Biochimica et Biophysica Acta, 433 (1976) 509-519

BBA 77289

THE SURFACE MEMBRANE OF THE SMALL INTESTINAL EPITHELIAL CELL

I. LOCALIZATION OF ADENYL CYCLASE

HEINI MURER, ELVIRA AMMANN, JÖRG BIBER and ULRICH HOPFER*

Max-Planck Institut für Biophysik, Frankfurt/M. (G.F.R.), Laboratorium für Biochemie, Federal Institute of Technology, Zurich (Switzerland) and Department of Anatomy, School of Medicine, and Developmental Biology Center, Case Western Reserve University, Cleveland, Ohio 44106 (U.S.A.)

(Received September 15th, 1975)
(Revised manuscript received January 12th, 1976)

SUMMARY

The subcellular distribution of adenyl cyclase was investigated in small intestinal epithelial cells. Enterocytes were isolated, disrupted and the resulting membranes fractionated by differential and sucrose gradient centrifugation. Separation of luminal (brush border) and contra-luminal (basolateral) plasma membrane was achieved on a discontinuous sucrose gradient.

The activity of adenyl cyclase was followed during fractionation in relation to other enzymes, notably those considered as markers for luminal and contraluminal plasma membrane. The luminal membrane was identified by the membrane-bound enzymes sucrase and alkaline phosphatase and the basolateral region by $(Na^+ + K^+)$-ATPase. Enrichment of the former two enzymes in purified luminal plasma membrane was 8-fold over cells and that of $(Na^+ + K^+)$-ATPase in purified basolateral plasma membranes was 13-fold. F^--activated adenyl cyclase co-purified with $(Na^+ + K^+)$-ATPase, suggesting a common localization on the plasma membrane. The distribution of K^+-stimulated phosphatase and 5′-nucleotidase also followed $(Na^+ + K^+)$-ATPase during fractionation.

INTRODUCTION

Adenyl cyclase has been localized in the plasma membrane of many different cell types [1–3]. This site is consistent with the "second messenger" concept for cyclic AMP, as only a plasma membrane-bound enzyme can directly interact with extracellular hormones [4, 5]. For epithelial cells the localization of adenyl cyclase poses a

* To whom reprint requests should be addressed at the Department of Anatomy, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106 (U.S.A.).

Abbreviation: HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid.

specific problem, since two distinct plasma membranes are present. The enzyme could be located in the luminal, the contraluminal or in both regions of the plasma membrane. In the small intestine the two parts of the surface membrane can be distinguished by morphologic, enzymatic and functional criteria [6–10].

Interestingly, the cyclic AMP levels of small intestinal epithelial cells are influenced by the addition of agents to the luminal as well as the contraluminal (serosal) side. Cholera toxin constitutes a prominent example of a substance that is effective when placed in the intestinal lumen [11–13]. Peptide hormones, on the other hand, reach the epithelial cell normally at the contraluminal side. Both agents are macromolecules [14] and, therefore, would not be expected easily to traverse the barrier separating luminal and serosal compartments. Hence, localization of adenyl cyclase is an important problem in the context of the stimulating effect exerted by cholera toxin and peptide hormones.

In a previous study on this problem, adenyl cyclase was found in a membrane fraction presumably derived from the contraluminal side of the epithelial cell [15]. However, objections to the methods employed in that particular study and therewith to the conclusions regarding the localization of the enzyme can be raised. Thus, because of the use of scrapings it was not possible to rule out that adenyl cyclase activity was derived from cells other than mature enterocytes. Furthermore, different procedures had been employed in isolation of luminal and of contraluminal plasma membrane, which makes it difficult to evaluate whether the enzyme had been preferentially inactivated by one of them. Additionally, other research groups have reported divergent results with respect to absence or presence of alkaline phosphatase in contraluminal plasma membranes [7, 8, 10] and K^+-stimulated phosphatase in brush border membranes [6, 8, 16], the latter enzyme thought to be a partial activity of $(Na^+ + K^+)$-ATPase [17].

The present studies were undertaken to resolve the uncertainties in the localization of plasma membrane enzymes, especially of adenyl cyclase.

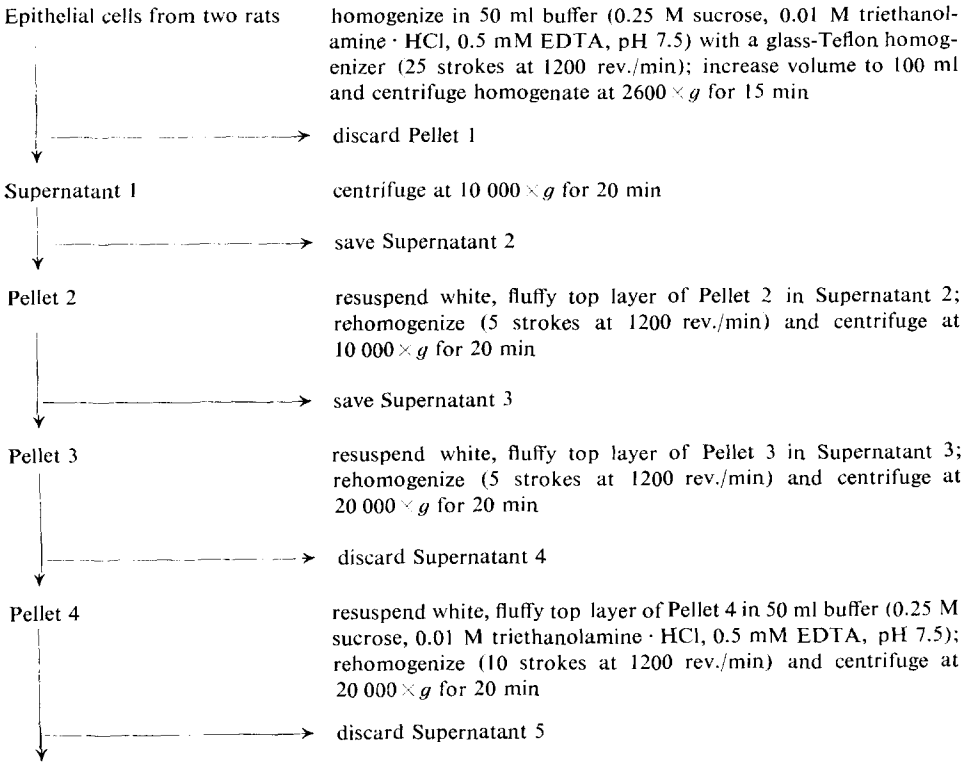
METHODS AND MATERIALS

Isolation and fractionation of small intestinal epithelial cells

Cells were isolated from male Sprague-Dawley rats (200–250 g body weight) as described earlier [10], with the modification that 0.5 mM EDTA was added to the sucrose/triethanolamine buffer. The isolated cells were disrupted in a glass-teflon homogenizer (clearance 0.095–0.115 mm, Braun GmbH, Melsungen, Germany). Breakage of cells was monitored by phase microscopy and homogenization was continued until at least 70 % of the nuclei had been released. Fractionation of the homogenate was carried out according to Scheme 1. The membrane fraction, settling between 200 000 and 400 000 $g \cdot$ min (all centrifugal forces refer to r_{av}), was labeled "crude" plasma membrane. Further purification was achieved with flotation of the membrane through a sucrose gradient. For this purpose the "crude" plasma membrane from two animals was homogenized in 4.5 ml of 50 % sucrose (w/v)/10 mM triethanolamine · HCl/0.5 mM EDTA (pH 7.5). The homogenization was carried out with 20 strokes at 1000 rev./min in a glass-teflon homogenizer. The membrane suspension was placed at the bottom of a gradient made up with 3.7 ml of 40 %, 2.7 ml of 30 % and 1.7 ml of 20 % sucrose (w/v). The sucrose solutions were buffered

SCHEME I

PREPARATION OF A "CRUDE" PLASMA MEMBRANE FRACTION FROM SMALL INTESTINAL EPITHELIAL CELLS



with 5 mM triethanolamine · HCl and 0.5 mM EDTA, adjusted to pH 7.5 with NaOH. The sucrose gradient was spun for 90 min at 150 000 × *g* in the swing-out bucket IEC SB 283. Most of the membranes collected at the interfaces as whitish bands. The membranes at the interfaces, the solutions between bands, and an occasional pellet were removed separately with tuberculin syringes, diluted with Tris/HEPES/mannitol buffer (1 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid adjusted with Tris hydroxide to pH 7.5 plus 0.1 M D-mannitol), homogenized, and pelleted at 200 000 × *g* for 30 min. Dilution with Tris/HEPES/mannitol buffer, homogenization, and collection of the membranes were repeated once more. The final membrane fractions were suspended in Tris/HEPES/mannitol buffer.

Enzyme activities and protein concentration

All enzyme activities were measured on the day of membrane preparation. Assays were carried out at 37 °C. Sucrase activity (EC 3.2.1.48) was determined according to Hopfer et al. [18].

The acid phosphatase (EC 3.1.3.2) assay was carried out in 50 mM sodium citrate buffer (pH 5.0) with 5.5 mM *p*-nitrophenyl phosphate as substrate. The

reaction was stopped by dilution with 1 volume of 1 M NaOH and the extent of hydrolysis determined spectrophotometrically.

K^+-stimulated *p*-nitrophenyl phosphatase (EC 3.1.3.-) was measured by a modification of the method of Garraham et al. [19]. 0.02 ml sample was incubated with 0.5 ml buffer containing 50 mM Tris · HCl (pH 7.8), 10 mM $MgSO_4$, 5 mM EDTA, 6 mM of the di-Tris salt of *p*-nitrophenyl phosphate and either 90 mM KCl or 90 mM NaCl. The specific K^+-stimulation of *p*-nitrophenyl phosphate hydrolysis was estimated from the difference by duplicates of the sample incubated either with KCl or with NaCl. Preliminary experiments had established that K^+-specific hydrolysis was maximal at 90–150 mM KCl.

(Na^+ + K^+)-ATPase (EC 3.6.1.3) was assayed as reported by Quigley and Gotterer [9]. Duplicate samples were incubated in presence and absence of 1 mM ouabain which inhibits rat small intestinal (Na^+ + K^+)-ATPase to about 90% [20]. The release of inorganic phosphate from ATP was measured with Merckotest® No. 3331.

For glucose-6-phosphatase (EC 3.1.3.9) a modification of the method of Hübscher et al. [21] was applied. 0.025 ml sample was incubated with 0.5 ml buffer of 60 mM maleate, adjusted to pH 6.7 with NaOH, 2 mM KF and 4 mM EDTA. Duplicates of samples were incubated in presence and absence of 40 mM D-glucose and the difference in inorganic phosphate release taken as specific glucose 6-phosphatase. The reaction was stopped by addition of 0.5 ml 20% trichloroacetic acid.

For cytochrome *c* oxidase (EC 1.9.3.1) the method of Erecinska et al. [22] was employed.

5'-Nucleotidase (EC 3.1.3.5) was measured with a modification of the method of Kinne et al. [23]. 0.1 ml sample was incubated with 0.5 ml medium containing 50 mM Tris · HCl (pH 7.6), 10 mM $MgCl_2$ and either 5 mM 5' AMP or 5 mM 3' AMP. The reaction was stopped by the addition of 0.5 ml 20% trichloroacetic acid and inorganic phosphate determined with Merckotest® No. 3331. The release of inorganic phosphates from 5' AMP above that from 3' AMP was taken as specific 5'-nucleotidase.

Adenyl cyclase (EC 4.6.1.1) was estimated in a medium containing 30 mM Tris · HCl (pH 7.4), 4 mM $MgCl_2$, 0.1 mM EDTA, 3 mM ATP, 10 mM creatine phosphate with 0.1 ml creatine kinase, and 10 mM theophylline. Basal and fluoride (10 mM NaF)-activated activity was measured. The reaction was terminated by heat denaturation (3 min in boiling water). After centrifugation cyclic AMP was measured in the supernatant with an Amersham test combination (TRK, 432).

Alkaline phosphatase (EC 3.1.3.1) was measured with *p*-nitrophenyl phosphate as substrate. 0.1 ml sample was incubated with 1 ml buffer, containing 50 mM glycine, adjusted with NaOH to pH 10.5, 2 mM $MgSO_4$, 2.5 mM $ZnSO_4$ and 5.5 mM *p*-nitrophenyl phosphate. The reaction was stopped by addition of 1 ml 1 M NaOH and the extent of hydrolysis determined spectrophotometrically.

Protein determination was carried out according to Lowry et al. [24] with crystalline bovine serum albumin as standard.

Materials

p-Nitrophenyl phosphate, glucose 6-phosphate, ATP, ouabain and theophylline were purchased from Sigma Chemical Co. (St. Louis, Mo.). All other chemicals were obtained from E. Merck (Darmstadt, Germany).

RESULTS

The isolation and separation of brush border and basolateral plasma membrane from enterocytes was carried out in two stages. After disruption of the cells, the resulting organelles and membrane fragments were fractionated by differential centrifugation. This procedure yielded a "crude" plasma membrane fraction. The osmotic pressure of the buffer during the first stage was high enough to preserve the integrity of most intracellular organelles so that they could be removed from the homogenate by a moderate centrifugal force. The "crude" plasma membrane fraction was not contaminated by nuclei and intact brush borders as assessed by phase microscopy. The distribution of mitochondria and lysosomes in the various fractions was measured using cytochrome *c* oxidase and acid phosphatase, respectively, as markers. Table I gives yield and specific activities of membrane-bound and organelle-associated

TABLE I

DISTRIBUTION OF ENZYMES DURING FRACTIONATION OF ENTEROCYTES ACCORDING TO SCHEME I

Values are the averages of three experiments with a S.D. less than 7 % for recovery. Specific activities (mean ± S.D.) are given in parentheses. All enzyme activities are given in μmol of substrate turned over per min per mg protein, except for adenyl cyclase which is expressed in pmol of cyclic AMP formed per min per mg protein.

	Alkaline phosphatase	Sucrase	$(Na^+ + K^+)$-ATPase	K^+-stimulated *p*-nitrophenyl phosphatase
Homogenate	100 % (0.206 ± 0.045)	100 % (0.128 ± 0.038)	100 % (0.058 ± 0.015)	100 % (0.019 ± 0.006)
Combined pellets*	62 % (0.234 ± 0.050)	66 % (0.165 ± 0.024)	34 % (0.038 ± 0.016)	36 % (0.014 ± 0.004)
"Crude" plasma membrane	25 % (0.504 ± 0.016)	24 % (0.304 ± 0.016)	58 % (0.324 ± 0.016)	55 % (0.108 ± 0.003)
Recovery	87 %	90 %	92 %	91 %

	5'-Nucleotidase	F^--activated adenyl cyclase	Acid phosphatase	Cytochrome *c* oxidase	Glucose-6-phosphatase
Homogenate	100 % (0.105 ± 0.008)	100 % (38 ± 12)	100 % (0.035 ± 0.006)	100 % (0.134 ± 0.015)	100 % (0.082 ± 0.023)
Combined pellets*	38 % (0.073 ± 0.021)	32 % (22 ± 4)	48 % (0.032 ± 0.016)	78 % (0.210 ± 0.016)	63 % (0.105 ± 0.018)
"Crude" plasma membrane	49 % (0.465 ± 0.035)	48 % (178 ± 16)	3 % (0.012 ± 0.004)	5 % (0.062 ± 0.014)	8 % (0.078 ± 0.016)
Recovery	87 %	80 %	51 %	83 %	71 %

* The combined pellet fraction includes all material from pellets 1, 2 and 3, except for that in the "crude" plasma membrane, as well as the pellet obtained from Supernatant 4 upon centrifugation at 200 000 · *g* for 30 min.

TABLE II

DISTRIBUTION OF ENZYMES DURING FRACTIONATION OF "CRUDE" PLASMA MEMBRANE ON THE SUCROSE GRADIENT

Values are the averages of three experiments with a S.D. less than 4 % for recovery. Specific activities (mean ± S.D.) are given in parentheses. All enzyme activities are expressed in μmol of substrate turned over per min per mg protein, except for adenyl cyclase which is given in pmol of cyclic AMP formed per min per mg protein.

	Alkaline phosphatase	Sucrase	$(Na^+ + K^+)$-ATPase	K^+-stimulated *p*-nitrophenyl phosphatase	5′-Nucleotidase	F^--activated adenyl cyclase
"Crude" plasma membrane	100 % (0.504 ± 0.016)	100 % (0.304 ± 0.016)	100 % (0.324 ± 0.016)	100 % (0.108 ± 0.003)	100 % (0.465 ± 0.035)	100 % (178 ± 16)
Upper band (20–30 % interface)	2 % (0.106 ± 0.015)	3 % (0.075 ± 0.011)	26 % (0.752 ± 0.018)	28 % (0.234 ± 0.030)	30 % (1.154 ± 0.036)	34 % (496 ± 25)
Middle band (30–40 % interface)	18 % (0.296 ± 0.018)	20 % (0.206 ± 0.036)	58 % (0.568 ± 0.064)	54 % (0.193 ± 0.046)	51 % (0.761 ± 0.048)	46 % (255 ± 18)
Lower band (40–50 % interface)	64 % (1.645 ± 0.085)	65 % (1.002 ± 0.012)	< 1 % (< 0.005)	< 1 % (< 0.005)	< 1 % (< 0.005)	< 1 % (< 10)
Pellet*	21 % (0.405 ± 0.042)	23 % (0.277 ± 0.018)	6 % (0.074 ± 0.008)	5 % (0.022 ± 0.014)	4 % (0.071 ± 0.011)	6 % (40 ± 16)
Recovery	105 %	111 %	90 %	87 %	85 %	86 %

* The pellet fraction contains all material from the sucrose gradient not at the interfaces. The solutions outside the three bands from the sucrose gradient, as well as any pellet, were collected, diluted with water and sedimented at 200 000 × *g* for 30 min.

enzymes for the "crude" plasma membrane fraction and the combined pellets of all other membrane fractions. Several putative plasma membrane enzymes were enriched in the membranes settling slightly slower than the mitochondria: alkaline phosphatase, sucrase, $(Na^+ + K^+)$-ATPase, K^+-stimulated *p*-nitrophenyl phosphatase, 5′-nucleotidase, F^--activated adenyl cyclase. The specific activities of mitochondrial and lysosomal enzymes were decreased, compared to the homogenate, while that of glucose-6-phosphatase, a marker of the endoplasmic reticulum, was unchanged. Only about 50 % of the lysosomal enzyme acid phosphatase was recovered in the particulate fractions. Another 20–25 % of the activity was solubilized and could be detected in Supernatant 4. This finding suggests that, in spite of all precautions, some lysosomal damage occurred during homogenization.

The plasma membrane enzymes tested in Table I fall into two classes according to the relative amount collected in the "crude" plasma membrane fraction. About a quarter of the total sucrase and alkaline phosphatase, present in the homogenate, was found in the "crude" plasma membrane. In contrast, the yield of $(Na^+ + K^+)$-ATPase, K^+-stimulated *p*-nitrophenyl phosphatase, 5′-nucleotidase and adenyl cyclase in this fraction was considerably more and ranged from 48 % to 58 %. This difference was not due to inactivation as the total recovery of all these six enzymes was at least 80 % of the activity of the homogenate. Further purification of the "crude" plasma membrane was achieved by flotation of the membranes through a discontinuous sucrose density gradient. The membranes accumulated at the interfaces as whitish bands which could easily be removed from the tubes. The centrifugation effectively separated the two classes of plasma membrane enzymes noted above. The yields and specific activities of enzymes are given in Table II. Membranes collected between 20 % and 30 % sucrose (upper band) were enriched in $(Na^+ + K^+)$-ATPase, K^+-stimulated phosphatase, 5′-nucleotidase and adenyl cyclase, although most of the activities were recovered at the 30-40 % interface (middle band). Very little sucrase or alkaline phosphatase activity was detectable in the upper band and only 18–20 % in the middle band. Most of the latter two activities were recovered between 40 % and 50 % sucrose (lower band). Interestingly, this band showed little contamination with those enzymes enriched in the upper band of the gradient. The sucrose gradient step also reduced the levels of contaminating intracellular membranes as measured by glucose-6-phosphatase and cytochrome *c* oxidase. The upper band was devoid of both these enzymes (limits of detection: 0.001 μmol/min per mg protein) and also the lower band was highly depleted in comparison to homogenate. The specific activities of glucose-6-phosphatase and cytochrome *c* oxidase in the lower band were about 0.055 and 0.010 μmol/min per mg protein, respectively. On the basis of recovered enzymes the lower band must be derived mainly from the brush-border region and the upper band from the basolateral region of the enterocyte. The overall purification of plasma membrane enzymes in upper and lower band is summarized in Table III. Sucrase and alkaline phosphatase were enriched about 8-fold in the purified brush border membrane (lower band), and $(Na^+ + K^+)$-ATPase 5′-nucleotidase, and F^--activated adenyl cyclase 11- to 13-fold in the purified basolateral plasma membrane (upper band).

Although only the activity of the F^--activated adenyl cyclase is given in Tables I and II, the basal activity of this enzyme was also assayed throughout the purification procedure. These results were more variable than those of the fluoride-

TABLE III

ENRICHMENT OF PLASMA MEMBRANE ENZYMES IN UPPER AND LOWER BAND FROM THE SUCROSE GRADIENT

	Relative enzyme activities					
	Alkaline phosphatase	Sucrase	$(Na^+ + K^+)$-ATPase	K^+-stimulated phosphatase	5′-Nucleotidase	F^--activated adenyl cyclase
Homogenate	1	1	1	1	1	1
Upper band	0.5	0.6	13	12	11	13
Lower band	8	8	<0.1	<0.3	<0.1	<0.3

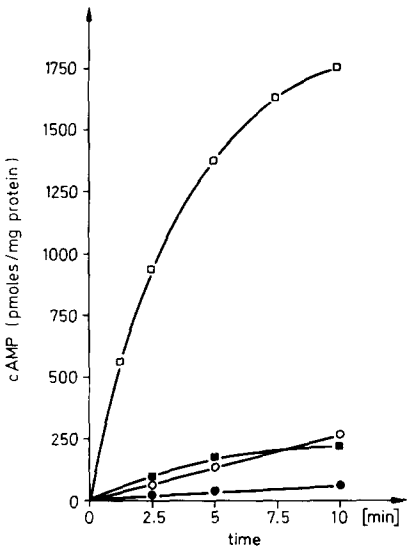


Fig. 1. Fluoride activation of adenyl cyclase in homogenate (solid symbols) and purified basolateral plasma membrane (open symbols) from small intestinal epithelial cells. Basal activity: ●, ○; activity with 10 mM NaF: ■, □.

activated enzyme. Fig. 1 shows that fluoride stimulation was preserved during the isolation procedure of the plasma membranes.

DISCUSSION

Although the work of Quigley and Gotterer [9] and Stirling [25] had suggested that $(Na^+ + K^+)$-ATPase was not located in the brush border region of the intestinal epithelial cell, convincing evidence was only obtained in the studies of Fujita et al. [6, 7] and recently in those of Lewis et al. [26]. The success of Fujita et al. in separating brush border from basolateral plasma membrane is probably related to the use of a buffer with a tonicity high enough to preserve the integrity of intracellular organelles. This procedure appears to minimize artifactual cross-contamination of membrane fractions.

The published fractionation schemes for intestinal cells were found to be inadequate for the purpose of this investigation, namely the localization of adenyl cyclase, because they are based either on intestinal scrapings or are too lengthy. Scrapings cannot be used as starting material in the case of a ubiquitous enzyme such as adenyl cyclase. It would be difficult to relate the findings with isolated membrane fractions to a specific cell type. In addition, adenyl cyclase is quite labile and is inactivated during a lengthy isolation procedure [15]. Both these problems were avoided in this study by starting with isolated epithelial cells and the use of a rapid discontinuous sucrose gradient centrifugation.

The use of a single marker enzyme for plasma membranes is not always reliable [27]. Therefore, the activities of K^+-stimulated *p*-nitrophenyl phosphatase and 5'-nucleotidase were measured in addition to those of $(Na^+ + K^+)$-ATPase and

the brush border enzymes. Because of variations in yield between preparations, all enzymes were assayed in the same membrane preparation.

The most critical parameter in the procedure appears to be the initial homogenization of the isolated cells. In other series of fractionation experiments (not included in this report) with the same method, but different homogenizers, a considerable lower fraction of the brush border membrane was found in the "crude" plasma membrane. However, separation of the brush border enzymes and the basolateral plasma membrane enzymes relative to each other as well as the distribution of the enzyme in the sucrose gradient were similar in all experiments.

This investigation shows clearly that adenyl cyclase is purified parallel to the following enzymes: $(Na^+ + K^+)$-ATPase, K^+-stimulated *p*-nitrophenyl phosphatase and 5′-nucleotidase. The specific activity of these enzymes in the purified basolateral plasma membrane compares favorably to previous studies [6–8, 10]. These results thus strongly support an exclusive localization of adenyl cyclase in the basolateral region of the enterocyte, in agreement with the conclusion of Parkinson et al. [15]. Recent immunohistochemical staining for cyclic AMP also points to the contraluminal surface region as the source for this nucleotide [28]. The small amounts of adenyl cyclase in the isolated brush border membrane fraction could be due to cross-contamination with basolateral plasma membrane.

The membrane fractionation method reported here offers several advantages over previously published schemes and will be useful in further investigations of the plasma membrane properties of the intestinal epithelial cell. The advantages are (a) that brush border and basolateral plasma membrane are purified with the same procedure, (b) that the total time for isolation of membranes is only about 6 h so that the membranes can be prepared and assayed the same day, (c) that, in terms of equipment, only centrifuges, usually readily available, are needed in this method and (d) that adenyl cyclase, 5′-nucleotidase or K^+-stimulated phosphatase can be used instead of or in addition to $(Na^+ + K^+)$-ATPase to monitor the effectiveness of the fractionation process with respect to enrichment of basolateral plasma membrane.

ACKNOWLEDGEMENTS

We thank Professor G. Semenza for his interest and Drs. C. Cooper and H. Gerhman for reading the manuscript. The work was supported in part by Swiss Nationalfonds Grants 3.511.71 and 3.0090.73 and NIH Grant AM-18265.

REFERENCES

1 Solyom, A. and Trams, E. G. (1972) Enzyme 13, 329–372
2 Schwartz, I. L., Schlatz, L. J., Kinne-Saffran, E. and Kinne, R. (1974) Proc. Natl. Acad. Sci. U.S. 71, 2595–2599
3 Ray, T. K. and Forte, J. G. (1974) Biochim. Biophys. Acta 363, 320–339
4 Robison, G. A., Butcher, R. W. and Sutherland, E. W. (1971) Cyclic AMP, Academic Press, New York, pp. 17–46
5 Sutherland, E. W., Robison, G. A. and Butcher, R. W. (1968) Circulation 37, 279–306
6 Fujita, M., Matsui, H., Natano, K. and Nakao, M. (1971) Biochim. Biophys. Acta 233, 404–408
7 Fujita, M., Matsui, H., Natano, K. and Nakao, M. (1971) Biochim. Biophys. Acta 274, 336–347
8 Douglas, A., Kerley, R. and Isselbacher, K. J. (1972) Biochem. J. 128, 1329–1338

9 Quigley, J. P. and Gotterer, S. G. (1969) Biochim. Biophys. Acta 173, 456–464
10 Murer, H., Hopfer, U., Kinne-Saffran, E. and Kinne, R. (1974) Biochim. Biophys. Acta 345, 170–179
11 Schäfer, D. E., Lust, W. D., Sircar, B. and Goldberg, N. E. (1970) Proc. Natl. Acad. Sci. U.S. 67, 851–856
12 Kimberg, D. V., Field, M., Johnson, J., Henderson, A. and Gershon, E. (1971) J. Clin. Invest. 50, 1218–1230
13 Sharp, G. W. G. and Hynie, S. (1971) Nature 229, 266–268
14 Lo Spalluto, J. J. and Finkelstein, R. A. (1972) Biochim. Biophys. Acta 257, 158–166
15 Parkinson, D. K., Ebel, H., DiBona, D. R. and Sharp, G. W. G. (1972) J. Clin. Invest. 51, 2292–2298
16 Boyd, C. A. R., Parsons, D. S. and Thomas, A. V. (1968) Biochim. Biophys. Acta 150, 723–726
17 Glynn, I. M. and Karlish, S. J. D. (1975) Annu. Rev. Physiol. 37, 13–55
18 Hopfer, U., Nelson, K., Perrotto, J. and Isselbacher, K. J. (1973) J. Biol. Chem. 248, 25–32
19 Garraham, P. J., Pouchan, M. I. and Rega, A. F. (1969) J. Physiol. 202, 305–327
20 Berg, G. G. and Szekerczes, J. (1966) J. Cell. Physiol. 67, 487–500
21 Hübscher, G. and West, G. R. (1965) Nature 205, 799–800
22 Erecinska, M., Sierakowska, H. and Shugar, D. (1969) Eur. J. Biochem. 11, 465–471
23 Kinne, R., Schmitz, J. E. and Kinne-Saffran, E. (1971) Pflügers Arch. 329, 191–206
24 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, J. (1951) J. Biol. Chem. 193, 265–275
25 Stirling, C. E. (1972) J. Cell Biol. 53, 704–714
26 Lewis, B. A., Elkin, A., Michell, R. H. and Coleman, R. (1975) Biochem. J. 152, 71–84
27 DePierre, J. W. and Karnovsky, M. L. (1973) J. Cell Biol. 56, 275–303
28 Ong, S. H., Whitley, T. H., Stow, N. W. and Steiner, A. L. (1975) Proc. Natl. Acad. Sci. U.S. 72, 2022–2026