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Molecular Weights of the Thy-1 Glycoproteins from Rat Thymus and Brain in the Presence and Absence of Deoxycholate

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1. The Thy-1 membrane glycoproteins from rat thymus and brain bound deoxycholate to 24% of their own weight as measured by equilibrium dialysis. The binding occurred co-operatively at the critical micelle concentration of deoxycholate, suggesting that the glycoproteins bind to a micelle, and not to the detergent monomer. 2. From sedimentation-equilibrium and deoxycholate-binding data the molecular weights of the glycoprotein monomers were calculated to be 18700 and 17500 for thymus and brain Thy-1 glycoproteins respectively. The molecular weight of the polypeptide part of the glycoprotein is thus 12500. 3. In the absence of deoxycholate, brain or thymus Thy-1 glycoprotein formed large homogeneous complexes of mol.wt. 270000 or 300000 respectively. The sedimentation coefficient of these was 12.8S. The complex was only partially dissociated by 4M-guanidinium chloride. 4. After cleavage of brain or thymus Thy-1 glycoprotein with CNBr, two peptides were clearly identified. They were linked by disulphide bonds and both contained carbohydrate. This cleavage suggests there is only one methionine residue per molecule, which is consistent with the above molecular weights and the known amino acid composition.

The Thy-1 glycoproteins are major membrane molecules of mouse and rat thymocytes and brain, and were first identified by their antigenic determinants (for a review, see Williams *et al.*, 1976). In the rat, Thy-1 glycoprotein has been purified from both thymus and brain, and in deoxycholate both forms had mol.wts. of about 28000 as estimated from their hydrodynamic properties (Barclay *et al.*, 1975; Letarte-Muirhead *et al.*, 1974, 1975). The apparent molecular weight as estimated by polyacrylamide-gel electrophoresis in sodium dodecyl sulphate was 24000 and 25000 for brain and thymocyte Thy-1 glycoproteins respectively (Barclay *et al.*, 1975; Letarte-Muirhead *et al.*, 1975), and a similar value has been obtained for mouse thymocyte Thy-1 glycoprotein (Trowbridge *et al.*, 1975). Despite quite good agreement, these estimates do not give an accurate value for molecular weight, since that determined in deoxycholate includes bound detergent, and the polyacrylamide-gel technique is not necessarily valid for glycoproteins (Grefrath & Reynolds, 1974).

The molecular weights of membrane molecules in detergents can be unequivocally determined in the ultracentrifuge by using sedimentation-equilibrium methods (Tanford *et al.*, 1974) if the amount of bound detergent is known. Also, measurement of detergent binding is of interest, since it appears that membrane proteins that are normally associated with lipid bind large amounts of non-ionic or weakly ionic detergents, whereas other proteins do not (Helenius & Simons, 1975; Tanford & Reynolds, 1976).

In the present studies the association of brain and thymus Thy-1 glycoproteins with deoxycholate has been investigated, and their molecular weights were measured in the presence or absence of detergent. Cleavage of the molecules with CNBr has also been investigated in an attempt to measure the number of methionine residues and thus deduce a minimum molecular weight from amino acid composition. Both approaches gave a lower molecular weight for Thy-1 glycoprotein than was expected.

Materials and Methods

Chemicals

CNBr and sodium deoxycholate were from Koch–Light, Colnbrook, Bucks., U.K. [^{14}C]Deoxycholate (124μCi/mg) and iodo[2-^{14}C]acetic acid (303μCi/mg) were from The Radiochemical Centre, Amersham, Bucks., U.K. Visking dialysis tubing was from Medicell International, London EC4N 4SA, U.K.

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Thy-1 glycoproteins

Rat brain Thy-1 glycoprotein was purified as described by Barclay *et al.* (1975), and thymocyte Thy-1 glycoprotein, which binds to lentil lectin, as described by Letarte-Muirhead *et al.* (1975), except that the lectin affinity chromatography was used before gel filtration, which was done twice to achieve complete purification. The purifications were in deoxycholate (twice recrystallized), and this was removed by ethanol precipitation (Barclay *et al.*, 1976). Precipitated Thy-1 glycoprotein was dissolved in 10mM-Tris/HCl buffer (pH8.0)/0.5mM-NaN_3, and dialysed against this before ultracentrifugation or equilibrium dialysis. The concentration of Thy-1 glycoprotein was measured by amino acid analysis, from which the total weight of glycoprotein could be calculated from the known carbohydrate and amino acid composition (Barclay *et al.*, 1976).

Equilibrium dialysis

Sodium deoxycholate for equilibrium dialysis was recrystallized four times from acetone/water (4:1, v/v). The purity of this and the [^{14}C]deoxycholate was checked by chromatography on Eastman–Kodak 13179 silica-gel sheets in the solvent acetic acid/carbon tetrachloride/di-isopropyl ether/3-methylbutan-1-ol/propanol/benzene (1:4:6:8:2:2, by vol.; Hoffman, 1962). Both the labelled and the unlabelled deoxycholate gave only one spot. The deoxycholate at various concentrations was dissolved in 10mM-Tris/HCl (pH8.0)/0.5mM-NaN_3, and [^{14}C]deoxycholate added to a concentration of 10^6d.p.m./0.1ml.

Dialysis tubing was soaked overnight in 0.5% (w/v) deoxycholate, washed in water, boiled in 10% (v/v) ethanol for 5min, washed in water again, and stored in 10mM-Tris/HCl (pH8.0)/3mM-NaN_3 at 4°C before use.

Equilibrium dialysis was in micro-cells with a maximum volume of 100μl on each side of the dialysis tubing, and Thy-1 glycoprotein was added at about 0.8mg/ml. Dialysis was for 2 days at 21°C, and preliminary experiments showed that, in this time, [^{14}C]deoxycholate reached equilibrium, in agreement with other results (Robinson & Tanford, 1975). Also with Thy-1 glycoprotein, the detergent binding was the same whether the [^{14}C]deoxycholate was added in the same well as the glycoprotein or in the opposite one. Five samples from each well were taken by using 5μl disposable pipettes, and radioactivity was counted in an LKB–Wallac liquid-scintillation counter with a scintillation fluid consisting of 10g of PPO (2,5-diphenyloxazole), 0.1g of α-NPO [2-(naphth-1-yl)-5-phenyloxazole], 160g of naphthalene, 770ml of xylene, 700ml of 1,4-dioxan and 460ml of ethanol.

Ultracentrifugation

The sedimentation-equilibrium methods of Tanford *et al.* (1974) were used and the molecular weight M (excluding detergent and solvent components) can be calculated from:

$$M(1-\phi'\rho) = (2RT/\omega^2)(\mathrm{d}\ln c/\mathrm{d}r^2) \quad (1)$$

where ω is the angular velocity, ρ is the solution density, $\mathrm{d}\ln c/\mathrm{d}r^2$ the concentration distribution at equilibrium and ϕ' the effective partial specific volume. The value for ϕ' can be obtained from the approximation:

$$1-\phi'\rho = (1-\bar{v}_P\rho)+\delta_D(1-\bar{v}_D\rho) \quad (2)$$

where $\bar{v}_P$ is the partial specific volume of the glycoprotein and can be calculated from the composition (Cohn & Edsall, 1943; Gibbons, 1972), $\bar{v}_D$ that of the detergent, which is 0.778ml/g for deoxycholate (Tanford *et al.*, 1974), and δ_D is the weight proportion of detergent bound per weight of glycoprotein and is determined by equilibrium dialysis under the same conditions of buffer and temperature as used for the ultracentrifuge experiments.

The ultracentrifugation was carried out at 21°C in a Beckman–Spinco model E analytical ultracentrifuge equipped with a mechanical speed control. AN-D and AN-H rotors were used for high angular velocities, and the heavier AN-H rotor for velocities below 12000rev./min. Schlieren and Rayleigh interference optical systems were used for sedimentation-velocity and -equilibrium experiments respectively.

The sedimentation-equilibrium experiments were of the meniscus-depletion type (Yphantis, 1964), and use was made of cells with sapphire windows and 12mm centre-pieces of two-sector aluminium-filled Epon, or six-channel carbon-filled Epon. Volumes were 0.10–0.12ml, giving column heights of approx. 3mm. The solvent and solution menisci were matched as closely as possible by delivery of the fluid from a micro-syringe. No FC-43 or silicone oil was used at the cell base, as detergent solutions were to be used. The attainment of equilibrium was assessed in the usual way (Yphantis, 1964; Teller, 1973). Baseline deviations were measured from fringe-pattern photographs obtained after shaking the cell to destroy solute gradients and then accelerating to 10000rev./min (Teller, 1973). In no case was the deviation of the baseline more than 0.002cm from a mean horizontal straight line.

The interference patterns were measured on a Nikon model 6C profile projector. At each radial position the ordinates of five consecutive light and dark fringes were measured. For the baseline photographs, abscissa increments of 0.05cm were used.

The data were analysed by using a computer program written in FORTRAN (P. W. Kuchel, unpub-

lished work; the program is, however, available on request from P. W. K.). The 'routine' corrected the fringe-displacement data with respect to a baseline obtained by fitting the baseline data to a degree-6 orthogonal polynomial by the method of Forsythe (1957). An unweighted least-squares linear regression was performed on eqn. (1) to yield a slope from which the mean molecular weight and S.D. of the mean was computed. Fringe displacements less than 0.1 mm from the meniscus concentration were not used (Yphantis, 1964).

CNBr cleavage

To a freeze-dried 100 μg sample of Thy-1 glycoprotein was added 10 μl of 70% (v/v) formic acid containing CNBr at 320 mg/ml. The Thy-1 glycoprotein solubilized in this was incubated at 20°C for 24 h. The digest was then diluted with 10 vol. of water, and two portions containing 30 μg and two of 15 μg were freeze-dried. To these samples was added 2% (w/v) sodium dodecyl sulphate, 10% (v/v) glycerol, and either 2 mM-iodoacetamide or 1% (w/v) dithiothreitol, and they were then boiled for 3 min; the reduced samples were then alkylated with iodoacetate. The samples were then electrophoresed on polyacrylamide slab gels (in a Bio-Rad model 220 apparatus; Bio-Rad Laboratories, Bromley, Kent, U.K.) in sodium dodecyl sulphate, with a separating gel of 15% (w/v) acrylamide and stacking gel of 3% (w/v) (Laemmli, 1970). Marker proteins (bovine serum albumin, immunoglobulin G light chain, myoglobulin, cytochrome *c* and insulin) were also run to measure apparent molecular weights, and after electrophoresis the gels were fixed and stained for protein with Coomassie Blue and for carbohydrate with periodic acid/Schiff stain (Letarte-Muirhead *et al.*, 1975). Gels were photographed with a yellow filter on Agfapan 25 film.

[^{14}C]Alkylation of Thy-1 glycoprotein

Thy-1 glycoprotein was dissolved in 7 M-guanidinium chloride/0.5 M-Tris/HCl, pH 8.0, at 7.5 mg/ml. A 40-fold molar excess of dithiothreitol was added (ninefold compared with protein cysteine) and the solution kept under N_2 for 1 h at 20°C. Iodo[2-^{14}C]-acetic acid (57 mCi/mmol) was then added at an eightfold molar excess over glycoprotein, and, after mixing for 2 min, unlabelled iodoacetate was added to give a 1.1 molar excess over all possible thiol groups. After incubation at 4°C for 25 min, excess dithiothreitol was added to reduce any remaining alkylating reagent. For analysis, 5 μg samples were electrophoresed on polyacrylamide disc gels (6 mm × 94 mm) in sodium dodecyl sulphate as described above for slab gels.

Results

Binding of deoxycholate

Fig. 1 shows the binding of deoxycholate by thymocyte and brain Thy-1 glycoprotein plotted against the concentration of free deoxycholate. The glycoproteins bound little deoxycholate at low concentrations of free detergent, but binding increased sharply at about the critical micelle concentration of deoxycholate. At concentrations above 2.5 mg of deoxycholate/ml (6.3 mM) no increase in binding occurred.

If all the data for binding at 2 mg/ml or greater are pooled, then the mean value for binding of deoxycholate to thymocyte Thy-1 glycoprotein is 24 ± 1 (S.E.M.) μg/100 μg of Thy-1 glycoprotein, from ten values with three different Thy-1 glycoprotein preparations. For brain Thy-1 glycoprotein, fewer experiments were carried out, but a similar mean of 26 μg/100 μg of glycoprotein was obtained. This was not significantly different from the value for thymocyte Thy-1 glycoprotein, and a δ_D of 0.24 was used in the calculations.

Calculation of $1 - \phi'\rho$

Values of $\bar{v}$ for brain and thymocyte Thy-1 glycoproteins were calculated from the amino acid and carbohydrate compositions (Barclay *et al.*,

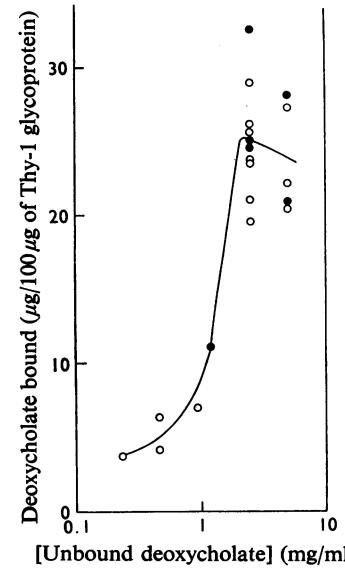


Fig. 1. *Binding of deoxycholate to Thy-1 glycoprotein, measured by equilibrium dialysis*
Binding of deoxycholate to thymocyte (○) and brain (●) Thy-1 glycoprotein is shown. The binding was determined as described in the Materials and Methods section.

Table 1. *Sedimentation-equilibrium results*

For details see the Materials and Methods and Results sections. Errors for individual molecular-weight values were in all cases <5% on the basis of goodness-of-fit.

Source of Thy-1 glycoprotein	Buffer	Protein concn. (approx.) (mg/ml)	Angular velocity (rev./min)	Molecular weight
Thymus	20mM-Tris/HCl (pH8.0), 0.5mM-NaN_3, 5mg of deoxycholate/ml	0.4	39460	18400
		0.2	37020	19100
		0.2	37020	19100
		0.2	39460	18000
Brain	20mM-Tris/HCl (pH8.0), 0.5mM-NaN_3, 5mg of deoxycholate/ml	0.2	37020	17200
		0.4	39460	17800
Thymus	20mM-Tris/HCl (pH8.0), 0.5mM-NaN_3, 5mg of Brij-96/ml in ^2H_2O:^1H_2O (6:19)	0.2	50740	18900
Thymus	20mM-Tris/HCl (pH8.0), 0.5mM-NaN_3	0.4	10589	290000
		0.4	10589	320000
Thymus	20mM-Tris/HCl (pH8.0), 0.5mM-NaN_3, 0.1M-NaCl	0.4	8766	310000
		0.2	10589	260000
Brain	20mM-Tris/HCl (pH8.0), 0.5mM-NaN_3	0.4	10589	280000

1976). The values obtained were 0.697ml/g for thymocyte Thy-1 glycoprotein and 0.703ml/g for brain Thy-1. The differences are due to differences in carbohydrate composition. By using these values and a δ_D of 0.24, the value of $1-\phi'\rho$ under the conditions for ultracentrifugation ($\rho = 1.004$) was calculated to be 0.353 for thymocyte Thy-1 glycoprotein, and 0.347 for brain Thy-1 glycoprotein.

Ultracentrifugation

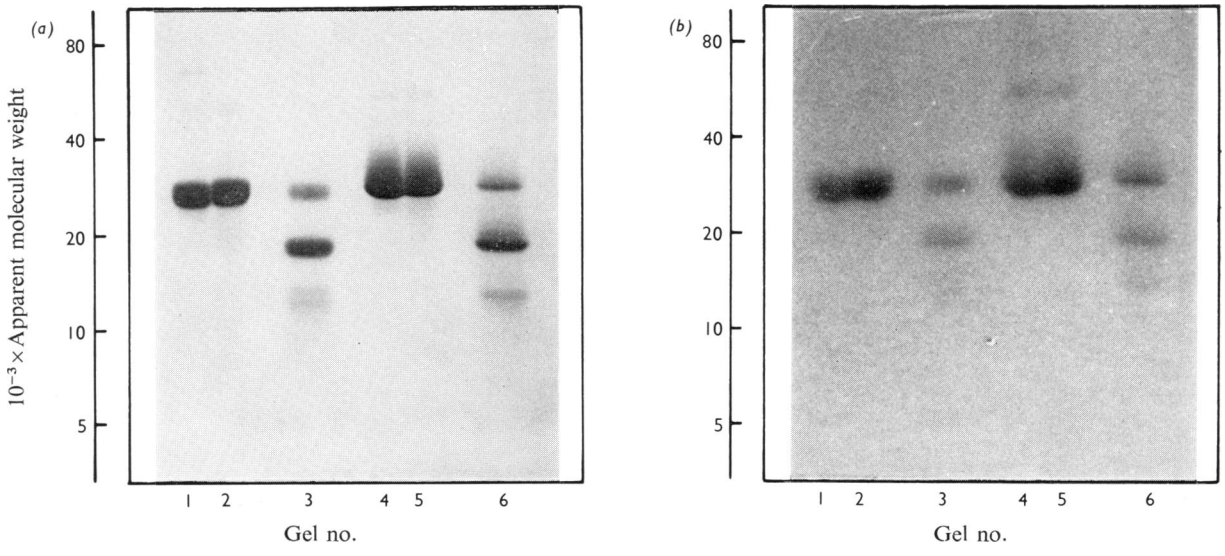
The series of high-speed-sedimentation-equilibrium experiments on brain and thymus Thy-1 glycoprotein gave data that were analysed in accordance with eqn. (1). In all circumstances except those referred to below, the graphs were linear within the experimental errors of the method (Teller, 1973). Table 1 summarizes these experiments, and Fig. 2 shows representative plots for thymocyte Thy-1 glycoprotein centrifuged in the presence of deoxycholate (Fig. 2*a*) and also in its absence (Fig. 2*b*). Under both of these conditions it is clear that the material is homogeneous with respect to molecular weight, since the graphs are linear.

In the first four rows of Table 1 results for three separate preparations of thymocyte Thy-1 glycoprotein in deoxycholate (5mg/ml) are shown. The molecular weights are in good agreement, and the mean was 18700. For brain Thy-1 glycoprotein in deoxycholate, one preparation was used, and from two centrifugation runs the mean value was 17500. The difference between these values is thought to be significant.

To check the molecular weight further, the alternative experimental design of Reynolds & Tanford (1976) was used with thymocyte Thy-1 glycoprotein. This method requires no knowledge of δ_D, but relies on the second term of eqn. (2) being reduced to zero by adjusting the density of the medium with ^2H_2O. Accordingly a sedimentation-equilibrium run was performed in Brij-96 (5mg/ml; $\bar{v} = 0.973$; Tanford *et al.*, 1974) in 20mM-Tris/HCl (pH8.0)/0.5mM-NaN_3 in a solvent of $^1H_2O/^2H_2O$ (19:6, v/v). The final buffer density was measured by weighing a standard volume and was found to be 1.03g/ml. The interferogram indicated that some material (of unknown composition) had floated towards the meniscus, but analysis of the pattern from the position where the fringes were horizontal to the base of the cell yielded satisfactory data. The analytical plot (eqn. 1) was linear, with a slope that corresponded to a mol.wt. of 18900 (line 7 in Table 1).

In the absence of deoxycholate and regardless of the presence or absence of 0.1M-NaCl, thymocyte or brain Thy-1 glycoprotein exists as a homogeneous oligomer (Fig. 2*b* and Table 1). For thymocyte Thy-1 glycoprotein the average mol.wt. was 300000, and for brain Thy-1 glycoprotein the value was 270000 (see the last five rows of Table 1). From these values and those for monomer Thy-1 glycoprotein it was calculated that the oligomer contains 16 molecules of Thy-1 glycoprotein. In a preliminary reference to these results (Barclay *et al.*, 1976), it was stated that the oligomer was somewhat heterogeneous. However, when the preparation was dialysed against the reference buffer before sedimentation (probably equilibrating traces of ethanol), results as in Fig. 2(*b*) were consistently obtained.

The sedimentation coefficient of the oligomer in the absence of deoxycholate was also determined so that its shape might be inferred. This was per-



EXPLANATION OF PLATE 1

Polyacrylamide-gel electrophoresis of Thy-1 glycoprotein before and after treatment with CNBr

All the samples were electrophoresed on 15% (w/v) polyacrylamide gels. Gels 1, 2 and 3 are electrophoretograms for brain Thy-1 glycoprotein, and 4, 5 and 6 for thymocyte Thy-1 glycoprotein; 15 μg samples were used for protein stain (*a*) and 30 μg samples for carbohydrate stain (*b*). Treatment of samples was as follows: 1 and 4, not treated with CNBr, non-reduced; 2 and 5, treated with CNBr, non-reduced; 5 and 6, treated with CNBr, followed by reduction and alkylation.

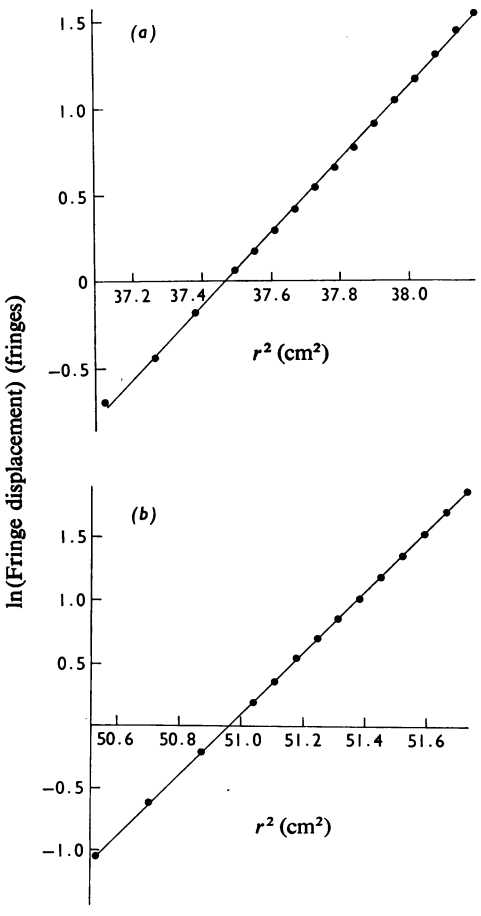


Fig. 2. *Sedimentation equilibrium of thymus Thy-1 glycoprotein in the presence and absence of deoxycholate*
Plots of ln(fringe displacement) versus r^2 (r, radial position) (eqn. 1) were obtained from data of sedimentation-equilibrium experiments. The temperature was 21°C, and the relevant buffer conditions are given in Table 1. (*a*) Results in the presence of deoxycholate, with an angular velocity of 37020rev./min (line 2, Table 1). (*b*) Results in the absence of deoxycholate, with an angular velocity of 10589rev./min (line 8, Table 1).

formed at 21°C at 39460rev./min in 20mM-Tris/HCl (pH8.0)/0.5mM-NaN_3, with an initial glycoprotein concentration of 0.9mg/ml. The material sedimented as a single peak as detected by schlieren optics, but accurate evaluation of any asymmetry was not possible, owing to the low concentration. A plot of ln(maximum ordinate) versus time was linear, and from this the sedimentation coefficient was computed (Schachman, 1959). A value of 12.8S was obtained, and this corresponds to a frictional ratio of 1.35 for the 300000-mol.wt. oligomer, a value consistent with the absence of marked asymmetry in the aggregate.

To see whether Thy-1 glycoprotein was aggregated or not at low deoxycholate concentrations, an experiment was carried out with thymocyte Thy-1 glycoprotein in deoxycholate (0.5mg/ml), which is well below the critical micelle concentration. It was immediately obvious that the Thy-1 glycoprotein was in a high-molecular-weight form, and thus the experiment was as for those in the absence of deoxycholate (Fig. 2*b*). The analytical plot was not linear (results not shown), but yielded a mean mol.wt. of 230000. Thus the small amount of deoxycholate bound at 0.5mg of deoxycholate/ml (see Fig. 1) was insufficient to dissociate the oligomers.

Finally the effect of 4M-guanidinium chloride [in 20mM-Tris/HCl (pH 8.0)/0.5mM-NaN_3] was measured in a high-speed equilibrium run as described by Munk & Cox (1972). The analytical plots (results not shown) were non-linear, with slopes that increased from the lowest to the highest concentrations. The heterogeneous mixture was in the range 30000–200000 mol.wt., indicating that 4M-guanidinium chloride does not dissociate the 300000-mol.wt. oligomer as effectively as does deoxycholate.

CNBr cleavage of Thy-1 glycoprotein

The products of thymocyte and brain Thy-1 glycoproteins after cleavage with CNBr are shown in Plate 1 after analysis by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. No effect was seen unless CNBr cleavage was followed by reduction. When this was done, bands appeared at positions corresponding to apparent mol.wts. of 18000 and 11000 (as well as some uncleaved Thy-1 glycoprotein), implying that the cleavage products are linked by at least one disulphide bond. All three bands stained for carbohydrate as well as protein (Plate 1), and could be labelled by iodo[^{14}C]acetate after reduction (Fig. 3).

The cleavage of both thymocyte and brain Thy-1 glycoprotein consistently occurred to the extent of about 70%, and changes in conditions involving time, concentration of CNBr and Thy-1 glycoprotein, temperature, solvent (100% formic acid; 70% trifluoroacetic acid; 1% sodium dodecyl sulphate/70% formic acid; and 3M-urea/70% formic acid were used), and prior reduction of Thy-1 glycoprotein did not increase this to 100%.

The smaller peptide appeared as a doublet after digestion of brain Thy-1 glycoprotein, and it could be argued that there are two peptides in this band. A more likely explanation is that the carbohydrate is heterogeneous, for the reason that when brain Thy-1 glycoprotein is electrophoresed on 10% (w/v)

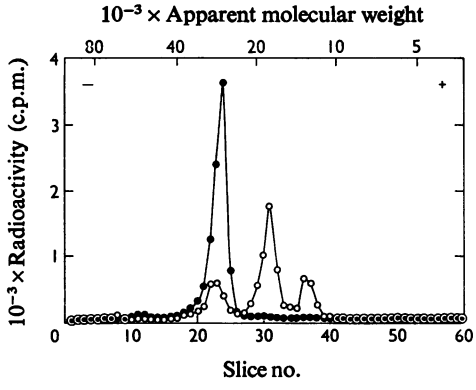


Fig. 3. [^{14}C]*Alkylation of Thy-1 glycoprotein after cleavage with CNBr*

Thymocyte Thy-1 glycoprotein was reduced and alkylated with iodo[2-^{14}C]acetic acid before (●) and after (○) treatment with CNBr. Samples (5μg) were then electrophoresed on 15% (w/v) polyacrylamide gels; after electrophoresis the gels were sliced into 1.5mm sections. They were added to 1.0ml of NCS solubilizer (Hopkin and Williams, Romford, Essex, U.K.)/water (9:1, v/v) and heated at 45°C for 2h. Toluene (10ml) containing 0.5% of PPO (2,5-diphenyloxazole) and 0.03% dimethyl-POPOP [1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene] was added and samples were counted for radioactivity in an LKB–Wallac liquid-scintillation counter.

acrylamide gels the intact molecule also appears as a doublet (A. N. Barclay & D. G. Campbell, unpublished work).

So far it has not been possible to separate the CNBr peptides, which remained aggregated in many solvents. No other peptides are seen, and polyacrylamide-gel electrophoresis and separation experiments suggest that they do not exist. After CNBr digestion Thy-1 glycoprotein was fractionated on Sephadex G-75 in 6M-urea/formate, pH3.3. The peptides, as shown in Plate 1, ran at the front, and lower-molecular-weight fractions were pooled, desalted on Sephadex G-75 and freeze-dried. These were then analysed for amino acids, which were not detected, except for glycine, which is a common amino acid contaminant of buffers.

Attempts were made to determine the number of *N*-termini produced by the CNBr cleavage, since this could establish the number of peptides regardless of size. The dansyl chloride method of Gray (1972) was used, but no *N*-termini were found either for native Thy-1 glycoprotein or for unfractionated CNBr-treated Thy-1 glycoprotein. The reasons for this remain to be established.

To summarize, CNBr cleavage of brain and thymocyte Thy-1 glycoprotein gave two peptides linked by at least one disulphide bond. The apparent molecular weights of these were 18000 and 11000, but these values probably bear little relation to the true molecular weights of the peptides.

Discussion

The experiments were done to measure the correct molecular weight of the monomer of Thy-1 glycoprotein and to see if the molecule behaves as one that is associated with lipid in the membrane. The mol.wts. of 18700 and 17500 for thymocyte and brain Thy-1 glycoprotein were somewhat lower than expected, but it should be noted that earlier estimates were made with unreliable procedures. From polyacrylamide-gel electrophoresis in sodium dodecyl sulphate, values of about 25000 were obtained (Letarte-Muirhead *et al.*, 1975), but this method has clearly been proved unreliable for heavily glycosylated molecules in studies on the major glycoprotein of human erythrocytes (Grefrath & Reynolds, 1974). For Thy-1 glycoprotein in deoxycholate, estimates of molecular weight from sucrose-gradient sedimentation and gel filtration at 5°C gave values of about 28000 for thymocyte or brain Thy-1 glycoprotein (Letarte-Muirhead *et al.*, 1974; Barclay *et al.*, 1975). This includes bound detergent, and if one assumes that 20% is deoxycholate as measured above, then a mol.wt. of 22000 would be obtained. This calculation is invalid, however, unless the hydrodynamic properties are determined at the same temperature as for equilibrium dialysis (21°C). From present studies it appears that Thy-1 glycoprotein binds to a deoxycholate micelle, and the size of this can vary with temperature (Small, 1968).

Given mol.wts. of 18700 and 17500 and the fact that 32% and 29% respectively of brain and thymocyte Thy-1 glycoprotein is carbohydrate (Barclay *et al.*, 1976), then the polypeptide has mol.wt. 12500 and consists of 110 amino acids. From the amino acid analysis this should include one methionine residue (1.2 for brain and 1.0 for thymocyte Thy-1 glycoprotein). This was supported by the fact that two peptides could be identified after CNBr cleavage.

The behaviour of Thy-1 glycoprotein in the presence and absence of deoxycholate puts it in the category of molecules that bind lipid in membranes. These molecules bind large amounts of weakly ionic or non-ionic detergents, whereas with water-soluble molecules such binding is exceptional (Helenius & Simons, 1975). Thy-1 glycoprotein bound 24μg of deoxycholate/100μg of glycoprotein, and on a protein basis (the detergent presumably binds to the polypeptide) this is 34μg/100μg. The binding curve with increasing deoxycholate concentration is virtually identical with that seen with cytochrome b_5 (Robinson & Tanford, 1975), and both these molecules appear to bind to a micelle of deoxycholate

rather than a detergent monomer. Without detergent, Thy-1 glycoprotein associates to form a homogeneous and roughly spherical oligomer containing 16 molecules of glycoprotein. This presumably occurs to remove hydrophobic portions from a hydrophilic environment, and 4M-guanidinium chloride did not completely dissociate the aggregate. Cytochrome b_5 also associates in the absence of detergent (Spatz & Strittmatter, 1971), and this behaviour is general for membrane molecules that bind lipid and need detergent for solubilization (Tanford & Reynolds, 1976).

The amino acid composition of Thy-1 glycoprotein is not unusually hydrophobic, and most of the molecule is probably found outside the membrane (Williams *et al.*, 1976). Other molecules of this type, for example cytochrome b_5 (Spatz & Strittmatter, 1971), the major human erythrocyte glycoprotein (Tomita & Marchesi, 1975), histocompatibility antigen (Ewenstein *et al.*, 1976) and viral glycoproteins (Brand & Skehel, 1972), all appear to have a small sequence of hydrophobic polypeptide by which the molecule is integrated into the membrane-lipid bilayer. This 'tail' is readily cleaved-off with proteolytic enzymes, releasing a water-soluble fragment that is more resistant to further proteolysis. With Thy-1 glycoprotein there is no evidence for similar behaviour (Williams *et al.*, 1976), and the nature of the hydrophobic portion of this glycoprotein remains to be established.

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