Biochimica et Biophysica Acta, 733 (1983) 154–162
Elsevier

BBA 71803

PREPARATION OF A HIGHLY PURIFIED SURFACE MEMBRANE FRACTION FROM RABBIT POLYMORPHONUCLEAR LEUCOCYTES BY HIGH-VOLTAGE FREE-FLOW ELECTROPHORESIS

D.I.H. STEWART *, C.H. EMES and N. CRAWFORD **

Department of Biochemistry, Institute of Basic Medical Sciences, Royal College of Surgeons of England, 35/43 Lincoln's Inn Fields, London, WC2A 3PN (U.K.)

(Received January 17th, 1983)
(Revised manuscript received May 30th, 1983)

Key words: Membrane fractionation; Cell surface; Sorbitol gradient; Free-flow electrophoresis; 5'-Nucleotidase; (Rabbit polymorphonuclear leukocyte)

A surface membrane fraction of high purity and good yield has been prepared from homogenates of rabbit peritoneal polymorphonuclear leucocytes, using a preliminary sorbitol density gradient sedimentation followed by preparative high voltage electrophoresis in a thin flowing buffer film. Enrichment values for the plasma membrane marker enzyme 5'-nucleotidase and ^{125}I-labelled *Lens culinaris* lectin, after the latter had been applied at the whole cell level, were 18-fold and 6-fold, respectively. Contamination of the surface membrane fraction by other organelles was negligible and approximately 1 mg of surface membrane protein can be obtained from $2 \cdot 10^9$ leucocytes. A triacylglycerol-rich, protein-poor fraction that lacks any definable structure in electron microscopy separates discretely from the surface membrane vesicles during electrophoresis. It is considered that this may be a contaminant not previously recognized as present in membrane fractions prepared by more conventional procedures.

Introduction

The response of polymorphonuclear leucocytes to chemotactic and phagocytic stimuli are membrane-mediated events involving not only integral surface membrane constituents but also, through transmembrane linkages, the various cytoskeletal elements which lie subadjacent to the membrane's cytoplasmic face [1]. In order to study, in these cells, some of the changes in the surface membrane structural and regulatory features during phagocytosis and other motile phenomena, a highly purified plasma membrane fraction is a prerequisite. Conventional techniques for the separation of leucocyte membranes from homogenates involve differential centrifugation and/or density gradient centrifugation using a variety of gradient media [2–4]. However, these techniques generally result in heterogeneous vesicle preparations containing surface membrane derived features contaminated to various degrees with intracellular membranes, the removal of which can often be achieved only by further gradient sedimentation under rate sedimentation or isopycnic conditions [5]. Recently, a three-step differential centrifugation procedure has been reported for the isolation of plasma membrane from human neutrophils

* Present Address: Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada.
** To whom reprint requests should be addressed.

Abbreviations: EGTA, ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*'-tetraacetic acid; PMSF, phenylmethylsulphonyl fluoride; 5'-nucleotidase, 5'-ribonucleotide phosphohydrolase, EC 3.1.3.5.

which had been homogenized by nitrogen cavitation [2]. This method gives initially a mixed membrane fraction containing plasma membrane and endoplasmic reticulum. Further resolution of these membrane elements is achieved by a continuous density gradient procedure using Dextran, yielding a surface membrane fraction which is up to 10-fold enriched in surface-specific markers relative to homogenate.

Differences in electrophoretic mobility of the various components of a mixed membrane fraction, due to differences in membrane vesicle charge characteristics, offers a possible way in which surface membranes of cells can be prepared free of other contaminating elements [6,7]. During the last few years, the development of free-flow electrophoresis as a preparative technique [8–10] has meant that a variety of cells and subcellular organelles can now be separated on the basis of their unit charge densities [11,12].

We present here a free-flow electrophoresis method that we have been using routinely to prepare a highly purified leucocyte surface membrane fraction in good yields from a mixed population of membrane vesicles harvested from a sorbitol gradient of separated homogenate.

Materials

Carrier-free $Na^{125}I$ and [2-^{3}H]adenosine 5'-monophosphate, ammonium salt, were obtained from Amersham International, U.K. 4-Methylumbelliferyl phosphate and 4-methylumbelliferyl-2-acetamide-2-deoxy-β-D-glucopyranoside were purchased from Koch-Light Laboratories, Colnbrook, U.K. Chloramine T was obtained from Hopkin & Williams Ltd., Chadwell Heath, U.K. *Lens culinaris* lectin, oyster glycogen, oxidized cytochrome *c*, sorbitol, leupeptin and pepstatin were obtained from Sigma Chemical Co. Ltd., Poole, U.K.

Methods

Preparation of polymorphonuclear leucocyte cell suspension. Peritoneal polymorphonuclear leucocytes were elicited in rabbits (3.5–5.0 kg) by intraperitoneal injection of 500 ml of sterile 0.1% (w/v) glycogen in 0.154 M saline and the cells were harvested after 8–16 h by washing out the peritoneal cavity with sterile saline [13]. The cells were washed twice with ice-cold 20 mM phosphate-buffered saline (pH 7.3; 300 mosmol), and resuspended in the same buffer to a final concentration of $1.2 \cdot 10^8$ cells/ml. In some experiments, the whole cells were surface-labelled with ^{125}I-labelled lectin prepared by the iodination of *L. culinaris* lectin with ^{125}I using the chloramine T procedure [14]. Initially, the lectin was kindly provided by Dr. M. Crumpton of the Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, London, but later this was purchased from Sigma Chemicals.

Cell disruption. The cells from the peritoneal cavity were washed a further two times with homogenization buffer (0.2 M sorbitol/1 mM EDTA/20 mM Tris-HCl (pH 7.4; 235 mosmol)) and finally resuspended in 4 ml of the same buffer but now containing the following protease inhibitors: 2 mM EGTA, 0.2 mM PMSF, 20 μg/ml leupeptin and 2 μg/ml pepstatin. Homogenization was performed by applying ten passes of a Potter-Elvehjem tube with a motor-driven Teflon pestle (Tri-R type, Camlab Ltd.). The homogenate was centrifuged at $800 \times g$ for 5 min at 4°C. A further 2 ml of the homogenization buffer, also containing the same cocktail of protease inhibitors, was added to the pellet and the homogenization was repeated with another three passes of the pestle. After repeating the low-speed centrifugation, the supernatants were pooled.

Analytical procedures. Protein was generally determined by the method of Lowry et al. [15], but if the samples contained sorbitol, the microtannin turbidometric method of Mejbaum-Katzenellenbogen and Dobryszycka [16] was used. Aliquots for enzyme analyses were dialyzed overnight against 0.154 M NaCl/20 mM Tris HCl (pH 7.3).

5'-Nucleotidase was assayed (in the presence of 1 mM *p*-nitrophenyl phosphate) as a marker for surface membrane [17]. The endoplasmic reticulum-containing fraction was identified by a glucose-6-phosphatase assay in the presence of NaF and EDTA and an acid phosphatase [19] assay. The latter enzyme is also localized in primary granules. The enzyme *n*-acetyl-β-glucosaminidase was assayed [20] as a marker for tertiary granules, and the distribution of malate dehydrogenase,

which is localized in the cytosol and mitochondria, was also determined [21]. Lactate dehydrogenase was assayed [22] as a marker for the cytosol compartment of the cell.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis was performed on 10% (w/v) resolving gels with 5.3% (w/v) stacking gels by the method of Laemmli [23].

Organic-phase extracts [24] of membranous material were examined by thin-layer chromatography with a hexane/diethylether/acetic acid (80 : 20 : 1) solvent system on Merck Silica-Gel 60 plates and quantitated for cholesterol [25], phospholipid [26] and triacylglycerols [27].

Periodic acid-Schiff staining of polyacrylamide gels was performed by the method of Glossman and Neville [28].

Deoxyribonucleic acid was determined by the ethidium bromide binding assay of Blackburn et al. [29]. Specificity was achieved by pretreatment with RNAase. Samples for transmission electron microscopy were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 1 h at 37°C. After pelleting by centrifugation, they were post-fixed with osmium tetroxide, dehydrated through a range of alcohols and embedded in Epon, and ultrathin sections were stained with uranyl acetate and examined by electron microscopy.

Isolation of surface membrane fraction. A sorbitol gradient, linear over the density range 1.08–1.23, was made using a gradient former with 8 ml of 13% (w/v) sorbitol and 8 ml of 55% (w/v) sorbitol, each solution containing 1 mM EDTA and buffered with 20 mM Tris-HCl to pH 7.4. We have found that sucrose solutions, or contaminants therein, compete for or displace *L. culinaris* from binding sites on the polymorph surface. Of a number of alternative sugars tried, sorbitol appeared not to release the ^{125}I-labelled lectin from the whole cells, and was therefore used instead of sucrose in both the homogenization buffer and in the density gradients.

The pooled supernatants from the homogenization were applied in volumes of up to 4 ml to the upper surface of sorbitol gradients which were then centrifuged at 70 000 × *g* for 50 min in an M.S.E. 3 × 25 swing-out rotor. The linearity of the gradient was determined by refractive index measurements of fractions from an unloaded gradient, centrifuged under the same conditions. The gradient fractions were designated: S_0, the non-linear portion of the gradient; S_1, in the density range 1.08–1.13; S_2, a turbid band in the density range 1.13–1.18; S_3, a slightly opaque band in the range 1.18–1.23, and S_4 being the pellet which was resuspended in a small volume of homogenization buffer. The S_2 fraction was diluted to 50 ml with ice-cold homogenization buffer and the membrane sedimented by centrifugation at 100 000 × *g* for 90 min at 4°C to yield a pellet designated PS_2.

Purification of surface membrane by free-flow electrophoresis. The 100 000 × *g* mixed-membrane pellet (PS_2) was resuspended in the electrophoresis chamber buffer (0.275 M sorbitol/10 mM triethanolamine/acetic acid (pH 7.3; 300 mosmol)) to a protein concentration of 2–3 mg/ml. This material was then applied to an Elphor VAP 5 free-flow electrophoresis apparatus (M.S.E./Bender and Hobein) [6]. The voltage was maintained at a constant 1100 V which generally gave a current of 70–110 mA at a chamber temperature of 5.5–6.5°C. The sample was injected at a flow rate of 1.5–2.0 ml/h and the chamber buffer flow rate was 3.8 ml/h per fraction. The electrode buffer was 100 mM triethanolamine/acetic acid (pH 7.3). Fractions around the peaks were pooled and the membranes were collected by centrifugation at 100 000 × *g* for 90 min at 4°C.

Results

The procedure for cell disruption was optimized by varying the number of passes made with the tight-fitting pestle homogenizer. In each experiment, a high-speed supernatant was prepared from the homogenate by centrifuging at 150 000 × *g* for 90 min, and this was assayed for lactate dehydrogenase. Maximum release of lactate dehydrogenase was achieved at 10–15 passes of the pestle (Fig. 1) and no further release occurred up to 25 passes. Light microscopy of the pellets revealed that after ten passes, few, it any, intact cells remained and the isolated nuclei seemed well preserved. Electron micrographs showed good preservation of granule integrity. A low-speed centrifugation of homogenate after ten passes removed all the nuclei and larger cell debris.

Centrifugation of homogenates on sorbitol

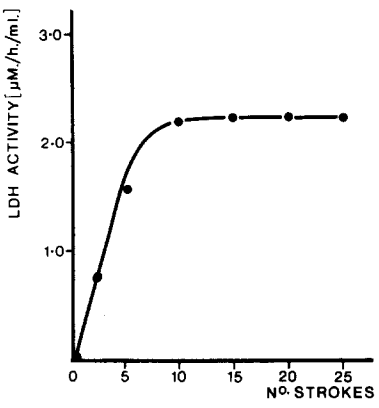

Fig. 1. The homogenization procedure monitored by the release of lactate dehydrogenase (LDH).

gradients produced a discrete turbid zone (S_2) within the density range 1.13–1.18. The nature of this zone was investigated and shown to contain surface elements by analysis of the distribution and enrichment of the marker enzyme 5′-nucleotidase and the surface lectin, ^{125}I-labelled *L. culinaris* lectin, which had been applied at the whole cell level. In addition to S_2 there were generally two other bands in the gradients which also contained ^{125}I-labelled lectin and 5′-nucleotidase (Table I): S_3 and S_4. The S_4 pellet fraction represents a small quantity of aggregated cell debris. The S_3 fraction was always very low in particulate content and, although it contained a reasonably high quantity of surface membrane markers, it was considered unsuitable for further purification because in all experiments, it also contained 2–3-times more contamination by non-plasma membrane markers than did fraction S_2 (Table I). Furthermore, when subjected to free-flow electrophoresis, the resuspended 100 000 × *g* pellet from this S_3 fraction produced a single peak which, following free flow, showed no enrichment in the activities of 5′-nucleotidase, ^{125}I-labelled lectin or acid phosphatase.

The S_2 mixed membrane fraction was routinely taken from the gradient, sedimented and resuspended in chamber buffer (PS_2). When run on the free-flow electrophoresis apparatus, this PS_2 sample always resolved into two discrete bands (Fig. 2a); F_1 was the most, and F_2 the least, electronegative of the two fractions. Analysis of these fractions in over 20 preparations showed that profiles for ^{125}I-labelled lectin and 5′-nucleotidase activity exclusively localized with the most electronegative peak F_1 (Figs. 2a and 2b).

Table II shows the enrichment of the specific activities of 5′-nucleotidase and ^{125}I-labelled lectin

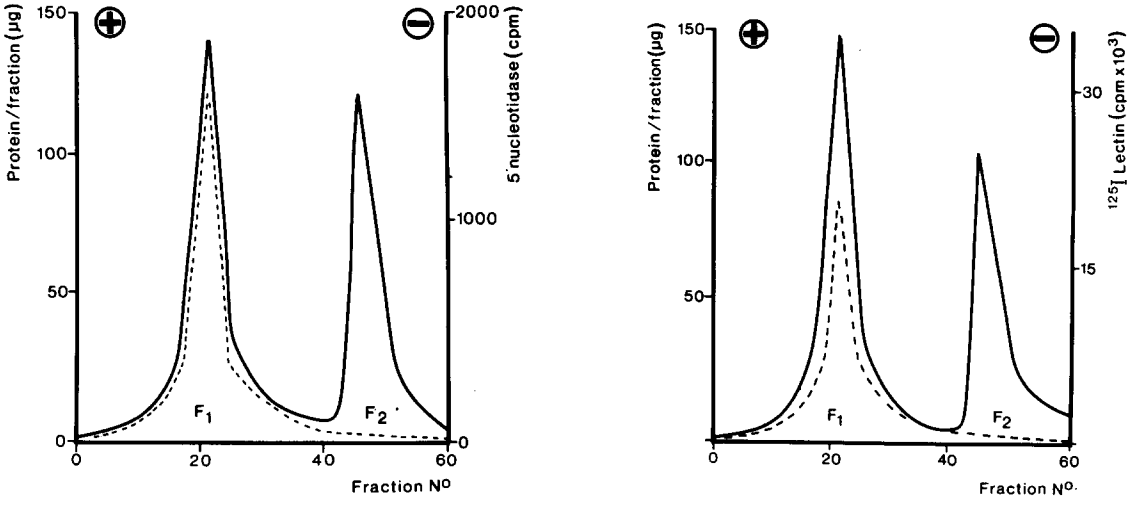

Fig. 2. (a) Free-flow electrophoresis profile showing 5′nucleotidase activity (— — —) and protein distribution (———). The enzyme activity is expressed as cpm for P_i released from ^{32}P-labelled AMP/mg protein per h. (b) Free-flow electrophoresis profile showing ^{125}I-labelled *Lens culinaris* lectin (— — —) and protein distribution (———).

TABLE I

SPECIFIC ACTIVITIES AND PERCENTAGE DISTRIBUTION OF MARKER ENZYMES AND ^{125}I-LABELLED LECTIN IN A TYPICAL GRADIENT FRACTIONATION

Specific activity (S.A.) given in μmol/min per mg protein for malate dehydrogenase, glucose-6-phosphatase and 5′-nucleotidase, nmol P_i/min per mg protein for the phosphatases and cpm/mg protein for the ^{125}I-labelled *Lens culinaris* lectin. % recovery is the sum of recoveries for each fraction (total activity in gradient) with respect to homogenate (100%). grad., gradient. Values are averages of three determinations.

Fraction	Malate dehydrogenase		Glucose-6-phosphatase		Acid phosphatase		Alkaline phosphatase		5′-Nucleotidase		^{125}I-labelled Lectin		Protein	
	S.A.	% in grad.	S.A.	% in grad.	S.A.	% in grad.	S.A.	% in grad.	S.A.	% in grad.	S.A.	% in grad.	mg	% in grad.
S_0	108	62	0.4	14	10	33	4.1	68	0.20	4	400	8	33	51
S_1	68	11	3.0	24	5	4	1.5	12	0.30	2	1000	5	8	12
S_2	45	6	1.4	10	3	2	0.5	3	0.450	21	6200	25	7	11
S_3	77	10	4.6	37	26	21	0.8	6	0.500	27	8200	37	8	12
S_4	41	11	1.5	15	41	41	1.1	11	0.690	46	3800	24	10	15
% recovery		89		93		95		89		107		81		97

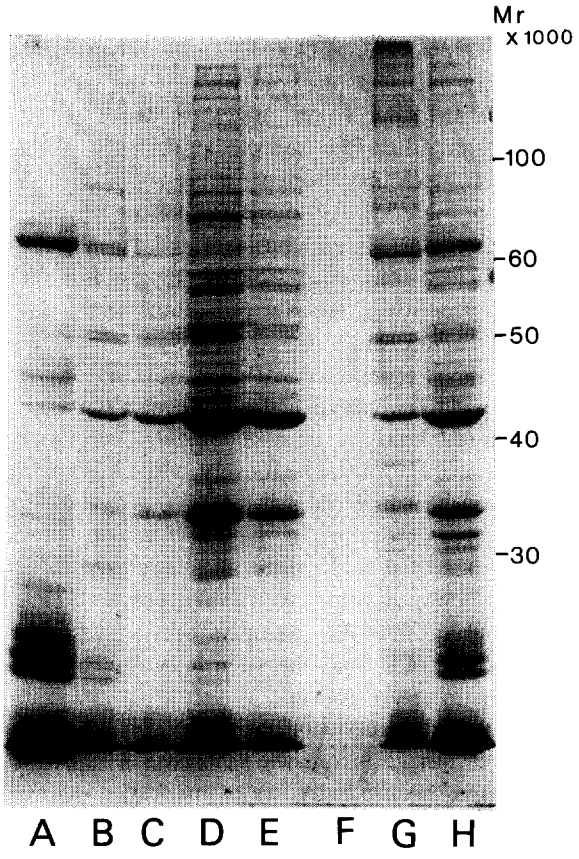

Fig. 3. SDS-polyacrylamide gel electrophoresis (10% gel) separation of polypeptides present in fractions taken at different stages during the surface membrane preparation. A–E, sorbitol density gradient fractions, S_0, S_1, S_2, S_3 and S_4. F, free-flow electrophoresis peak fraction F_2; G, free-flow electrophoresis peak fraction F_1; H, whole cell homogenate.

in the pooled F_1 fractions expressed with respect to the activities found for the homogenate, H_2. The free-flow electrophoresis procedure increased the enrichment values achieved at the sorbitol gradient stage by a factor of two or more. The final enrichement of ^{125}I-labelled lectin in all the F_1 fractions studied was, however, generally less than that of the 5′-nucleotidase activity. This is attributed to redistribution artifacts due to difficulties in the low-speed washing procedures for removal of the unbound ^{125}I-labelled lectin before cell disruption.

The polypeptide patterns of the 100 000 × *g* pellet (PF_1) obtained from pooled fractions taken across the most electronegative peak (F_1 in Figs. 2a and 2b) and the gradient fraction S_2 from which it was derived, are shown in Fig. 3. Notable is the absence in PF_1 of a number of low-molecular-weight species (under 30 000). Since these polypeptides were not present in the supernatant after

TABLE II

ENRICHMENT AND RECOVERY VALUES FOR MARKER ENZYMES IN FRACTIONS TAKEN AT DIFFEENT STAGES OF THE MEMBRANE PURIFICATION

Specific activities are expressed as in Table I and converted to relative specific acitivites ('Enrichment') with respect to homogenate values. % recovery with respect to initial homogenate (100%). S_2, mixed membrane fraction from the sorbitol gradient; PS_2, pellet from the sorbitol gradient fraction; PF_1, pellet from the most anodal fraction (F_1) in the free-flow electrophoresis profile. n.d. = not detected. Values are means for the number of determinations listed in column 2. PF_1 enrichment figures are means ± S.D.

Enzyme, etc.		Fraction					
		S_2		PS_2		PF_1	
		% recovery	Enrich-ment	% recovery	Enrich-ment	% recovery	Enrichment
5′-Nucleotidase (EC 3.1.3.5)	(4)	23	3.5	25	8.0	23	18.0 ± 0.8
^{125}I-labelled *L. culinaris* lectin	(4)	21	3.0	14	4.0	11	6.2 ± 0.9
Malate dehydrogenase (EC 1.1.1.37)	(2)	5	0.12	1	0.9	1	0.01
Acid phosphatase (EC 3.1.3.2)	(2)	2	1.8	1	1.2	1	1.2
Alkaline phosphatase (EC 3.1.3.1)	(2)	3	0.65	3	0.95	3	3.3
Glucose-6-phosphatase (EC 3.1.3.9)	(2)	9	1.0	5	1.1	2	1.3
N-Acetylglucosaminidase (EC 3.2.1.30)	(2)	n.d.	n.d.	n.d.	n.d.	1	0.04
Lactate dehydrogenase (EC 1.1.1.28)	(3)	1	0.01	1	0.01	1	0.01
Protein		11	–	2	–	0.5	–

the pelleting of PF_1, they are believed to represent soluble-phase constituents trapped initially in the gradient fraction but subsequently dispersed in a non-discrete manner in the electrophoresis chamber during the run.

Fractions were also taken from the least electronegative peak F_2 (Figs. 2a and 2b) and these were pooled and centrifuged at $100\,000 \times g$ for 90 min (PF_2). This PF_2 sample, when run on SDS polyacrylamide gels, showed a complete absence of any stainable polypeptides using the Coomassie brilliant blue staining procedure, although very faint bands were present using the silver staining procedure. We attempted to investigate the nature of this PF_2 sample by the following experiments. The periodic acid-Schiff staining of the SDS gels failed to demonstrate the presence of any glycoproteins in the PF_2 sample. Tests for the presence of nucleic acids in PF_2 using ethidium bromide fluorescence were always negative. Analysis of organic-phase extracts of PF_2 and PF_1 by thin-layer chromatography and specific quantitative assays showed that, on a comparative basis, the PF_2 fraction was enriched at least 30-fold in triacylglycerols compared to PF_1. However, unlike PF_1, the content of cholesterol and phospholipid was low or virtually negligible in PF_2. Taken together, these results suggest that the PF_2 sample represents a triacylglycerol-rich component present in the S_2 membrane material. Although it has been reported that triacylglycerols can be liberated from membranes during long-term storage, we do not feel that this fraction is so derived, since the whole preparative procedure, including the free flow electrophoresis, is completed within a working day. It is considered that this PF_2 fraction may represent either stored lipid micelles or triacylglycerol-rich, protein-poor lipoproteins loosely associated with the plasma membrane vesicles.

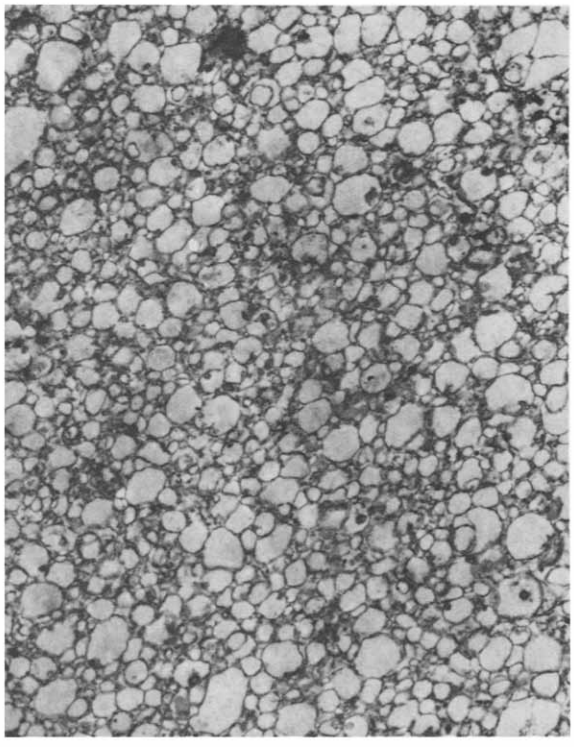

Fig. 4. Electron microscopy of purified membrane fraction PF_1. Original magnification, ×40000; as presented, ×28000.

Electron microscopy of the PF_1 fraction shows the presence of sealed membrane vesicles with no apparent contamination by granular elements (Fig. 4). Examination of PF_2 samples prepared by the same procedure showed no such organized vesiculated membrane structures.

The overall purification of the surface-membrane fraction PF_1 from the whole cells based on the relative specific activities of markers is shown in Table II, from which it can be seen that the PF_1 fraction, in which the lectin surface label is exclusively located, is on average 18-fold enriched in 5′-nucleotidase with respect to homogenate activities. This fraction contains negligible amounts of *N*-acetylglucosaminidase and antimycin-insensitive NADH-cytochrome *c* reductase (EC 1.6.99.3) both of which are markers for intracellular elements (lysosomes and endoplasmic reticulum) in these and other cells. Lactate dehydrogenase activities were always undetectable, suggesting no significant contamination by cytosolic constituents after the free-flow electrophoresis.

Discussion

Density gradient centrifugation of a leucocyte homogenate on 10–55% (w/v) sorbitol gradients yields one major and one minor fraction (S_2 and S_3) containing plasma membrane markers. The higher density fraction, S_3, has much less particulate material than S_2 and may either be the result of some genuine in vivo association between plasma membrane elements and intracellular membranes, or may be an artifact of the cell

disruption procedure which causes nonspecific aggregation of membranes of different cellular origin. In either event, the contaminating non-surface membrane material cannot be removed from this S_3 fraction by free-flow electrophoresis.

The low-density membrane fraction, S_2, showed little, if any, contamination by endoplasmic reticulum and mitochondrial marker enzymes and separated into two discrete fractions when electrophoresed (F_1 and F_2). Analysis of the F_1 fractions showed that this peak accounted for almost all of the ^{125}I-labelled lectin and 5′-nucleotidase activity in the material applied to the chamber. Neither marker was detectable in the F_2 fraction.

The significant enrichment in alkaline phosphatase activity (3.3-fold) in PF_1, compared to the homogenate activity, as well as a smaller but also significant enrichment in glucose-6-phosphatase activity (1.3-fold), suggests that a small amount of endoplasmic reticulum-like material co-purifies with the plasma membrane. Although, in common with other workers in this field (e.g., Ref. 30), we have used glucose-6-phosphatase as a marker for endoplasmic reticulum, in our hands this enzyme in leucocytes does not fulfil the criteria established for the liver enzyme (pH 5 lability and inhibition by excess glucose). It should perhaps, as in other cells, be regarded as a nonspecific phosphatase which has some value as an intracellular membrane marker. It has been suggested [31] that some alkaline phosphatase activity can be associated with a specific organelle known as a phosphasome. Should this be the case in rabbit leucocytes, this activity may not be separable from plasma membrane vesicles by electrophoresis. Alternatively, a genuine in vivo structural association between certain domains of the endoplasmic reticulum and the plasma membrane could also account for this co-electrophoresis. The slight enrichment in specific activity for acid phosphatase in the PF_1 surface membrane fraction when compared to homogenate levels (Table II) is not considered to be important, since this enzyme is known to have a bimodal distribution in granulocytes between primary granules and plasma membrane vesicles.

On the basis of the biochemical evidence presented here, supported by the vesicular appearance in electron microscopy, we believe that our fraction F_1, isolated from a mixed membrane population, is a highly purified membrane fraction. This fraction can be prepared in high yields, since the electrophoresis stage is fully preparative and it is suitable for detailed analytical investigation. The F_2 peak, seen consistently in our electrophoretic profiles, but varying in amount, is devoid of surface markers and shows no identifiable vesicles in electron microscopy. This fraction, however, which separates discretely from the plasma membrane during electrophoresis, shows no clearly identifiable polypeptides in SDS-polyacrylamide gel electrophoresis although it is rich in triacylglycerols. Its origin at the whole cell level is unknown, but we believe it is not generated artifactually from the F_1 plasma membrane during our electrophoresis procedures, since when F_1 is pooled and re-electrophoresed, only a single peak is seen with the same electrophoretic mobility and analytical characteristics as that originally isolated. In view of the reported relative abundance of triacylglycerols in polymorphonuclear leucocytes [32], it is perhaps not surprising such a triacylglycerol-enriched fraction is indentifiable and it may represent a genuine lipid rich subcellular entity worthy of further study now that it can be separated from other elements. The inclusion of this triacylglycerol-rich component in leucocyte membranes isolated only by density gradient procedures may well account for the wide variation in the literature in leucocyte membrane lipid data.

In conclusion, the overall purification of plasma membrane in reasonable yield (about 1 mg of purified membrane from $2 \cdot 10^9$ cells) achieved in this study, using a combination of gradient sedimentation and free flow electrophoresis, is 18–20-fold with respect to 5′-nucleotidase specific activities. The enrichment values for this membrane marker enzyme and for the lectin applied at the whole cell level are substantially higher than those generally reported for more conventional gradient separation methods, though we have reported similar enrichment values for membranes harvested by a nylon fibre adhesion procedure [13]. Although 5′-nucleotidase appears to be substantially confined to plasma membranes in guinea-pig polymorphonuclear leucocytes [30], is has been shown cytochemically to be present also in the primary granules of rabbit leucocytes [3] and in both plasma membranes and cytosol of human neutrophils [31],

though lower in activity than other species. With free-flow electrophoresis procedures, it is not possible to compile complete balance sheets and recovery data for the various constitutents as done in the case of density gradient methods. During electrophoresis, any soluble-phase constituents trapped within a particulate fraction may become widely dispersed across the 90-fraction profile emerging from the chamber. Recoveries are thus grossly distorted and have little meaning.

In the present study, comparison of the recovery data (Table II) suggests that there is some loss of lectin in both the centrifugal step to sediment the particles (PS_2) and in the electrophoresis. The recovery values for 5′-nucleotidase do not change through these procedures which, taken together with the progressive enrichment from 3-fold to 18-fold, with respect to homogenate, substantiates its value as a surface marker enzyme for this species.

Acknowledgements

We are grateful to Mr. Steve Gschmeissner of the Department of Anatomy, Royal College of Surgeons, London, for preparation of samples for electron microscopy and to Dr. Ron Hutton of the Department of Haemophilia and Thrombosis, Royal Free Hospital, London, for providing differential cell counts. We would like to thank the Cancer Research Campaign for continuing financial support. One of us (D.I.H.S.) was the recipient of an MRC studentship in research.

References

1 Weissmann, G., Smolen, J.E. and Korchak, H.M. (1980) N. engl. J. Med. 303, 27–34
2 Klempner, M.S., Mikkelson, R.B., Corfman, D.H. and Andre-Schwartz, J. (1980) J. Cell Biol. 86, 21–28
3 Bainton, D.F. and Farquar, M.G. (1968) J. Cell Biol. 39, 286–298
4 De Pierre, J.W. and Karnovsky, M.L. (1973) J. Cell Biol. 56, 275–303
5 Avruch, J. and Wallach, D.F.H. (1971) Biochim. Biophys. Acta 233, 334–347
6 Menashi, S., Weintroub, H. and Crawford, N. (1981) J. Biol. Chem. 256, 4095–4115
7 Hannig, K. (1980) J. Chromatogr. 159, 183–191
8 Zeiller, K., Pascher, G. and Hannig, K. (1976) Immunology 31, 863–880
9 Hannig, K. and Heidrich, H.-G. (1977) in Cell Separation Methods (Bloemendal, H., ed.), 93–115, Elsevier/North-Holland Biomedical Press, Amsterdam
10 Heidrich, H.-G., Russmann, L., Bayer, B. and Jung, A. (1979) Z. Parasitoenkunde 58, 151–159
11 Hannig, K. (1972) in Techniques of Biochemical and Biophysical Morphology (Glick, D. and Rosenbaum, R., eds.), Vol. 1, pp. 191–198, Wiley, New York
12 Hannig, K. and Heidrich, H.-G. (1974) Methods Enzymol. 31, 746–761
13 Stewart, D.I.H. and Crawford, N. (1981) FEBS Lett. 126, 175–179
14 Majerus, P.S. and Brodie, G.N. (1971) J. Biol. Chem. 247, 4253–4257
15 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
16 Mejbaum-Katzenellenbogen, W. and Dobryszycka, W.M. (1959) Clin. Chim. Acta 4, 515–522
17 Avruch, J. and Wallach, D.F.H. (1971) Biochim. Biophys. Acta 490, 27–34
18 Hubscher, G. and West, G.R. (1965) Nature 205, 799–801
19 Fernley, H.N. and Walker, P.G. (1965) Biochem. J. 97, 95–103
20 Peters, T.J., Muller, M. and De Duve, C. (1972) J. Exp. Med. 136, 1117–1139
21 Lowry, O.H., Roberts, N.R. and Kapphahn, J.H. (1957) J. Biol. Chem. 224, 1047–1064
22 Wroblewski, F. and Ladue, J.S. (1955) Proc. Soc. Exp. Biol. Med. 90, 210–213
23 Laemmli, U.K. (1970) Nature 227, 680–685
24 Bligh, E.G. and Dyer, W.J. (1959) Can. J. Bioc. Physiol. 37, 911–917
25 Crawford, N. (1959) Clin. Chim. Acta 4, 494–502
26 King, E.J. (1932) Biochem. J. 26, 292–297
27 Snyder, F. and Stephens, N. (1959) Biochim. Biophys. Acta 34, 244–249
28 Glossman, H. and Neville, D.M. (1971) J. Biol. Chem. 246, 6339–6346
29 Blackburn, M.J., Andrews, T.M. and Watts, R.W.E. (1971) Anal. Bioc. 51, 1–10
30 De Pierre, J.W. and Karnovsky, M.L. (1974) J. Biol. Chem. 240, 7121–7129
31 Rustin, G.J.S. and Peters, T.J. (1979) Br. J. Haematol. 41, 533–543
32 Elsbach, P. (1959) J. Exp. Med. 110, 969–980