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GLYCOPROTEINS AND ANTIGENS OF MEMBRANES PREPARED FROM RAT THYMOCYTES AFTER LYSIS BY SHEARING OR WITH THE DETERGENT TWEEN-40

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Summary

In purification of cell surface antigens an efficient method for preparing membrane from large numbers of cells is needed. Such a method is described for preparing membranes from rat thymocytes after lysis in the non-ionic detergent Tween-40. Cell surface antigens were recovered at a yield of 30—50%, and a purification of 30—40-fold. By contrast enzyme markers for the other cell organelles were present in the membrane fraction in very low yield.

The membrane obtained with the detergent method was compared with that resulting from the best of previously described methods involving cell lysis by shearing. The detergent method compared favourably for simplicity as well as for yield and purification, and both membrane preparations contained similar protein and glycoprotein constituents.

The main glycoprotein bands of membranes from thymocytes and thoracic duct lymphocytes were identified after polyacrylamide gel electrophoresis in dodecyl sulphate. In thymocyte membrane, three main bands at apparent molecular weights of 150 000, 84 000 and 25 000 were seen, and of these the 84 000 glycoprotein did not bind to the lentil lectin. In thoracic duct lymphocyte membrane the 25 000 glycoprotein was absent and a band at 95 000 was intensified in comparison with thymocytes.

Introduction

In the purification of cell surface molecules from lymphocytes it is important that there should be a simple and efficient method for preparation of membrane. In most cases this has involved lysing cells by shearing followed by differential centrifugation. The best yields have been obtained by methods involving nitrogen cavitation or shearing by pumping through a pressure controlled orifice (see Crumpton and Snary for review [1]).

In the purification of rat Thy-1 antigen, membrane particles were prepared by the use of the detergent Tween-40 [2,3]. In screening for solubilisation of Thy-1 it was discovered that this detergent released the antigen in a form that stayed in suspension after centrifugation at $9 \cdot 10^4 \times g \cdot \text{min}$, but pelleted at $6 \cdot 10^6 \times g \cdot \text{min}$. The yield was about 50% at 10-fold purification. In these studies the nature of the membrane prepared by detergent was not fully characterised and this has now been done for rat thymocytes. In this paper the results are reported and contrasted with membrane prepared after shearing with the continuous flow pump. Parameters examined include the standard enzyme markers established for lymphocyte membrane preparations [1,4], and the constituent polypeptide and glycoprotein bands seen after electrophoresis on polyacrylamide gels in dodecyl sulphate. Also three cell surface antigens have been assayed, namely, Thy-1, a T lymphocyte-specific xenoantigen, and a leucocyte-common xenoantigen. Thy-1 is a major glycoprotein of rodent thymocytes [3], and the other two antigens were immunodominant in immunisations of rabbits with thymocyte membrane [5] or thoracic duct lymphocytes [6]. They both appear to be large glycoproteins and are not found in tissues other than lymphoid.

In the course of these studies the major glycoproteins of rat thymocytes and thoracic duct lymphocytes have also been defined, and the binding of thymocyte glycoproteins to lentil lectin studied.

Materials and Methods

Chemicals. Standard reagents were from Sigma Chemical Co. London; Schiff reagent from Difco Labs., Surrey; Coomassie Blue from Michrome, Edward Gurr Ltd., London.

Animals. Wistar rats from Oxfordshire Laboratory Animal Colonies, Oxon. were used.

Buffers. Tris/saline; 0.025 M Tris · HCl (pH 7.4)/0.15 M NaCl/0.01 M NaN_3/0.001 M $MgSO_4$/0.002 M $CaCl_2$.

Biochemical assays. Enzymic activities of 5'-nucleotidase (EC 3.1.3.5), glucose-6-phosphatase (EC 3.1.3.9), acid phosphatase (EC 3.1.3.1) and succinate dehydrogenase (EC 1.3.99.1) were measured as in ref. 7, 8, and 9 respectively. Protein was assayed by the Folin and Ciocalteau method with bovine serum albumin as standard [10], and samples with sucrose were precipitated with trichloroacetic acid. Phospholipid was extracted as in ref. 11 and the phosphorus determined as in ref. 12.

Assays for antigens. Cell surface antigens were assayed by inhibition of radioactive binding assays; these included Thy-1 xenoantigen [3], a T-lymphocyte-specific xenoantigen [5], and a leucocyte-common xenoantigen [6].

Thymocyte membranes. All procedures were at 0—4°C.

Rat thymocytes or thoracic duct lymphocytes were prepared and washed as before [3,13] with protein-free buffers, and counted with a Coulter counter model FN.

Steps in the membrane preparation are shown in Fig. 1 which covers all details except cell disruption and centrifugation of crude membrane on sucrose gradients.

For cell disruption by the shearing method cells were suspended at 10^8/ml in Tris/saline buffer (see above) and pumped through a small orifice at controlled pressure as in refs. 1 and 14. The cell number was reduced by 50% and the viability of the remaining cells was <5% compared with an initial 90%.

In the detergent method cells were suspended at $5 \cdot 10^8$/ml in Tris/saline and added to an equal volume of 4% Tween-40 in Tris/saline (warmed to dissolve, then cooled to 0°C). The mixture was stirred for 60 min and then homogenised with four strokes in a Potter-Elvehjem homogeniser volume 120 ml with a motor-driven teflon pestle. Viability by dye exclusion was reduced to 2% but the number of remaining nuclei equalled the starting cell number.

The crude membrane fractions prepared as in Fig. 1 were suspended to 5 mg/ml in 10 mM Tris · HCl (pH 7.4) and 3 ml per gradient was loaded on to continuous or stepwise sucrose gradients. Continuous gradients were prepared from 17 ml of 20%, and 17 ml of 50% (w/v) sucrose using a mixing device. For stepwise gradients three steps of 11 ml each were layered on to each other. For the shearing method these contained 40, 35, and 20% (w/v) sucrose while in the detergent method steps of 40, 28, and 10% were used. All sucrose solutions were in 10 mM Tris · HCl (pH 7.4). To each gradient 4—5 ml of crude membrane was added and centrifuged for 15 h at 25 000 rev./min in a SW27 rotor on a Beckman L5-65 ultracentrifuge. The gradients were fractionated through a long needle inserted into the bottom of the tube and 2-ml fractions were collected. The sucrose concentrations were measured with an Abbe refractometer, and the position of the membrane roughly assayed spectrophotometrically by turbidity readings at 340 nm. With the step gradients, fractions containing material at the density interfaces were pooled.

Lentil lectin affinity chromatography. Membrane was prepared from 10^{11} thymocytes with the detergent method and most of the sucrose removed by diluting appropriate gradient fractions with four volumes of 20 mM Tris · HCl (pH 8.0), and pelleting membrane by centrifugation at $5 \cdot 10^6 \times g \cdot$ min. To the pellets was added 60 ml of 2% (w/v) deoxycholate in the above buffer, plus 1.25 mM iodoacetamide and 1 mM diisopropyl fluorophosphate, and the mixture was homogenised and stirred for 60 min on ice. Further diisopropyl fluorophosphate was added to 1 mM and a high speed supernatant obtained by centrifugation at $5 \cdot 10^6 \times g \cdot$ min. This was passed through a column of 10 ml of Lentil lectin Sepharose 4B (10 mg lectin/ml), which was washed, and eluted with α-methyl glucopyranoside as previously described [3].

Polyacrylamide gel electrophoresis in sodium dodecyl sulphate. This was done on a BioRad slab gel apparatus using a 10% (w/v) acrylamide separating gel and a 3% (w/v) stacking gel as in ref. 15. Gel thickness was 1.5 mm for protein analysis, and 3.0 mm for glycoproteins. Samples were concentrated for electrophoresis either by precipitation with trichloroacetic acid, followed by washing with acetone, or by ultracentrifugation at $5 \cdot 10^6 \times g \cdot$ min. After acid precipitation a mixture of 2% dodecyl sulphate, 8 M urea and 1% dithiothreitol was added to the pellets followed by heating at 100°C for 3 min. To pellets obtained by centrifugation a mixture of 2% dodecyl sulphate (or greater to give dodecyl sulphate : protein of 10 : 1 (w/v)), 15% glycerol and 1% dithiothreitol was added before heating. Gels were stained for protein with Coomassie Blue and carbohydrate using the periodic acid-Schiff method

[3,16,17]. The apparent molecular weights of the glycoproteins were estimated as in ref. 16 using the following marker proteins: H chain (52 000) and L chain (23 000) of Rabbit IgG, bovine serum albumin (68 000), ovalbumin (43 000), chymotrypsinogen (25 600) and phosphorylase (94 000).

Results

The shearing and detergent methods

A flow sheet summarising both methods is given in Fig. 1. Apart from the sucrose gradient steps which are discussed below, the only points requiring explanation are the first steps in the detergent method where cells were stirred in Tween-40, and then given a brief homogenisation. It was found that in small scale preparations stirring alone gave good membrane yields, but with large preparations the yield dropped. This was remedied by a brief homogenisation, which was added in the belief that some agitation may be needed to release membrane destabilised by detergent. In a trial on cells without detergent this homogenisation caused no cell lysis, while in detergent virtually all cells were lysed.

Nuclei were not disrupted at all in detergent, while in the shearing method about 50% were destroyed as recommended by Snary et al. [14].

Distribution of the crude membrane fraction on sucrose gradients

Crude membrane was centrifuged to equilibrium on continuous sucrose gradients, and the distribution of protein and the membrane markers Thy-1 and 5′-nucleotidase are shown in Fig. 2. For the shearing method (Fig. 2a) a broad

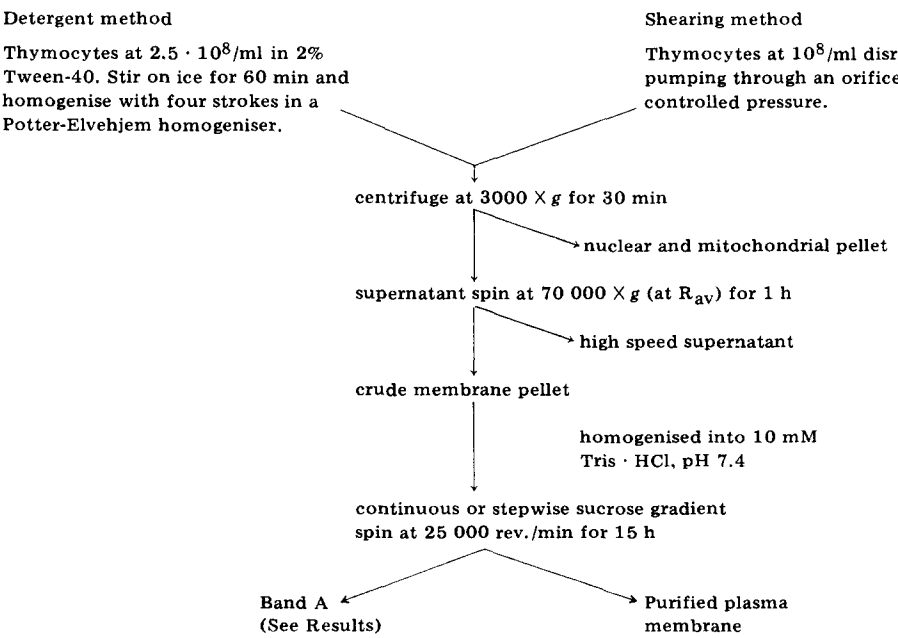

Fig. 1. Flow sheet for the preparation of membranes.

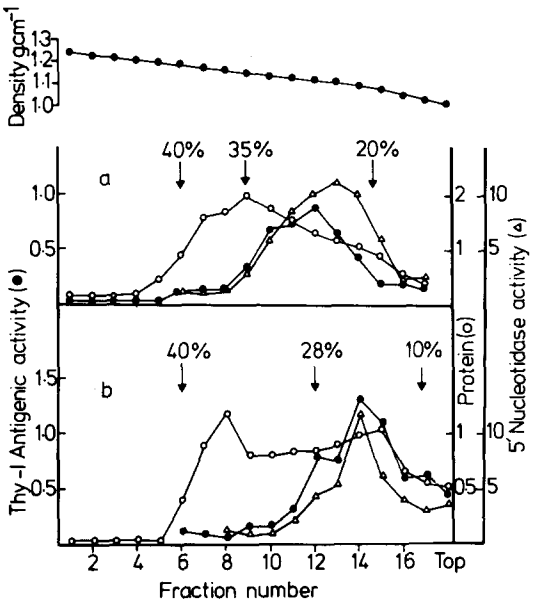

Fig. 2. Distribution of membrane markers on a sucrose density gradient. Crude membranes prepared by the shearing method (a), or the detergent method (b) were layered onto density gradients of 50—20% (w/v) sucrose. The gradients were centrifuged at 25 000 rev./min for 16 h in a Beckman SW27 rotor and then fractionated into 2-ml samples. These were analysed for sucrose concentration (density), protein (○, mg/fraction), 5'-nucleotidase (△, μM product/mg protein per h), and Thy-1 antigenic activity (●, units × 10^{-5}/mg protein).

peak of protein was obtained, and as shown by the specific activity of the membrane markers, the plasma membrane was found at a density of 1.155—1.08 g/cm (35—20% sucrose). The membrane is not clearly separated from non-membrane protein which is found at densities between 1.18 and 1.155 g/cm (40—35% sucrose).

For the detergent preparation (Fig. 2b) the material with high activity for Thy-1 and 5'-nucleotidase was at a density of 1.12—1.04 g/cm (28—10% sucrose), which is lower than in the shearing method. Due to this lower density the membrane is more clearly resolved from the non-membrane protein, which has about the same density in both preparations (compare Figs. 2a and 2b).

On the basis of the results in Fig. 2, stepwise gradients were constructed to give a plasma membrane fraction and a non-membrane protein fraction called Band A. The appropriate steps were 40, 35, and 20% for the shearing method, and 40, 28, and 10% for the detergent method.

Band A was found at the higher density interfaces and plasma membrane between the lower density steps. It was noted that in the detergent method the purified membrane fraction existed in the gradient as a yellow turbid band with some white flocculent material above. The specific activity of Thy-1 antigen was the same in both these fractions and thus they were pooled to constitute the purified membrane fraction.

TABLE I

ENZYME ACTIVITIES AND THY-1 ANTIGEN THROUGHOUT MEMBRANE PREPARATIONS BY THE SHEARING AND DETERGENT METHODS

Membrane was prepared from 10^{10} thymocytes. Protein content is given as percent of broken cells which contained 240 ± 20 (S.D.) mg protein. Enzymes were assayed on all fractions and activity related to that of broken cells put at 100%. The absolute activity in μmol of product released per h per 10^{10} cells ± S.D. was: 5′-nucleotidase, shearing method (S) 240 ± 26, detergent method (D), 270 ± 35; acid phosphatase, S, 170 ± 10, D, 170 ± 10; glucose-6-phosphatase, S, 200 ± 25, D, 200 ± 25; and succinate dehydrogenase, S, 10 ± 1.7, D, 30 ± 4. RSA is the relative specific activity and equals the specific activity (μM/h per mg) of each fraction divided by that of broken cells. For Thy-1 antigen 100% activity equalled $5 \cdot 10^5 \pm 0.6 \cdot 10^5$ units per 10^{10} cells and relative specific activity is as for the enzymes. Results are the mean values from three experiments and in all cases but one the standard deviation of each value in the Table was 20% or less of the value. The S.D. for 5′-nucleotidase in the nuclear pellet, with the shearing method was 30% of the mean value shown.

		Protein (%)	5′-Nucleotidase		Acid phosphatase		Glucose-6-phosphatase		Succinate dehydrogenase		Thy-1 antigen	
			%	RSA	%	RSA	%	RSA	%	RSA	%	RSA
Broken cells	S	100	100	1	100	1	100	1	100	1	100	1
	D											
Nuclear	S	48	12	0.2	21	0.4	20	0.3	77	1.6	27	0.5
and mitochondrial pellet	D	42	36	0.9	37	1.0	25	0.7	74	2.0	14	0.4
High speed supernatant	S	67	64	0.9	60	0.8	54	0.7	11	0.1	4.5	0.1
	D	64	46	0.7	61	1.0	70	1.0	11	0.1	2.4	0.1
Crude membrane	S	5.0	30	7.0	21	4.3	9.7	1.8	35	7.5	68	10
	D	5.0	25	4.7	12	1.9	10	1.9	13	2.2	71	9.9
Band A	S	1.0	2.3	2.3	2.7	2.6	0.2	0.2	16	12.5	7.3	4.1
	D	1.4	2.8	2.0	3.9	2.7	2.0	1.7	13	7.7	8.6	5.0
Purified membrane	S	1.5	30	20	5.2	3.7	2.9	1.5	3.0	1.8	61	25
	D	1.0	12	12	3.0	3.8	2.1	2.3	0.9	1.0	59	51

Enzymatic and antigenic markers

Four enzyme markers were used: 5′-nucleotidase for plasma membrane, acid phosphatase for lysosomes, glucose-6-phosphatase for endoplasmic reticulum, and succinate dehydrogenase for mitochondria. The use of these for rat lymphocyte membrane preparations has been well established [4]. Thy-1 antigen was also used as a membrane marker. This was appropriate since 600 000 molecules of Thy-1 are detectable at the cell surface, and only a little extra antigen can be detected in broken cells [3,13].

Table I shows all these activities and protein content for various steps in the preparation of membranes by the shearing and detergent methods. The recovery of enzymatic and antigenic activities are expressed as a percentage of the activity of the broken cells, and the relative specific activity or purification factor, which is normalised to the broken cells put at 1. The absolute activities for the enzymes are given in the footnote to Table I.

For all the assays the results for both preparations were quite similar though not identical. In a typical experiment using 10^{10} cells (240 mg protein), 3.8 mg of protein was obtained in the shearing method, while for the detergent method 2.3 mg of protein was recovered. The lower value for the detergent method could have been due to increased purity or loss of membrane material. The results for Thy-1 antigen suggested increased purity while those for 5′-nucleotidase favoured membrane loss. Taken together the results may suggest selective loss of some components, although this is not obvious from the analysis of polypeptide components by electrophoresis on polyacrylamide gels in sodium dodecyl sulphate (see below).

The results for markers of lysosomes, endoplasmic reticulum, and mitochondria, suggested that both membrane preparations were relatively free of these contaminants, and that Band A contains a considerable amount of mitochondria. These results can be compared with and are broadly similar to

TABLE II

ANTIGENS AND 5′-NUCLEOTIDASE IN MEMBRANES PREPARED BY THE SHEARING AND DETERGENT METHODS

For each fraction the percent activity is related to the activity of broken cells put at 100%. The absolute activities, in units per 10^{10} broken cells (240 mg protein) were, T-lymphocyte-specific xenoantigen, $6.4 \cdot 10^4$ (range $0.6 \cdot 10^4$), leucocyte-common xenoantigen, $1.2 \cdot 10^5$ (range $0.1 \cdot 10^5$), Thy-1 antigen $5.2 \cdot 10^5$ (range $0.6 \cdot 10^5$). For 5′-nucleotidase the absolute activity, in μmol of product released per h per 10^{10} broken cells was (shearing method, S) 240 (range 30), (detergent method, D) 270 (range 30). The relative specific acitivity (RSA) of each fraction is the specific activity divided by that of broken cells. The results are from one to two experiments where the values did not differ by more than 16%.

		T-Lymphocyte specific xenoantigen		Leucocyte-common xenoantigen		Thy-1 antigen		5′-nucleotidase	
		%	RSA	%	RSA	%	RSA	%	RSA
Crude membrane	S	60	10	50	6	67	9	33	6
	D	60	10	50	6	70	10	25	5
Band A	S	2	2	2	2	7	4	2.4	2
	D	10	8	5	6	8	6	3	2
Purified membrane	S	40	20	30	17	60	27	30	18
	D	30	30	40	30	60	46	13	12

those of Misra et al. [4] and Ladoulis et al. [18] for membrane preparations of rat spleen and thymus cells. One difference is that the contamination of the membrane fraction with succinate dehydrogenase is greater in the present studies than obtained in refs. 4 and 18. Presumably, this could have been minimised in our experiments by taking a cut-off point of lower density for the membrane fraction. This was not done as the aim was to obtain a high yield for subsequent purification of membrane molecules rather than to reduce contamination to insignificant levels.

To evaluate the membrane fractions further two other cell surface antigens of thymocytes were examined. The results are shown in Table II where the activities of crude membrane, Band A and purified membrane are compared with those of broken cells. The Thy-1 and 5'-nucleotidase data are included for comparison, and it can be seen that all three antigenic activities are recovered in high yield in the purified membrane fractions with little activity being found in Band A. The specific antigenic activity is higher with the detergent method. In the shearing method the purification and recovery of 5'-nucleotidase were similar to the antigens, but with the detergent method the enzyme marker seems partially lost compared with the antigens.

Phospholipid content

Phospholipids were measured in both purified membranes. For the shearing method there was 0.65 mg per mg of protein, while with the detergent method the value was 1.38 mg per mg of protein. This higher value is due to having less

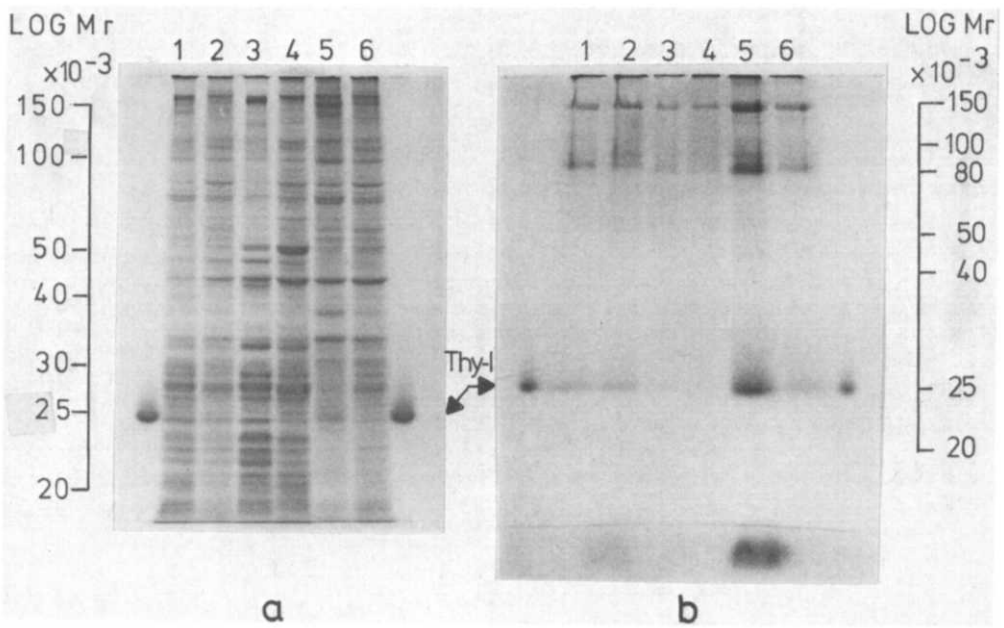

Fig. 3. Electrophoresis of membrane fractions on polyacrylamide gels in dodecyl sulphate. Samples from crude membrane (1, 2), Band A (3, 4) and purified membrane (5, 6) prepared by the detergent (1, 3, 5) or shearing (2, 4, 6) method were electrophoresed and stained for protein (a) or carbohydrate (b). For a, 30 μg of each sample was loaded and in b, 150 μg was used. On the sides of each gel 5 μg of pure Thy-1 from rat thymocytes was loaded.

protein in the detergent membrane, and on the basis of starting cell number the yield of phospholipid was 2.5 mg for the shearing method and 2.7 mg for the detergent method.

Protein and glycoprotein bands after polyacrylamide gel electrophoresis in dodecyl sulphate

The protein and carbohydrate bands after electrophoresis of crude membrane, Band A, and purified membranes on polyacrylamide gels containing dodecyl sulphate are shown in Figs. 3a and 3b, respectively. The protein stain shows a complex pattern and no systematic qualitative differences were observed between membrane prepared with the shearing or detergent method. Some regions seemed more heavily stained in the detergent membranes; for example, that corresponding to Thy-1 and the large glycoprotein region. This could be due to the higher specific activity of glycoproteins in this membrane

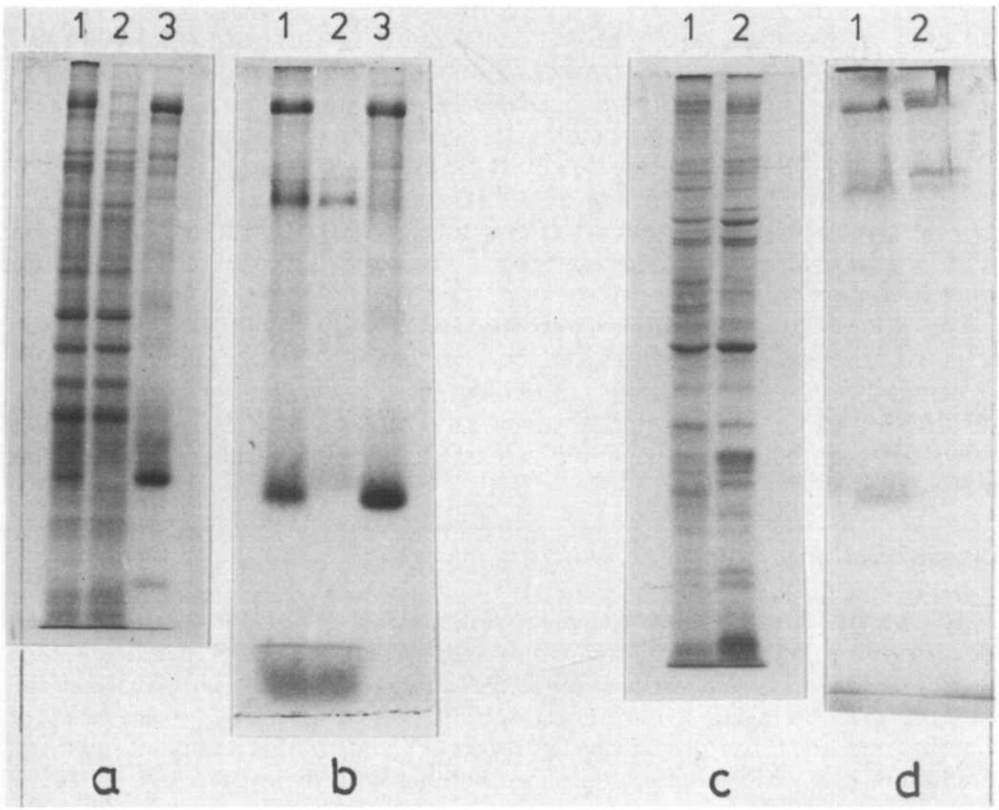

Fig. 4. Membrane glycoproteins of rat thymocytes and thoracic duct lymphocytes. Purified thymocyte membrane prepared by the detergent method was solubilised in deoxycholate and subjected to affinity chromatography on Lentil lectin. a and b show polyacrylamide gels in dodecyl sulphate stained for protein and carbohydrate and sample 1 is unfractionated membrane, 2, material unbound to the lectin column, and 3, material specifically eluted with α-methyl glucopyranoside. For a, 40 μg of protein was loaded on 1 and 2, and 20 μg on 3, while for b, the amounts were 160 μg for 1 and 2, and 80 μg for 3. In c and d are shown protein and glycoprotein profiles, respectively, of membrane from thymocytes (sample 1) and thoracic duct lymphocytes (sample 2) prepared by the shearing method. For c, 20 μg of protein was loaded while for d the amount was 150 μg.

(see below). The low molecular weight proteins in the crude membrane are found mainly in Band A, and in the pure membrane proteins are distributed from apparent molecular weights of 20 000—200 000.

The carbohydrate stain shows a much simpler pattern, and three main bands were seen at apparent molecular weights of 25 000, 84 000 and 150 000. These bands were found in purified membrane, Band A, and crude membrane, but the intensity was greatly increased in pure membrane compared with Band A. Also, staining was always more intense with membrane prepared with detergent compared with the shearing method. This is consistent with Table II since the 25 000 band is Thy-1 [3] and at least part of the 150 000 band is the leucocyte-common antigen (Sunderland, C.A. and Williams, A.F., unpublished).

As the glycoprotein pattern of rat thymocytes has not been clearly established before, further investigations involving affinity chromatography with Lentil lectin were carried out. The results are shown in Fig. 4a stained for protein, and Fig. 4b stained for carbohydrate. The distinction between the three main glycoprotein bands is clearly established since the 84 000 band did not bind to the lectin, while the 150 000 band and a part of the 25 000 band (Thy-1) did. Also, in these figures a number of more minor glycoprotein bands are seen. These are identified as bands staining weakly with periodic acid-Schiff (Figs. 4b, 2 and 3), or binding to Lentil lectin and staining for protein (Figs. 4a and 3). In this experiment it was important to add inhibitors of proteolysis when membrane was solubilised in deoxycholate since even at 0—5°C the 150 000 glycoprotein was readily autolysed to multiple bands in the range 90 000—150 000 molecular weight. This did not occur readily in unsolubilised membrane.

The thymocyte glycoproteins were also compared with those of membrane prepared from rat thoracic duct lymphocytes which contain about 75% mature T-lymphocytes in Wistar rats. From Fig. 4c it can be seen that in this membrane the 25 000 glycoprotein was absent, the 150 000 band was more heterogeneous, and a band of 95 000 apparent molecular weight was intensified compared with thymocytes.

Discussion

The results establish that the detergent method is suitable for preparation of membrane material from rat thymocytes for subsequent purification of glycoproteins and cell surface antigens. Yields are high and equal to the shearing method using a continuous pump, which is probably the best of shearing methods for preparative purposes [1]. With the detergent method, membrane can easily be prepared from batches of cells in the range of 10^{11}—10^{12}, and the advantages are simplicity, yield and the high degree of purity. While these advantages hold in purification procedures it is obvious that the method would not be suitable in studies where the aim is to prepare membrane as an organelle in a form analogous to that in the intact cell. After preparation in Tween-40 the lipid bilayer is likely to be grossly disrupted, and also selective loss of some components may occur.

Tween-40 detergent presumably disrupts cells by binding to and destabilising the membrane. The detergent has a $\bar{v}$ of approx. 0.88 ml/g [19] and the low

density of membrane prepared in its presence is probably due to detergent binding, in addition to the endogenous lipids which from the phospholipid analysis are not removed. It is not surprising that Tween-40 does not solubilise the membrane proteins since this is a detergent of the type known to be unsuitable for solubilisation of membrane proteins which bind to lipid [20,21]. The hydrophilic-lipophilic balance number is often used to assess the suitability of detergents for solubilisiation of hydrophobic membrane molecules and effective detergents usually have a value in the range 12—14, while for Tween-40 the hydrophilic-lipophilic balance number is 15.6 [20,21]. Although Tween-40 is ineffective for solubilisation of hydrophobic molecules it may release some molecules which are loosely associated with membrane [20] and this may contribute to the high specific activity of glycoproteins and antigens in membrane prepared with the detergent method.

Glycoproteins of rat thymocytes and thoracic duct lymphocytes have also been characterised in this study. Previously this has been examined by other workers [3,18], but the discontinuous polyacrylamide gels used here seemed to give clearer results. Three main glycoprotein bands were identified at apparent molecular weights of 25 000, 84 000 and 150 000 with a number of other minor bands also being present. The 25 000 glycoprotein band was absent from membrane of thoracic duct lymphocytes (Results and ref. 18). This molecule is the Thy-1 antigen and this is barely detectable serologically in peripheral lymphocytes [13]. At least a part of the 150 000 glycoprotein is the leucocyte common antigen (ref. 6 and Sunderland, C.A. and Williams, A.F., unpublished) while nothing is known of the 84 000 band except that as shown above it does not bind to Lentil lectin. The T-lymphocyte-specific antigen [5] has yet to be associated with a band on a gel. With regard to thoracic duct lymphocytes the intensified band at 95 000 molecular weight is of interest, but its relationship to the other major bands is yet to be established. Also at present it is not clear how many of the minor glycoprotein bands are distinct molecules, rather than artifacts of one sort or another.

Membranes from mouse thymocytes stained for carbohydrate have a virtually identical pattern to that shown for the rat above (Standring, R. and Williams, A.F., unpublished). Also these glycoproteins are probably the molecules which are dominant in surface labelling studies with ^{125}I and lactoperoxidase on mouse thymocytes. One large glycoprotein referred to as T200 has been studied in some detail by Trowbridge and co-workers [22,23], and this may be equivalent to the 150 000 band identified in these studies. Also in the mouse, surface labelling studies for carbohydrate with NaB^3H_4 and galactose oxidase have been carried out resulting in the identification of bands at 170 000 and 125 000 molecular weight [24]. These are presumably equivalent to the two bands staining with periodic acid-Schiff at similar positions, although there would appear to be a serious discrepancy between the apparent molecular weights of 125 000 in the mouse and 84 000 in the rat for the smaller of these two glycoproteins. In more recent experiments Gahmberg and Andersson [25] have labeled mouse thymocytes with NaB^3H_4 after periodate oxidation. This procedure labels sialic acid and in this case the apparent molecular weight of the smaller glycoprotein was reduced. In the galactose oxidase method the sialic acid is removed with neuraminidase prior to

surface labelling and this results in an increase of apparent molecular weight on polyacrylamide gels [25]. Labeling of the major glycoprotein at 25 000 apparent molecular weight (Thy-1) did not clearly occur in the experiments of Gahmberg et al. [24]. A band in this position on polyacrylamide gels was found with thymocytes of some mouse strains but not others. The reasons for this remains to be established.

At present it is difficult to determine the relationships between molecules studied by different workers with different techniques, but eventually purification of the major glycoproteins will clarify the confusion.

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