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PROTEIN COMPONENTS OF TWO DIFFERENT REGIONS OF AN INTESTINAL EPITHELIAL CELL MEMBRANE

REGIONAL SINGULARITIES

MICHIYA FUJITA, KOICHI KAWAI, SHIGETAKA ASANO and MAKOTO NAKAO

Department of Biochemistry, School of Medicine, Tokyo Medical and Dental University, Yushima-1, Bunkyo, Tokyo (Japan)

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SUMMARY

The two different regions of the plasma membrane, *i.e.* apical and basolateral membranes, of intestinal epithelial cells were analyzed as to their protein components. They showed very contrasting profiles on sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The apical membranes possessed several major components with apparent molecular weights larger than 108000, most of which were also periodic acid–Schiff reagent positive. In contrast, there were no protein components with corresponding molecular weights in the basolateral membrane. The electrophoretic profile of the latter was strikingly simple. The dominant band was assigned a molecular weight of 101000 and was periodic acid–Schiff reagent negative. No major components were shared by the two membranes.

INTRODUCTION

A considerable amount of knowledge has been accumulated on the electrophoretic profiles of plasma membrane proteins of animal cells, especially for erythrocytes. However, there have been few reports on possible differences among different regions of the plasma membrane of a single cell.

We have previously reported a differential isolation of apical and basolateral plasma membranes from mouse intestinal epithelium[1]. These were different in enzyme composition. The former was rich in alkaline phosphatase and digestive enzymes, while the latter showed a high (Na^+,K^+)-ATPase activity. In this paper we compare the electrophoretic patterns of protein components of the two membranes. The original aim of the present work was to investigate which proteins are shared by different regions of a plasma membrane.

METHODS

Preparation of membranes

The microvillous or apical plasma membranes and the basolateral plasma membranes were isolated from mouse small intestine as previously described[1]. The

purity of a preparation was checked by assaying alkaline phosphatase and ouabain-sensitive ATPase activities[1].

Solubilization of membranes

An aliquot of 10–20 μl of membrane suspensions containing 10–40 μg of protein as determined with the Lowry method[10] was mixed with 50 μl of the solubilizing mixture and immediately applied onto the gels. The latter was made up of 1.25 ml of 20% sodium dodecyl sulfate, 0.125 ml of β-mercaptoethanol and 1.25 ml of 0.2 M phosphate buffer (pH 7.1), made to a total volume of 10 ml with water.

Polyacrylamide gel systems

Because most plasma membrane proteins have been found to be larger than 30000 in apparent molecular weight[2], gel systems adequate to distinguishing proteins with molecular weights larger than 30000 were sought. Five different systems were used in the present work; System I was that of Neville[3] and the original method was closely followed. System II corresponded to the "half the normal amount" cross-linker system of Weber and Osborn[4], which contained 10% polyacrylamide and 0.135% methylene bisacrylamide. In the Systems III, IV and V either the polyacrylamide concentration or that of methylene bisacrylamide was further reduced. The compositions of the five systems are outlined below.

System	*Polyacrylamide* (%)	*Methylene bisacrylamide* (%)	*Ratio*
I	12.0	0.10	120
II	10.0	0.135	74
III	10.0	0.090	111
IV	6.7	0.090	74
V	6.7	0.060	111

Gels were cast in glass tubes, 12 cm long, with an internal diameter of 5 mm. A microsyringe was used to overlayer a gel solution with water. Samples were dissolved as described above and immediately applied onto the gels and subjected to electrophoresis usually with 3 mA per tube at room temperature for 8 h.

Staining of protein and carbohydrate

The developed gels were stained for protein with either Amido Black or Coomassie Brilliant Blue. In order to stain with the former, it was necessary to remove sodium dodecyl sulfate from the gel blocks prior to staining by constant stirring in 50% methanol containing 7% acetic acid for 2 h or leaving them in the same medium overnight.

Carbohydrate was stained by periodate oxidation followed with the Schiff reagent[5]. The gel blocks were freed of sodium dodecyl sulfate prior to staining as described above. Occasionally the gels previously stained with Amido Black were further stained with the periodic acid–Schiff reagent to locate a carbohydrate band with relation to that of protein. When such double-stained gels were scanned with a

densitometer, a 600-nm filter was used for the Amido Black stain and a 530-nm or 570-nm filter for the periodic acid–Schiff reagent stain. In some cases Amido Black staining followed periodic acid–Schiff reagent staining.

Calibration curves for molecular weight determination

Lysozyme, horse cytochrome *c*, trypsin, lactate dehydrogenase, phosphorylase *a* and β-galactosidase were products of Boehringer Mannheim. Bovine serum albumin was obtained from Pentex and ovalbumin from Miles Seravac.

Mobilities of these proteins relative to that of lysozyme (Systems I, II and III) or cytochrome *c* (other systems) were summarized in Table I and plotted in curves in Fig. 1a (Systems I and II) and Fig. 1b (other systems). It can be seen from Table I that relative mobilities are quite reproducible.

TABLE I

RELATIVE MOBILITIES OF SOME REFERENCE PROTEINS IN SODIUM DODECYL SULFATE–POLYACRYLAMIDE GEL SYSTEMS DIFFERENT IN POLYACRYLAMIDE AND METHYLENE BISACRYLAMIDE CONCENTRATIONS

Details of the composition of the systems are described in the text. The mobility of lysozyme (Systems I–III) or cytochrome *c* (other systems) was taken as unit R_F.

	System				
	I	*II*	*III*	*IV*	*V*
Trimer of bovine serum albumin				0.13 ± 0.01 (4)	0.17 ± 0.013 (5)
Dimer of bovine serum albumin		0.12 ± 0.01 (5)		0.21 ± 0.005 (10)	0.27 ± 0.009 (15)
β-Galactosidase	0.13 (2)	0.11 ± 0.00 (5)	0.15 (3)	0.26 (3)	0.31 (3)
Phosphorylase *a*	0.18 (2)	0.20 ± 0.005 (9)	0.24 (3)	0.32 ± 0.005 (12)	0.38 ± 0.007 (13)
Monomer of bovine serum albumin	0.31 (3)	0.34 ± 0.005 (16)	0.35 (3)	0.46 ± 0.007 (15)	0.51 ± 0.006 (16)
L-Glutamate dehydrogenase	0.38 (2)	0.36 ± 0.01 (11)			
Ovalbumin		0.46 ± 0.01 (3)		0.59 ± 0.007 (12)	0.64 ± 0.006 (14)
Lactate dehydrogenase	0.58 (2)	0.58 ± 0.01 (12)			
Trypsin	0.68 (2)	0.73 ± 0.01 (5)			
Lysozyme	1.00	1.00	1.00	(1.00)	(1.00)
Cytochrome *c* (horse)		1.04 (2)		1.00	1.00

As shown in Fig. 1a, the Systems I and II showed almost identical curves except that a bend at the apparent molecular weight of 100000 was slightly more pronounced in System I (●) than in the other system (○). With Systems III–V the curves, in this order, approached a straight line in the range of molecular weight smaller than 130000 and larger than 40000.

The molecular weight of bovine serum albumin was taken as 65000 (ref. 6).

Determination of R_F values and apparent molecular weights

Lysozyme or cytochrome *c* was co-electrophoresed with each sample and

relative mobilities were calculated for stained bands. The apparent molecular weights were then read on the calibration curves described above. When 10 μg of cytochrome *c* were added, it was visible during the electrophoresis. It should be remembered that the present gel systems were chosen to determine molecular weights larger than 30000 at the expense of accuracy below this value.

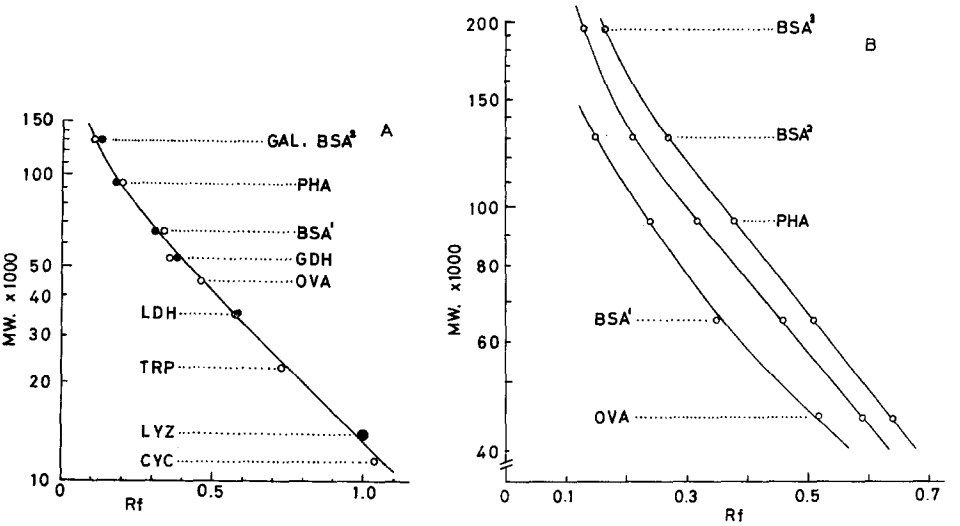

Fig. 1. Calibration curves for molecular weight determination with some reference proteins. (A) —●—, System I; —○—, System II. (B) From left to right, Systems III, IV and V. Average R_F values were plotted from Table I. Abbreviations: BSA^1, BSA^2 and BSA^3, monomer, dimer and trimer of bovine serum albumin, respectively; GAL, β-galactosidase; PHA, phosphorylase *a*; GDH, L-glutamate dehydrogenase; OVA, ovalbumin; LDH, lactate dehydrogenase; TRP, trypsin; LYZ, lysozyme; CYC, cytochrome *c* (horse).

RESULTS

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of apical and basolateral plasma membranes

The apical membrane and the basolateral membrane of small intestinal epithelial cells were solubilized and electrophoresed in sodium dodecyl sulfate–polyacrylamide gel systems as described in Methods.

A preliminary electrophoresis was performed in System V (see Methods). The two membranes revealed remarkable differences upon staining for protein (Fig. 2). Most of the major bands of the apical membrane were located relatively close to the origin and some of them that were closest to the origin will henceforth be referred to as A1 complex. The latter was followed by the most prominent band, A2, and three to four definite but less conspicuous ones.

Much in contrast with the apical membrane, the basolateral membrane had very few, if present, or no components comparable to those of the A1 complex and A2 (Fig. 2). In fact, even the most prominent band, named β, which was closer to the origin than any other band of this membrane, moved ahead of the A2 band.

Because the mobilities of the Bands B1 and B2 of the apical membrane were

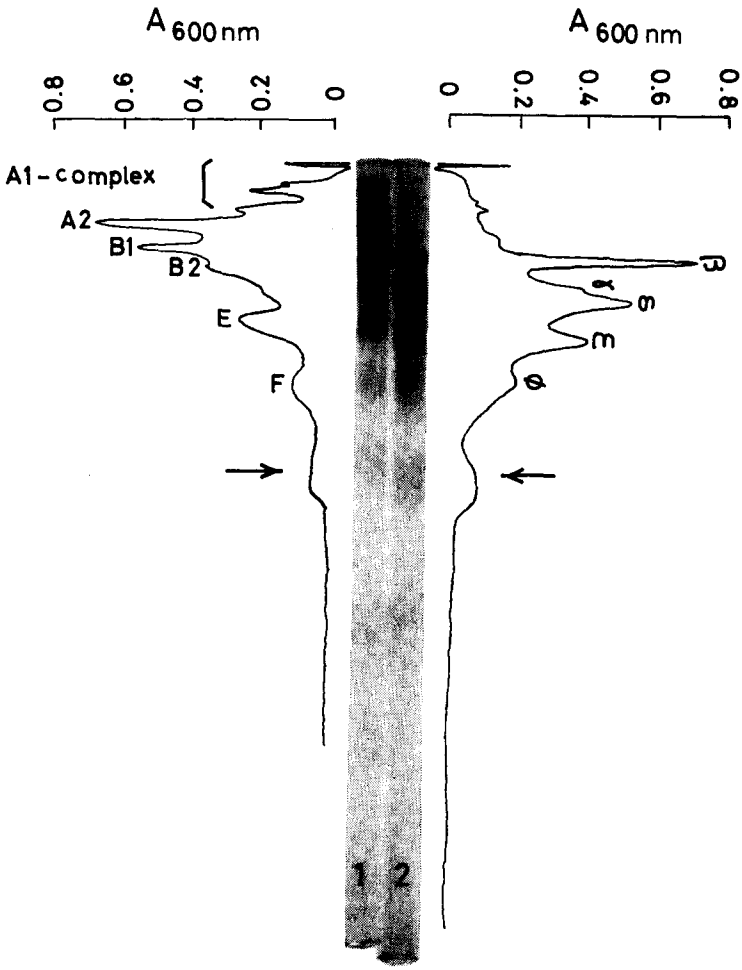

Fig. 2. Electrophoretic profiles of an apical membrane and a basolateral membrane fraction in gel System V. 33 μg protein of the former and 25 μg of the latter were applied. Electrophoresis was carried out with 1 mA per tube at room temperature for 16 h. After removal of sodium dodecyl slufate the gels were stained with Amido Black. The treatment of the gel blocks with a limited amount of the periodic acid–Schiff reagent before Amido Black destaining produced a slight periodic acid–Schiff reagent stain at the positions indicated by arrows. These were not stained with Amido Black.

close to that of the Band β, a further examination was carried out in order to determine their exact relative mobilities, in which cytochrome *c* was used as an internal reference protein as described in Methods.

Electrophoresis of the two membranes, separate and combined, made it clear that the band β of the basolateral membrane had a mobility about midway between those of the Bands B1 and B2 of the apical membrane (Figs 3 and 4).

Determination of apparent molecular weights

Apparent molecular weights of the protein components present in the stained

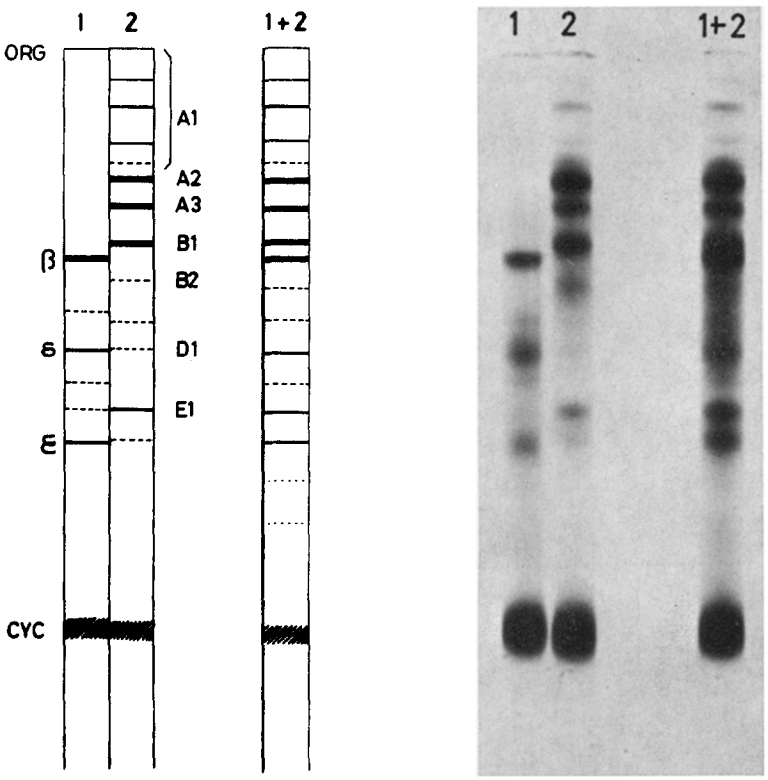

Fig. 3. Electrophoretic profiles of an apical and a basolateral membrane fraction with cytochrome *c* as internal reference in the System V. 1, Basolateral membrane; 2, apical membrane; 1+2, the two membranes mixed before electrophoresis. 37 μg protein of the basolateral and 66 μg protein of the apical membrane were applied. Electrophoresis was performed with 3 mA per tube at room temperature for 8 h. For more details see Methods. The diagram is presented to show the exact positions of the bands and their designations.

bands were read on the calibration curves given in Fig. 1, depending on the system used for the electrophoresis. The results are summarized in Table IIa and IIb.

The average apparent molecular weights estimated as above did not show a marked variance in the five different systems. The "overall average" was then calculated on the basis of those of different systems, weighing the number of experiments with each system.

The sharp and most prominent band, β, of the basolateral membrane showed an average apparent molecular weight of 101000 (allowing $\pm$2000 variance). The band had no counterpart in the other membrane. The components of the other membrane which had the closest molecular weights to that of the β were B1 and B2. Their average apparent molecular weights were 108000 and 93000, respectively.

The dominant band of the apical membrane, A2, had an apparent molecular weight of 146000. The A1 complex, which consisted of four to five minor bands, had molecular weights larger than 160000.

The apparent molecular weights of the components of the basolateral membrane

occurring in the bands δ and ε were 61000 and 40000, respectively, while that of the band E1 of the apical membrane was 44000.

None of the bands described above were shared by the two membranes. Although the bands with relative mobilities corresponding to those of β and δ occasionally occurred in the apical membrane, they were always minor bands, possibly revealing a contamination with the basolateral membrane. When the apical membrane fraction was heavily contaminated with the basolateral membrane, which could result from an incomplete purification of brush borders at a stage preceding the density-gradient centrifugation, the fraction showed considerable ouabain-sensitive

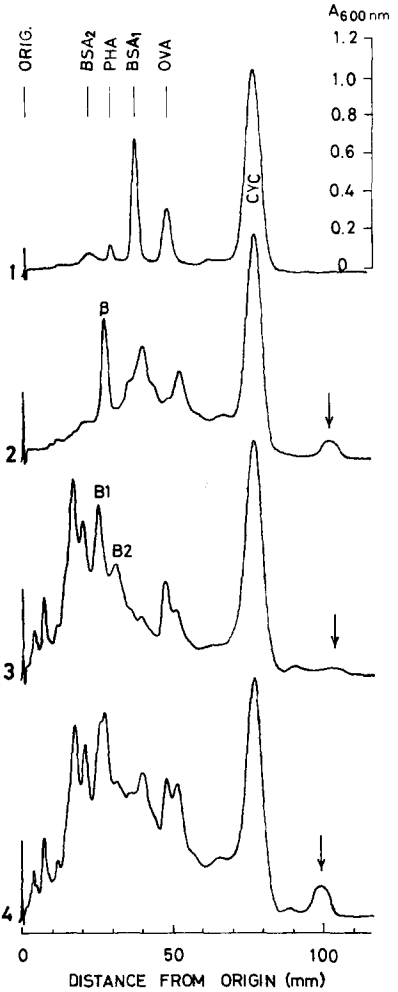

Fig. 4. Densitometric tracings of the apical and basolateral membranes electrophoresed in gel System V. Trace 1, the reference proteins; Trace 2, basolateral membrane; Trace 3, apical membrane; Trace 4, mixture of apical and basolateral membranes. It can be seen that Band β appears between those of B1 and B2 on trace No. 4. The arrows show the periodic acid–Schiff reagent-stainable components. The absorption in densitometry of those components is due to light scattering and not due to Amido Black staining. ORIG, origin. For other abbreviations see legend of Fig. 1.

TABLE II

RELATIVE MOBILITIES AND CORRESPONDING APPARENT MOLECULAR WEIGHTS OF THE PROTEIN COMPONENTS PRESENT IN THE STAINED BANDS IN FIVE DIFFERENT POLYACRYLAMIDE GEL SYSTEMS

(a) Basolateral plasma membrane. (b) Apical plasma membrane. The R_F values were calculated on the basis of the mobility of lysozyme (Systems I–III) or cytochrome *c* (other systems), which were added as internal reference as described in Methods. They are presented as average relative mobilities with S.E. and number of experiments. The figures in the brackets beneath the R_F values represent apparent molecular weights estimated with the calibration curves in Fig. 1. The figures in the column at the farthest right represent overall average molecular weights with the number of experiments weighed for each system.

	System					
	I	*II*	*III*	*IV*	*V*	*Av. mol. w.*
(a) Basolateral plasma membrane						
β	0.15 (3) (105000)	0.17 ± 0.004 (16) (101000 ± 2000)	0.22 ± 0.01 (4) (100000 ± 5000)	0.30 ± 0.005 (15) (100000 ± 2000)	0.35 ± 0.005 (17) (102000 ± 1000)	101000
δ	0.32 (3) (63000)	0.36 ± 0.01 (13) (58000 ± 1500)	0.39 ± 0.01 (4) (60000 ± 1500)	0.48 ± 0.005 (15) (62000 ± 600)	0.52 ± 0.006 (15) (63000 ± 1000)	61000
ε	0.51 (3) (40000)	0.54 ± 0.005 (14) (37000 ± 400)	0.56 (3) (42000)	0.64 ± 0.004 (14) (40000 ± 500)	0.67 ± 0.01 (15) (41000 ± 1000)	40000
(b) Apical plasma membrane						
A1	0.02 (3) 0.03 (3)	0.01 ± 0.002 (14) 0.04 ± 0.003 (12)	0.01 (2) 0.02 (2)	0.03 ± 0.006 (4) 0.09 ± 0.007 (8) 0.11 ± 0.006 (5)	0.04 ± 0.004 (13) 0.10 ± 0.003 (15) 0.13 ± 0.004 (6) 0.16 ± 0.003 (8) (> 200000) 0.20 ± 0.002 (14) (168000 ± 1600)	
A2	0.09 (3) (> 150000)	0.074 ± 0.001 (20) (≈ 160000)	0.10 (3) (≈ 180000)	0.19 ± 0.005 (13) (142000 ± 4000)	0.23 ± 0.003 (19) (148000 ± 1000)	146000
A3		0.105 ± 0.002 (13) (130000 ± 2500)		0.22 ± 0.007 (11) (126000 ± 4000)	0.27 ± 0.003 (19) (130000 ± 1000)	128000
B1	0.15 (3) (105000)	0.15 ± 0.004 (6) (110000 ± 2500)	0.19 (3) (110000)	0.28 ± 0.006 (11) (106000 ± 2000)	0.33 ± 0.005 (14) (108000 ± 1000)	108000
B2		0.20 ± 0.003 (8) (91000 ± 1500)		0.33 ± 0.01 (5) (92000 ± 3000)	0.38 ± 0.005 (12) (94000 ± 1000)	93000
E1		0.48 ± 0.004 (15) (43000 ± 400)	0.52 (3) (45000)	0.59 ± 0.007 (12) (45000 ± 5000)	0.63 ± 0.006 (12) (45000 ± 600)	44000

ATPase activity which was otherwise absent, and upon electrophoresis new major bands appeared at the positions corresponding to β and δ, similar to the pattern obtained when the two membranes were mixed after separation and before electrophoresis (Figs 3 and 4). Systems I, II and III were inadequate to resolve the proteins larger than the components of the A2 (Table II, a).

Periodic acid–Schiff reagent staining

The developed gels were stained for carbohydrate as described in Methods.

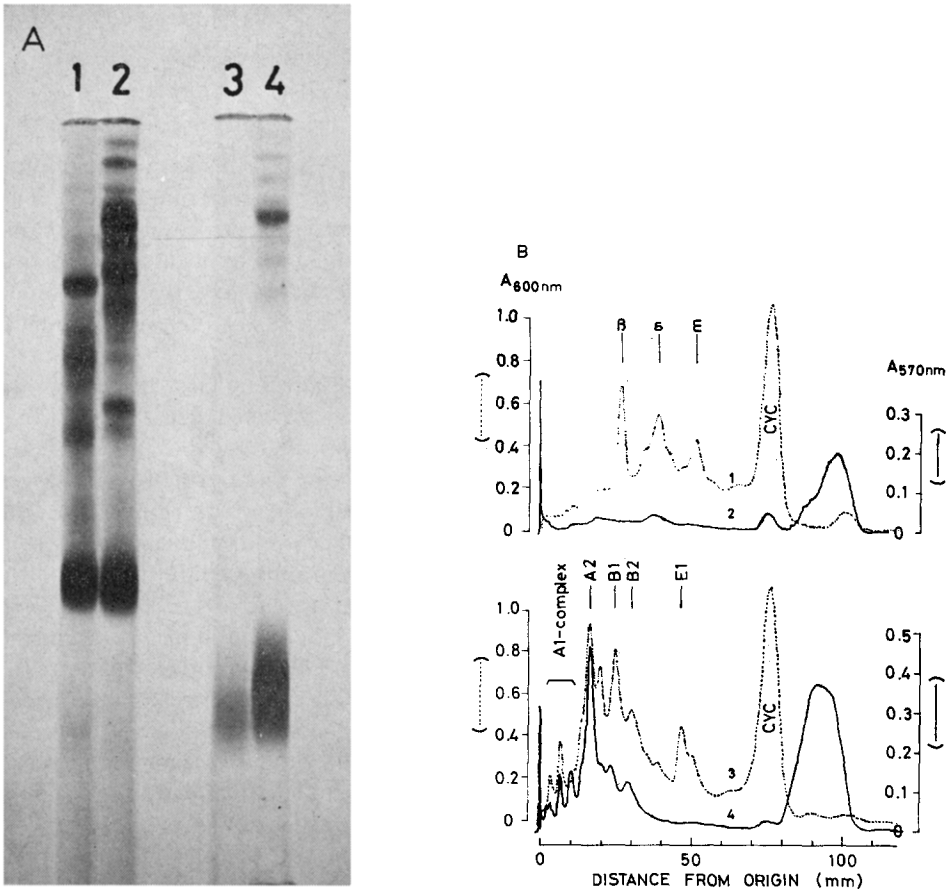

Fig. 5. Periodic acid–Schiff reagent staining of the developed gels in comparison with Amido Black staining. (A) 1 and 2, basolateral and apical membranes, respectively, both stained with Amido Black; 3 and 4, doubles of gel blocks 1 and 2, both stained with periodic acid–Schiff reagent. The apical membrane gel was most intensely stained with periodic acid-Schiff reagent at band A2 and less intensely at the bands corresponding to A1 complex, A3, B1 and B2. The intensely stained bands far ahead of cytochrome *c* were not stained with Amido Black. (B) Densitometric tracings of the developed gel blocks stained either for protein or for carbohydrate. The dotted lines show the protein stain (Amido Black) and the continuous ones the carbohydrate stain (periodic acid–Schiff reagent). The absorption at the position of cytochrome *c* was due to light scattering.

The apical and basolateral membranes showed very contrasting stains. The former was stained intensely, the latter hardly. Of the apical membrane all the components of the A1 complex and the Bands A2, A3, B1 and B2 took the stain (Fig. 5). Band A2 was most prominent, not only in protein stain but also in carbohydrate stain.

The identity of a band stained for carbohydrate and that for protein was confirmed by densitometry of the separately stained gel blocks (Fig. 5b) and of those double-stained for carbohydrate and protein as described in Methods.

In striking contrast with the apical membrane the basolateral membrane was

almost free of periodic acid–Schiff reagent stain except a faintly stained band corresponding to δ (Fig. 5b).

DISCUSSION

It was shown that the apical membrane and the basolateral plasma membrane of the intestinal epithelial cell were markedly different in enzymic composition[1]. The former was rich in alkaline phosphatase and digestive enzymes, the latter in ouabain-sensitive ATPase. This fact suggested different protein components in the two membranes. However, from the point of view that these are two regions of a single cell surface, it seemed to be of interest to know whether or not there is a common denominator component for the two regions.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis revealed very contrasting profiles for the two fractions as had been expected from the enzymic findings. There were no major proteins shared by both fractions.

The findings that in the apical membrane there were more proteins with larger apparent molecular weights than in the basolateral membrane may be related to the earlier finding that the former was thicker than the latter[1,7]. Plasma membranes from various cells including erythrocytes and hepatocytes were shown to have a number of protein components with apparent molecular weights larger than 110000 (ref. 2). In comparison with these findings it is remarkable that the basolateral membrane of the intestinal epithelial cells has such a strikingly simple size-distribution pattern for its protein components. This might be interpreted as an extensive regional differentiation or specialization of a cell membrane according to its physiological functions.

Although the most prominent protein band, A2, of the apical membrane, also stainable for carbohydrate, was assigned the molecular weight of 146000, this does not exclude the possibility that the molecular weight of the protein moiety might be much smaller. In order to check this possibility, however, further investigation is required.

It also remains to be determined whether or not the protein component(s) in Band β of the basolateral membrane represents, wholly or partly, the (Na^+, K^+)-ATPase molecule. It is of interest to note in this connexion that a recently reported and highly active (Na^+, K^+)-ATPase preparation[8] showed a major band with an apparent molecular weight of roughly 100000. The present electrophoretic pattern for the basolateral membrane (Fig. 2, Gel 2) bears a close resemblance to what has been claimed to be a purified (Na^+, K^+)-ATPase[9], although the assigned molecular weights were slightly different from those in the present paper.

Finally, it should be remembered that the molecular weights allotted to the stained bands in the present work were obtained on the basis of the curves presented in Fig. 1 and their reliability therefore depends on the adequacy of these reference proteins.

REFERENCES

1 Fujita, M., Ohta, H., Kawai, K., Matsui, H. and Nakao, M. (1972) *Biochim. Biophys. Acta* 274, 336–347

2 Neville, Jr, D. M. and Glossmann, H. (1972) *J. Biol. Chem.* 246, 6335–6338

3 Neville, D. M. (1972) *J. Biol. Chem.* 246, 6328–6334
4 Weber, K. and Osborn, M. (1969) *J. Biol. Chem.* 244, 4406–4412
5 Zacharius, R. M., Zell, T. E., Morrison, J. H. and Woodlock, J. J. (1969) *Anal. Biochem.* 30, 148–152
6 Peters, Jr, T. (1970) *Adv. Clin. Chem.* 13, 37–111
7 Sjöstrand, F. S. (1963) *J. Ultrastruct. Res.* 8, 517–541
8 Nakao, T., Nakao, M., Nagai, F., Kawai, K., Fujihira, Y., Hara, Y. and Fujita, M. (1972) *J. Biochem. Tokyo* 72, 1061–1063
9 Uesugi, S., Dulak, N. C., Dixon, J. F., Hexum, T. D., Dahl, J. L., Perdue, J. F. and Hokin, L. E. (1971) *J. Biol. Chem.* 246, 531–543
10 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* 193, 265–275