DISTRIBUTION OF SOME ADSORBED AND INTRINSIC ENZYMES BETWEEN THE MUCOSAL CELLS OF THE RAT SMALL INTESTINE AND THE APICAL GLYCOCALYX SEPARATED FROM THEM

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1. Introduction

The enterocyte brush border microvillous surface is covered by the glycocalyx which seems to be polyfunctional [1–7]. In particular, it gives a certain stability to the brush border and is responsible for adhesion, separation of food molecules according to size and charge [2,5]. One of the most important properties of the glycocalyx is its ability to adsorb various biologically active molecules including digestive pancreatic enzymes. However, our knowledge of it is still incomplete since so far it has not been preparatively separated.

We have recently succeeded in separating the apical glycocalyx from the rat brush border surface without disturbing the lipoprotein membrane and the lateral glycocalyx [8] as was shown by electron microscopy. This made it possible to investigate the distribution between the intestinal epithelium and the apical glycocalyx of some adsorbed enzymes: i.e., α-amylase (EC 3.2.1.1) and trypsin (EC 3.4.21.4); as well as some intrinsic enzymes: i.e., invertase (EC 3.2.1.48), alkaline phosphatase (EC 3.1.3.10), di- and tripeptidases (EC 3.4.13.11 and 3.4.11.4). It has been demonstrated that, in contrast to intrinsic enzymes, adsorbed pancreatic enzymes accomplishing mainly the intermediate stages of food hydrolysis are localized predominantly in the glycocalyx.

2. Materials and methods

2.1. *Materials*

Synthetic substrate *N*-benzoyl-DL-arginine-*p*-nitroanilide (*N*-BAPA) for the determination of trypsin was from Merck (FRG), *p*-nitrophenylphosphate came from Feinchemie K. H. Kallies KG (GDR), dipeptides and tripeptide were from Reanal (Hungary). The remaining substrates and reagents were supplied by Reachim (USSR).

2.2. *Experimental procedure*

After decapitation, the abdomen of a Wistar rat (male, 180–200 g) was opened and the proximal small intestine removed. After washing with 10 ml cooled saline, the 7 cm segment was tied at one end and rapidly filled with 3% agar solution (at 42°C) prepared in advance by heating in a boiling water bath then cooled to the required temperature. After filling, the intestinal segment was tied at the other end and placed in cooled saline, in order to gel the agar. Then, after a certain time (15–240 min) the intestinal segment was longitudinally cut with care and the agar gel (replica) removed carefully to avoid injury to the mucosa. The mucosa was scraped off and weighed as was the replica, both were then homogenized separately. The homogenates obtained were assayed for the above mentioned enzyme activities.

2.3. *Determination of enzyme activities*

The activity of α-amylase was determined by the decrease in starch concentration in the medium [9], that of trypsin by the release of *p*-nitroaniline from *N*-BAPA [10], that of invertase by the release of free hexoses [11], that of alkaline phosphatase by the

release of *p*-nitrophenol from *p*-nitrophenylphosphate, those of di- and tripeptidases by the release of glycine from glycine-containing peptides [12].

3. Results and discussion

Figure 1a shows the brush border surface of the intact intestinal mucosa with the glycocalyx. After removal of the replica (fig.1b), the apical glycocalyx became practically completely separated from the brush border surface, whereas the intermicrovillous glycocalyx remained. The figures also indicate that after the removal of the agar replica, the apical part of the lipoprotein membrane of the enterocytes is not distinguishable from the control.

The distribution of adsorbed pancreatic enzymes

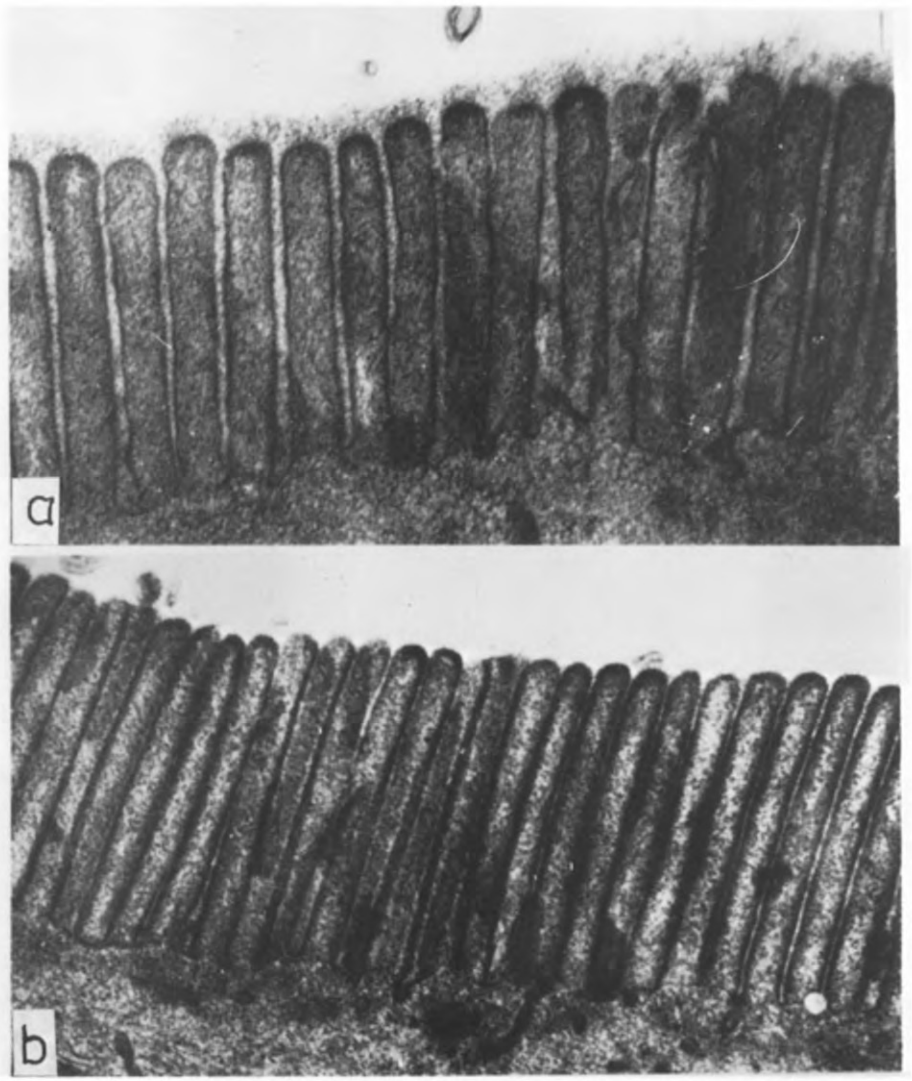

Fig.1. The brush border region of the rat intestinal cell: (a) longitudinal section of the apical zone of an intact cell; glycocalyx, on the external and side surfaces of microvilli (80 000 ×); (b) longitudinal section of the apical zone of a cell after the removal of the agar replica; glycocalyx on the external surface of microvilli is absent, undisturbed three-layered cell membrane and lateral glycocalyx are seen (80 000 ×).

between the mucosa and the replica separated from it showed that their activities were largely concentrated in the latter (86.5 ± 3.0% and 62.0 ± 3.3% for trypsin and α-amylase, respectively). These data confirm the assumption that the adsorbed enzymes, responsible for the intermediate intestinal hydrolysis of food substances, are located predominantly in the glycocalyx [5].

The content of invertase in the replica was negligible (~0–3%). As this enzyme is tightly bound to the lipoprotein membrane [13–17], from its amount in the replica together with electron microscopy, one could monitor the degree of desquamation of the intestinal epithelium and disruption of the apical part of the enterocytes during our experimental procedure.

An unexpected finding was that alkaline phosphatase, which is also accepted as a lipoprotein membrane-bound enzyme, is present in great amounts (17.9 ± 3.1%) in the apical glycocalyx. Such localization of this enzyme was proposed [1] on the basis of histochemical studies of the glycocalyx. However, it was not ruled out that this could have been an artefact. The mechanical separation of the apical glycocalyx from the remaining mucosa leaves no doubts as to the presence of significant amounts of alkaline phosphatase in it.

The contents of different di- and tripeptidases in the replica were, in most cases, not significantly different (table 1). Thus, the activity of tripeptidase in the apical glycocalyx was higher than that of glycyl–L-leucine dipeptidase. Since diglycylglycine and glycyl–L-leucine are cleaved in the small intestine mainly in the zone of membrane digestion [5,14,18], it may be concluded that glycyl–L-leucine dipeptidase appears to be concentrated in other zones of the cell surface than in the apical glycocalyx.

To evaluate the role of diffusion in the appearance of enzymes in the replica, their contents were compared in replicas that had been in contact with the mucosal surface for 15 and 240 min. The results were quite unexpected. There was an insignificant increase in the replica enzyme content after 240 min contact compared to 15 min contact (fig.2, table 1).

Comparison of these data and electron microscopic results demonstrate that only separation of the apical glycocalyx from the other structures of the brush border of the enterocytes took place in the experiments described here. Some enzymes within the apical glycocalyx (i.e., alkaline phosphatase and peptidases) may probably be bound to its structures rather tightly. It is not yet clear whether these enzymes are fully identical to the lipoprotein membrane-bound enzymes or whether they are glycocalyx isoforms.

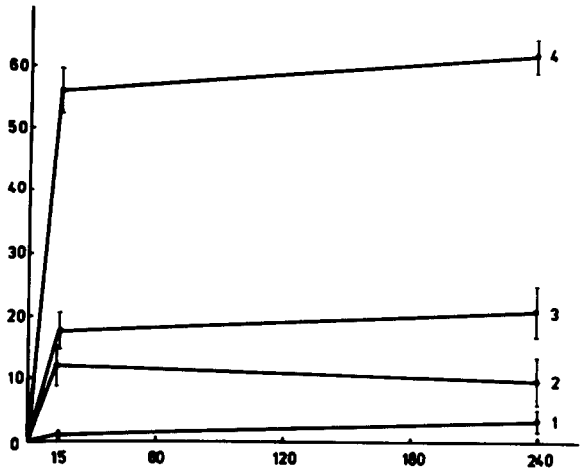

Fig.2. Percentage of the total activities (mucosa plus replica) of glycyl–L-leucine dipeptidase (1), glycylglycine dipeptidase (2), alkaline phosphatase (3) and α-amylase (4) in the agar replica plotted against the time of contact (15 and 240 min.) of the latter with the mucosal surface. Ordinate (%); abscissa, time of contact in min.

Table 1
Peptidase activities in apical glycocalyx separated from the small intestinal mucosa by agar replicas

Enzyme	Substrate L-form	Enzyme activity (%)[a]	
		15 min	240 min[b]
Dipeptidase	Gly–Leu	0.9 ± 0.3	3.5 ± 1.8
	Gly–Val	1.3 ± 0.4	4.4 ± 1.8
	Gly–Try	2.2 ± 1.0	9.2 ± 3.6
	Gly–Ala	2.3 ± 1.0	4.9 ± 2.4
	Gly–β–Phe	3.4 ± 2.5	2.6 ± 1.2
	Gly–Tyr	3.6 ± 2.1	6.3 ± 2.8
	Gly–His	4.4 ± 2.4	10.9 ± 2.1
Tripeptidase	Gly–Gly–Gly	5.5 ± 1.6	10.3 ± 3.9

[a] Enzyme activity is expressed as a percentage. The sum of the activities of the mucosal homogenates and the agar replicas taken as 100%

[b] Contact time of the small intestinal segment with the agar replica

Our technique will possibly permit the solution of some important problems concerned with membrane hydrolysis in the small intestine and other functions of the cell surface. This work presents results on the enzymology of the membrane surface and draws attention to the fact that not only adsorbed but also various intrinsic enzymes (di- and tripeptidases and especially alkaline phosphatase) are localized in the glycocalyx.

Analysis of the ultrastructure of the enterocyte surface allowed us to distinguish three zones:

1. Premicrovillous glycocalyx space;
2. Intermicrovillous glycocalyx space;
3. The three-layered lipoprotein membrane.

These zones may have essential functional distinctions [18].

References

[1] Ito, S. (1964) Anat. Rec. 148, 294.
[2] Ito, S. (1969) Fed. Proc. FASEB 28, 12–25.
[3] Ito, S. (1974) Trans. Roy. Soc. Lond. 268, 55.
[4] Komissarchik, Ja. Ju. and Ugolev, A. M. (1970) Dokl. Akad. Nauk SSSR 194, 731–733.
[5] Ugolev, A. M. (1972) Membrane digestion. Polysubstrate processes, organization and regulation, Nauka, Leningrad.
[6] Creamer, B. (1974) in: Intestinal Absorption. Biomembranes (Smyth, D. H. et al. eds) vol. 4A, pp. 2–24, Plenum, London, New York.
[7] Frank, L. M. and Wilson, P. D. (1977) in: International Review of Cytology, vol. 48, p. 55, Academic Press, London, New York, San Francisco.
[8] Ugolev, A. M., Parshkov, E. M., Egorova, V. V., Iezuitova, N. N., Mityushova, N. M., Smirnova, L. F., Timofeeva, N. M. and Tsvetkova, V. A. (1978) Dokl. Akad. Nauk SSSR 241, 491–494.
[9] Smyth, B. W. and Roe, J. M. (1949) J. Biol. Chem. 179, 53.
[10] Nagel, W., Willig, F., Peschke, W. and Schmidt, F. H. (1965) Hoppe-Seyler's Z. Physiol. Chemie 340, I.
[11] Ugolev, A. M. and Iezuitova, N. N. (1969) in: The study of the digestive apparatus in man, pp. 192–196, Nauka, Leningrad.
[12] Ugolev, A. M. and Timofeeva, N. M. (1969) in: The study of the digestive apparatus in man, pp. 178–181, Nauka, Leningrad.
[13] Ugolev, A. M. (1963) Prystenochnoye (contact) digestion. Izd. Akad. Nauk SSSR, Moscow, Leningrad.
[14] Ugolev, A. M. (1968) Physiology and Pathology of Membrane Digestion, Plenum, New York.
[15] Oda, T. and Seki, S. (1965) J. Electron Microsc. 14, 210–217.
[16] Johnson, C. F. (1969) Fed. Proc. FASEB 28, 26–29.
[17] Takesue, Y., Yoshida, T. O., Akaza, T. and Nishi, Y. (1973) J. Biochem. 74, 415–423.
[18] Peptide Transport and Hydrolysis (1977) CIBA Found. Symp. 50, Elsevier/Excerpta Medica/North-Holland, Amsterdam, Oxford, New York.