins were precipitated with satd. $(NH_4)_2SO_4$ (final concn. 50%). After dialysis against 0.32M-NaCl/0.01M-Tris/HCl, pH8.0, the globulin fraction was subjected to gel filtration on Bio-Gel P-300 (50.0cm×110.0cm) (Bio-Rad Laboratories, Montreal, Canada) equilibrated with 0.32M-NaCl/0.01M-Tris/HCl, pH8.0, when two major peaks were obtained. The 7S peak, representing IgG types, was subjected to ion-exchange chromatography on TEAE-cellulose (1.5cm×30.0cm) (Bio-Rad) by using a stepwise elution. Though IgG_2 was eluted with 0.001M-Tris/HCl, pH8.6, IgG_1 was eluted with buffers consisting of 0.001M-Tris/HCl, pH8.6, plus NaCl added in various increasing quantities, namely 0.089M, 0.14M and 0.32M. Immunoglobulin G fractions eluted with these buffers were all shown to be IgG_1 (Tewari & Mukkur, 1975) and showed complete antigenic identity with each other on Ouchterlony (1949) plates, which were developed by using monospecific antisera prepared in our laboratory (Tewari & Mukkur, 1975). For studies reported here,

all IgG_1 fractions were pooled and used for various enzymic digestions.

Preparation of anti-(bovine colostral IgG_1) antisera

Anti-(bovine colostral IgG_1) antisera were prepared by intradermal injection of rabbits with 1.0ml of purified colostral IgG_1 (1.0mg/ml) in complete Freund's adjuvant, followed by subcutaneous booster injections at monthly intervals. The rabbits were bled periodically.

Determination of bovine colostral IgG_1 concentration

IgG_1 concentration was determined by using an $E_{280}^{1\%}$ of 12.1 (1cm light-path) as determined previously (Tewari & Mukkur, 1975).

Enzymic digestions of bovine colostral IgG_1

(*a*) *Peptic digestion.* The procedures used for pepsin digestion of normal bovine colostral IgG_1 were essentially those of Nisonoff *et al.* (1961). The samples, at a concentration of 50mg/ml, were incubated with pepsin (crystallized; Worthington Biochemical Corp., Toronto, Ont., Canada) at a protein/enzyme ratio of 50:1, in 0.2M-sodium acetate, pH4.5, at 37°C in a water bath. The incubations were varied between 1h and 36h with periodic checks of pH and temperature in order to maintain constancy. The reactions were stopped by adjusting to pH8.0 with concentrated Tris solution. The digestion products were dialysed against 0.2M-sodium acetate, pH7.5, and either stored frozen until used or fractionated directly by chromatography.

(*b*) *Papain digestion.* Papain digestions were carried out essentially as described by Porter (1959) with slight modifications. Briefly, the reaction mixtures consisted of IgG_1 (50mg/ml) in 0.1M-sodium phosphate buffer, pH7.0, papain (1%, w/w; Dickson and Co., Orangeburg, NY, U.S.A.), 0.01M-cysteine and 2mM-EDTA. The enzymic digestions were carried out by incubations of the reaction mixtures at 37°C for 6, 12, 18, 24, 30 and 36h. The reactions were stopped by alkylation with 100% molar excess of sodium iodoacetate (Sigma Chemical Co., St. Louis, MO, U.S.A.) dissolved in 0.2M-Tris/HCl, pH8.0. The samples were then dialysed exhaustively against 0.1M-Na_2HPO_4, pH7.0, in the cold-room (4°C). The digestion products were either frozen until ready to use or fractionated directly by chromatography.

(*c*) *Tryptic digestion.* Tryptic digestions were performed as described by Edelman (1967). IgG_1 (100mg) dissolved in 2ml of 0.15M-NaCl containing 0.05M-$CaCl_2$ was incubated in a water bath at 37°C, and the pH adjusted to 8.0. Trypsin (2%, w/w; Sigma) was added in the reaction vial, and the reaction stopped by addition of soya-bean trypsin inhibitor (Nutritional Biochemical Corp., Cleveland, OH, U.S.A.) at an enzyme/inhibitor ratio of 5:1 (w/w). The samples were chilled at 4°C and were dialysed exhaustively against 0.1M-Na_2HPO_4, pH7.0, and stored frozen until ready for fractionation.

Analytical ultracentrifugation

A Beckman model E analytical ultracentrifuge equipped with electronic speed control was used. Sedimentation-velocity runs were made at 52000rev./min at 20°C in an An-H rotor. The method of Schachman (1957) was used to correct $s_{obs.}$ to $s_{20,w}$.

Sedimentation equilibrium runs were made either at 24000rev./min or 30000rev./min at 20°C. Then the molecular weights were determined by the meniscus-depletion method of Yphantis (1964). Partial specific volumes ($\bar{v}$) of the digestion fragments were calculated by the H_2O–^2H_2O method of Edelstein & Schachman (1967).

Immunological tests

The purity of isolated immunoglobulins and different enzymic-digestion fragments were tested by immunoelectrophoresis as described by Scheidegger (1955), and Ouchterlony's (1949) gel-diffusion technique. The latter was also used to determine the antigenic relationships between fragments obtained from the various different enzymic digestions.

Reduction and alkylation

Reduction of purified fragment $F(ab')_2$ obtained from peptic digestion of IgG_1 was carried out in 0.1M-Na_2HPO_4, pH7.0, by using 0.3M-cysteine for 12h under an N_2 atmosphere. Alkylation was performed by addition of a 100% molar excess of sodium iodoacetate, pH7.0.

Agglutination tests

The agglutination tests were performed with IgG_1 isolated from the colostrum of cows immunized with a *Staphylococcus aureus* vaccine as described by Mukkur & Tewari (1975), its peptic fragment $F(ab')_2$ and Fab', which was prepared by reduction of $F(ab')_2$ as described above. The agglutination tests were performed using a thick live suspension of *S. aureus* as antigen (10^9 bacteria/ml). Serial twofold dilutions of the above samples were made in 1% inactivated normal rabbit serum in 0.85% NaCl as diluent. A sample (0.5ml) of each of the serial dilutions was mixed with 0.5ml of the bacterial suspension. The control contained only the diluent and the bacterial suspension. The test tubes were incubated for 2h at 37°C in a water bath followed by an 18h incubation at room temperature (23°C). An agglutination test was recorded as positive if bacteria were uniformly spread at the bottom of the test tube, with or without serrated edges, and the supernatant was transparent. If the supernatant was partially turbid the test was considered doubtful; if the bacteria settled in the form of a button with a clear or translucent super-

natant, the agglutination test was recorded as negative.

Results and Discussion

Investigations of the aggregation behaviour of normal bovine colostral IgG_1 in different buffers

It was reported previously that both bovine colostral and serum IgG_1 aggregated in certain buffers, but not in others (Tewari & Mukkur, 1975). Therefore, in order to carry out the enzymic digestion of IgG_1 in an unaggregated state, it was necessary to ascertain that IgG_1 did not aggregate in buffers used in different types of enzymic digestions. To this end, sedimentation-velocity runs of different concentrations of IgG_1 (2.0–10.0mg/ml) were made in 0.2M-sodium acetate, pH4.5 (for peptic digestion), 0.1M-Na_2HPO_4, pH7.0 (for papain digestion), and 0.15M-NaCl/0.05M-$CaCl_2$, pH8.0 (for tryptic digestion). That no aggregation of IgG_1 occurred in these media was evident from the fact that IgG_1 showed a negative concentration dependence of sedimentation coefficients in these media.

(*a*) *Peptic digestion*

Hydrolysis of IgG_1 by pepsin. The pepsin digested mixtures from different incubation periods as described in the Materials and Methods section, were dialysed against 0.2M-sodium acetate, pH8.0, at 4°C. On analytical ultracentrifugation, only one peak with an $s_{20,w}$ of 5.3S was observed, regardless of the time of digestion, even though fragment Fc′ constituted about 25% of the total digest. This observation could be explained by the presence of non-covalent interactions between fragment $F(ab')_2$ and Fc′, and is supported, as described later, by our inability to resolve these fragments on molecular, sieve chromatography. As IgG_1 has an $s_{20,w}$ of 7.2S, it was clear that the protein had been split into a large fragment(s) and possibly small peptide fragment(s).

Immunoelectrophoretic patterns of various peptic digests showed the presence of three components. One component migrated toward the anode and another toward the cathode. The third component, which appeared as a faint line, was found to be undigested IgG_1 in each of these digestion mixtures, and migrated toward the cathode like IgG_1.

Isolation of fragments hydrolysed by pepsin. A portion (200mg) of a 2h peptic digest was dialysed against 0.32M-NaCl/0.01M-Tris/HCl, pH8.0, and filtered through a Bio-Gel P-200 column (2.5cm×100.0cm) equilibrated with the same buffer, and two major peaks were obtained (Fig. 1*a*). The first fraction (peak 1) was composed of undigested IgG_1 as determined by complete antigenic identity with untreated IgG_1, whereas the second fraction (peak 2) was found to be composed of the mixtures of $F(ab')_2$ and possibly Fc′ fragments as described below.

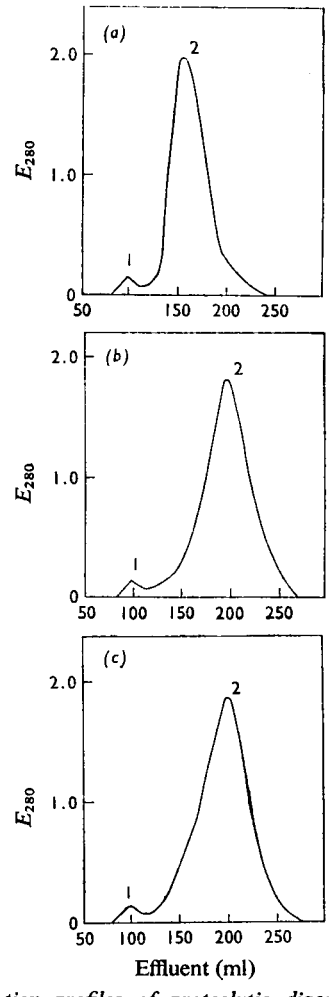


Fig.1. *Elution profiles of proteolytic digest of bovine colostral IgG_1*

A portion of bovine colostral IgG_1 was digested with pepsin (2h), papain (12h) and trysin (2h) and subjected to gel filtration on a Bio-Gel P-200 column (2.5cm×100.0cm) equilibrated with 0.32M-NaCl/0.01M-Tris/HCl, pH8.0. Profiles show: (*a*) pepsin digest; (*b*) papain digest; (*c*) trypsin digest.

Attempts to separate $F(ab')_2$ from Fc′ fragments by methods described previously for mammalian systems such as gel-filtration on Sephadex G-75 (Utsumi & Karush, 1965) and DEAE-cellulose chromatography (Porter, 1959) were not successful. Peak 2 (Fig. 1) was concentrated by pervaporation, dialysed against 0.025M-Na_2HPO_4/0.3M-NaCl, pH 4.2, and subjected to ion-exchange chromatography on a phosphocellulose column (2.5cm×35.0cm) by using discontinuous gradient elution. The first

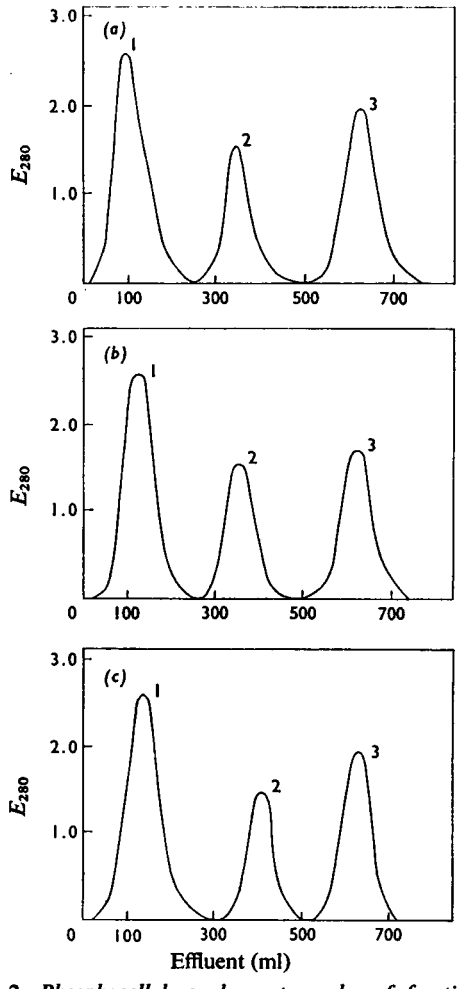


Fig. 2. *Phosphocellulose chromatography of fraction* 2 *obtained by gel filtration of various enzymic digests as shown in Plate* 2

Fraction 2 was obtained from the peptic digest and subjected to ion-exchange chromatography on phosphocellulose (2.5cm×30.0cm) by using a stepwise gradient elution. The solvents used were: (1) 0.025M-Na_2HPO_4/0.3M-NaCl, pH4.2; (2) 0.025M-Na_2HPO_4/0.5M-NaCl, pH4.2; (3) 0.1M-Na_2HPO_4, pH7.4. Profiles shown: (*a*) pepsin digest; (*b*) papain digest; (*c*) trypsin digest.

solvent used for elution was 0.025M-Na_2HPO_4/0.3M-NaCl, pH4.2 (300ml), followed by 0.025M-Na_2HPO_4/0.5M-NaCl, pH4.2 (300ml), as the second solvent. The third solvent used was 0.1M-Na_2HPO_4, pH7.4 (300ml). Three peaks, 1, 2 and 3, were obtained (Fig. 2*a*). Yields of these three fractions were calculated by summing the E_{280} in each fraction. The ratio of the yields for the three fractions (1:2:3) was 1.0:0.4:0.5 and the total recovery from the column was 70–74%.

On Ouchterlony (1949) gel-diffusion tests, fractions 1 and 2 were found to be antigenically identical to each other, and therefore were pooled together. Fractions 3 and 1, and 3 and 2, on the other hand, showed non-identity. However, a partial identity was observed between fraction 2 and IgG_1, and fraction 3 and IgG_1 (results not shown). The antiserum used for development was rabbit anti-(colostral IgG_1) antiserum. To find out the localization of antibody activity in these fragments, fractions 1, 2 and 3 were prepared by using colostral IgG_1 possessing anti-(*S. aureus*) activity. Although IgG_1 and pooled fractions 1 and 2 agglutinated *S. aureus*, fraction 3 did not. Therefore fractions 1 and 2 corresponded to the antigen binding fragment $F(ab')_2$, as reported by Nisonoff *et al.* (1961) in the rabbit system, whereas fraction 3 corresponded to the Fc′ fragment.

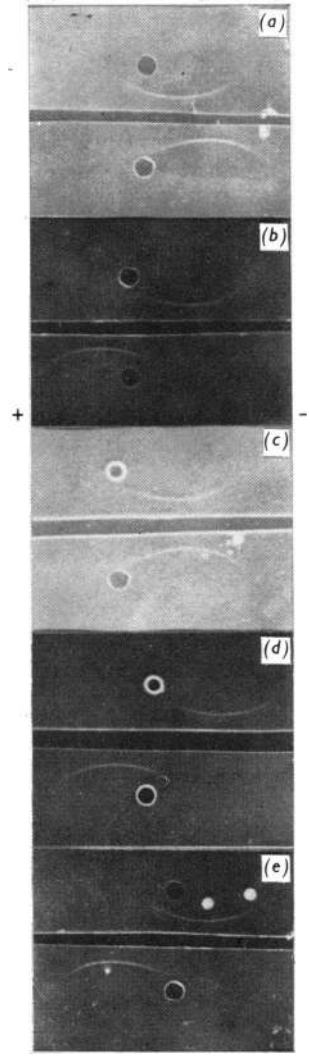
Immunoelectrophoretic patterns of IgG_1, $F(ab')_2$ and Fc′ fragments showed that the first two migrated toward the cathode whereas Fc′ fragment was composed of an anodally migrating component (Plate 1*a* and 1*b*).

Conversion of fragment $F(ab')_2$ to Fab′

Reduction and alkylation of fragment $F(ab')_2$ was carried out as described in the Materials and Methods section. The reaction mixture was dialysed against 0.1M-Na_2HPO_4, pH8.0, and filtered through a Bio-Gel P-60 column (1.5cm×30.0cm) equilibrated with the same medium. Two peaks were obtained. About 60% of the original $F(ab')_2$ (V_e/V_0 = 2.0) was reduced to Fab′, which was eluted from the column in the second peak ($V_e/V_0 = 4.0$).

Immunoelectrophoretic analysis revealed that both fragments $F(ab')_2$ and Fab′ migrated toward the cathode (Plate 1*c*). In addition, fragment $F(ab')_2$ and Fab′ were found to be antigenically identical as judged by Ouchterlony's (1949) gel-diffusion technique (results not shown). Agglutination tests conducted with fragment Fab′ showed that it did not agglutinate a *S. aureus* suspension, thus attesting to its already known monovalency.

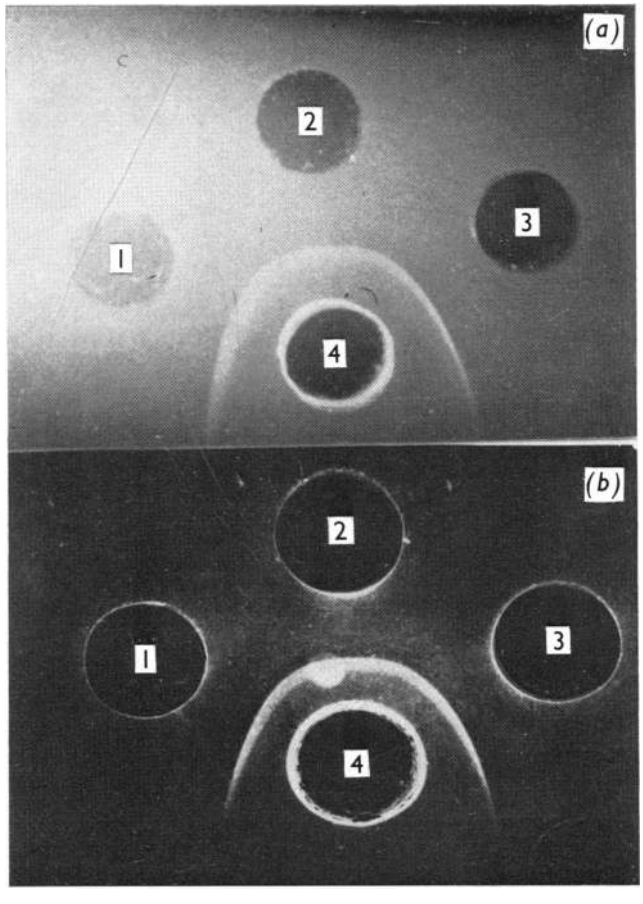
Molecular weight and sedimentation coefficient ($s_{20,w}$). Analytical ultracentrifugation of $F(ab')_2$, Fab′ and Fc′ revealed the $s_{20,w}$ values of 5.3, 3.9 and 3.2S respectively (Table 1). Although the partial specific volumes ($\bar{v}$) of fragment $F(ab')_2$ and Fc′ were calculated to be 0.768 and 0.753ml/mg respectively, that used for molecular weight determination of fragments Fab′ was assumed to be the same as that of fragment $F(ab')_2$. The mol.wts. thus calculated were 104000±200, 51900±340 and 42900±650 for fragments $F(ab')_2$, Fab′ and Fc′ respectively (Table 1). The nature of association of the fragment Fc′, whether covalent or non-covalent, was not determined in this study. One of the most interesting findings in our system was that unlike the rabbit (Nisonoff *et al.*, 1960) and the human system (Heimer



EXPLANATION OF PLATE 1

Immunoelectrophoretic patterns of IgG_1 and various enzymic fragments

(*a*) IgG_1 (top) and $F(ab')_2$ (bottom); (*b*) $F(ab')_2$ (top) and Fc' (bottom); (*c*) $F(ab')_2$ (top) and Fab' (bottom); (*d*) Fab (top) and Fc (bottom) produced by papain digestion; (*e*) Fab(t) and Fc(t). The central troughs contained rabbit anti-(bovine colostral IgG_1) antisera.



EXPLANATION OF PLATE 2

Ouchterlony analysis showing antigenic relationship between enzymically produced fragments

(*a*) Fab′ (well 1), Fab (well 2) and Fab(t) (well 3). Ouchterlony (1949) plates were developed with rabbit anti-(bovine colostral IgG_1) antisera (well 4). (*b*) Fc′ (well 1), Fc (well 2) and Fc(t) (well 3). Ouchterlony plates were developed with specific rabbit anti-(bovine colostral IgG_1) antisera (well 4).

Table 1. *Partial specific volumes, $s_{20,w}$ and molecular weights of normal bovine colostral IgG_1 and its peptic, papain and tryptic fragments*

The concentration of IgG_1 and its various fragments used for analytical ultracentrifugation to determine $s_{20,w}$ was 5mg/ml.

	Enzyme used for digestion	$s_{20,w}$ (S)	Partial specific volume	Molecular weight
IgG_1	—	7.2*	0.726	156000†
$F(ab')_2$	Pepsin	5.3	0.768	104000±200
Fab'	Pepsin	3.9	0.768‡	51900±340
Fc'	Pepsin	3.2	0.753	42900±650
Fab	Papain	3.7	0.768‡	50900±230
Fc	Papain	3.7	0.753§	50800±300
Fab(t)	Trypsin	3.7	0.768‡	50900±300
Fc(t)	Trypsin	3.7	0.753§	50800±450

* $s^0_{20,w}$.
† Determined by Tewari & Mukkur (1975).
‡ Assumed to be the same as that for $F(ab')_2$.
§ Assumed to be the same as that for Fc'.

et al., 1969), fragment Fc' was not completely digested regardless of the time of digestion, therefore suggesting a possible conformational difference between the Fc region(s) of bovine colostral IgG_1 and other species studied so far.

(*b*) *Papain digestion*

Hydrolysis of IgG_1 by papain. Five digestion mixtures from different incubation periods, prepared as described previously, were dialysed exhaustively against 0.1M-Na_2HPO_4, pH7.0, in the cold-room (4°). This medium was used, since IgG_1 was found not to undergo aggregation. When these digests were examined in the analytical ultracentrifuge, only one peak, with an $s_{20,w}$ of 3.7S, was obtained. Therefore it was clear that IgG_1 had been split into a large fragment or fragments of similar size with possibly some diffusible peptides.

Immunoelectrophoretic patterns of the digestion mixtures showed that three components were present, one migrating towards the anode and another towards the cathode. The third component, which appeared as a faint line, migrated towards the cathode like IgG_1 and was identified to be so by Ouchterlony (1949) analysis (results not shown).

Resolution of fragments hydrolyzed by papain. Attempts to fractionate this mixture by chromatography on CM-cellulose (Porter, 1959), Sephadex G-75 (Utsumi & Karush, 1965) and DEAE-cellulose (Porter, 1959) were not successful. However, resolution could be achieved by chromatography on phosphocellulose.

A portion (200mg) of IgG_1 digested for 12h in 0.1M-Na_2HPO_4, pH7.0, was loaded on a Bio-Gel P-200 column (2.5cm×9.00cm) equilibrated with the same medium. Approximately 3% of the reaction material that was eluted off the column first was found to be undigested IgG_1 (Fig. 1*b*) as judged by its complete antigenic identity with intact IgG_1. The second peak was dialysed against 0.025M-Na_2HPO_4/0.3M-NaCl, pH4.2, and subjected to ion-exchange chromatography on a phosphocellulose column (2.5cm×35.0cm) by using a stepwise gradient. The solvents used for elution were the same as described for pepsin digestion. Again three peaks were eluted, which were termed 1, 2 and 3, in order of elution from the column (Fig. 2*b*). Yields of these three fractions were measured by summing the E_{280} of each fraction. The ratio of the yields for the three fractions (1:2:3) was 1.0:0.6:0.8, and the total recovery from the column was 85–90%. Fractions 1 and 2 were antigenically identical and were therefore pooled together. These fractions were found to show a complete identity with fragment Fab' produced by reduction of peptic fragment $F(ab')_2$ and were therefore considered to constitute the Fab fragment. However, complete nonidentity was observed between Fab and Fc (fraction 3) fragments. As with the peptic products, partial identity was observed between fragments Fab and IgG_1, and Fc and IgG_1 (results not shown). Immunoelectrophoretically, fragment Fab migrated towards the cathode, whereas Fc fragment migrated towards the anode (Plate 1*d*).

Determinations of $s_{20,w}$ and molecular weights of papain fragments. The sedimentation coefficient ($s_{20,w}$) of fragments Fab and Fc determined in 0.1M-Na_2HPO_4, pH7.0, were found to be 3.7S for each of the fragments (Table 1). Assuming the partial specific volumes ($\bar{v}$) for fragments Fab and Fc to be the same as those calculated for pepsin-derived fragments i.e. 0.768ml/mg for Fab and 0.753ml/mg for Fc, the mol.wts. were calculated to be 50900± 230 and 50800±300 respectively (Table 1).

(*c*) *Tryptic digestion*

Hydrolysis of IgG_1 by trypsin. Seven digestion mixtures from different incubation periods, prepared as described above, were dialysed exhaustively against 0.1M-Na_2HPO_4, pH7.0, in the cold-room. This medium was used since IgG_1 was found not to undergo aggregation in it. When these digests (5mg/ml) were examined in the analytical ultracentrifuge, only one peak with an $s_{20,w}$ of 3.7S was observed. Therefore it was evident that IgG_1 had been split into a large fragment or fragments of similar size with possibly some diffusible peptides. As with papain digestion, immunoelectrophoretic patterns of the digestion mixtures revealed the presence of three components. One component migrated towards the anode, another towards the cathode and the third component, which appeared as a faint line and was identified as undigested IgG_1, migrated towards the cathode like intact IgG_1.

Resolution of fragments hydrolysed by trypsin. A portion (200mg) of IgG_1 digested for 2h in 0.015M-NaCl/0.05M-$CaCl_2$, pH8.0, was dialysed against 0.1M-Na_2HPO_4, pH7.0, and loaded on a Bio-Gel P-200 column (2.5cm×90.0cm) equilibrated with the same medium. Approx. 4% of the reaction material that was eluted off the column first (Fig. 1*c*) was found to be undigested IgG_1, as judged by its complete antigenic identity with intact IgG_1 (results not shown). The second peak was dialysed against 0.025M-Na_2HPO_4/0.3M-NaCl, pH4.2, and subjected to ion-exchange chromatography on phosphocellulose column (2.5cm×35.0cm) by using a stepwise gradient and the same solvents as described for pepsin and papain. Again three peaks were eluted (Fig. 2*c*), which were termed 1, 2 and 3, in order of their elution from the column. The ratio of the yields of these three fractions (1:2:3) as measured by summing the E_{280} was 1.0:0.5:0.9, and the total recovery from the column was 87–91%. Fractions 1 and 2 were antigenically identical and were pooled together. These fractions were found to show a complete antigenic identity with the peptic Fab′ and the papain-produced Fab fragments. They were therefore considered to constitute the Fab(t) fragment. As with peptic and papain fragments, Ouchterlony (1949) analysis revealed non-identity between Fab(t) and Fc(t) (fraction 3) fragments, whereas partial identity was shown between Fab(t) and IgG_1, and Fc(t) and IgG_1 (results not shown).

Immunoelectrophoretic patterns showed that fragment Fab(t) migrated towards the cathode where as fragment Fc(t) migrated towards the anode (Plate 1*e*).

Determinations of $s_{20,w}$ and molecular weights of fragments Fab(t) and Fc(t). The sedimentation coefficient ($s_{20,w}$) of fragments Fab(t) and Fc(t) were determined in 0.1M-Na_2HPO_4, pH7.0, at a concentration of 5mg/ml and were 3.7S for each of the fragments (Table 1). The determinations of the molecular weights of fragments Fab(t) and Fc(t) were carried out by sedimentation-equilibrium analysis by assuming the partial specific volumes ($\bar{v}$) to be the same as those for peptic fragments $F(ab')_2$ and Fc′ prepared by pepsin digestion, i.e. 0.768ml/mg for Fab(t) and 0.753ml/mg for Fc(t), and were 50900± 300 and 50800±450 respectively.

Antigenic relationship of fragments obtained from different enzymic digestions. Ouchterlony's (1949) gel-diffusion technique was used to test the antigenic relationship between fragments $F(ab')_2$ and Fab′ (peptic digestion), Fab (papain digestion) and Fab(t) (tryptic digestion) by using rabbit anti-(bovine colostral IgG_1) antisera. A complete identity was observed between fragments $F(ab')_2$, Fab and Fab(t) (Plate 2*a*). Similarly, Fc′ (peptic digestion), Fc (papain digestion) and Fc(t) (tryptic digestion) were also found to antigenically identical (Plate 2*b*). The antisera used for development were specific rabbit anti-(bovine colostral IgG_1) antisera. However, no precipitin lines were observed between fragments Fc′, Fc and Fc(t), if specific rabbit anti-(bovine colostral IgM) antisera were used, although fragments $F(ab')_2$, Fab′, Fab and Fab(t) again showed lines of complete antigenic identity (results not shown) thus pointing out the absence of light chain determinants on fragments Fc′, Fc and Fc(t).

Earlier, Kickhöfen *et al.* (1968) had suggested that a high-molecular-weight secretory immunoglobulin G found in bovine colostrum (γG_s) [later identified as IgG_1 (Duncan *et al.*, 1972; Tewari & Mukkur, 1975)] might be a functional counterpart of IgA, which is found in very small quantities in bovine colostrum (Mach *et al.*, 1969). However, this investigation revealed that colostral IgG_1 seemed, at least, structurally different from secretory IgA, since the latter is known to be relatively resistant to the action of proteolytic enzymes such as papain, chymotrypsin and trypsin (Ghetie & Mota, 1973; Tomasi & Bienenstock, 1968).

References

Butler, J. E. (1969) *J. Dairy Sci.* **52**, 1859–1897

Duncan, J. R., Wilkie, B. N., Hiestand, F. & Winter, A. J. (1972) *J. Immunol.* **108**, 965–971

Edelman, G. M. (1967) *Gamma Globulins, Proc. Nobel Symp. 3rd*, pp. 89–94

Edelstein, S. J. & Schachman, H. K. (1967) *J. Biol. Chem.* **242**, 306–312

Heimer, R., Clark, L. C. & Maurer, P. H. (1969) *Arch. Biochem. Biophys.* **131**, 1–11

Kickhöfen, B., Hammer, D. K. & Scheel, D. (1968) *Hoppe-Seyler's Z. Physiol. Chem.* **349**, 1755–1761

Ghetie, V. & Mota, G. (1973) *International Congress of Biochemistry 9th, Stockholm*, abstract 6d 15

Mach, J. P., Pahud, J. J. & Isliker, H. (1968) *Nature (London)* **223**, 952–955

Mukkur, T. K. S. & Froese, A. (1971) *Immunochemistry* **8**, 257–261

Mukkur, T. K. S. & Tewari, U. J. (1975) *Can. J. Microbiol.* in the press

Nisonoff, A., Wissler, F. C., Lipman, L. N. & Woernely, D. L. (1960) *Arch. Biochem. Biophys.* **89**, 230–236

Nisonoff, A., Markus, G. & Wissler, F. C. (1961) *Nature (London)* **189**, 293–297

Ouchterlony, O. (1949) *Acta Pathol. Microbiol. Scand.* **26**, 507–602

Porter, P. (1971) *Immunology* **23**, 225–237

Porter, R. R. (1959) *Biochem. J.* **73**, 119–127

Schachman, H. K. (1957) *Methods Enzymol.* **4**, 32–37

Scheidegger, J. J. (1955) *Int. Arch. Allergy Appl. Immunol.* **7**, 103–109

Tewari, U. J. & Mukkur, T. K. S. (1975) *Immunochemistry* **12**, 925–930

Tomasi, T. B. & Bienenstock, J. (1968) *Adv. Immunol.* **9**, 952–953

Utsumi, S. & Karush, F. (1965) *Biochemistry* **4**, 1766–1771

Yphantis, D. A. (1964) *Biochemistry* **4**, 297–317