STUDIES ON THE ARRANGEMENT OF A GLUCOSE SENSITIVE PHLORIZIN BINDING SITE IN THE MICROVILLI OF ISOLATED RAT KIDNEY BRUSHBORDER

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1. Introduction

The carrier mediated active transport of glucose in proximal renal convolution can be inhibited competitively by phlorizin [1]. Phlorizin has a much higher affinity for the transport site than D-glucose and turned out to be a better tool for the characterization of the receptor than glucose itself. Isolated brushborders show phlorizin binding which can be inhibited competitively by D-glucose [2–5].

The association constant for the receptor–glucose complex is similar to the kinetic parameters of the transepithelial transport rate of D-glucose in the proximal renal tubule [6, 7]. To isolate this receptor information about its localization in the microvillus structure is an important prerequisite. Therefore glucose sensitive phlorizin binding to brushborders disorganized specifically by papain and detergents into fragments was measured. The large fragments were sedimented and their amount of phlorizin binding sites was correlated with the sedimented activities of aminopeptidase and alkaline phosphatase. Aminopeptidase is known to be surface-localized but alkaline phosphatase is firmly attached to the microvilli matrix [8].

2. Methods

2.1. *Isolation of the brushborders*

The isolation of the brushborder fraction was achieved by differential centrifugation [9]. The purification of the membranes was analyzed by determination of marker enzymes, such as alkaline phosphatase, aminopeptidase and glucose-6-phosphatase.

2.2. *Disaggregation of brushborders*

Microvillus fragments which differ in size and enzymatic activity were obtained in the following ways:

a) by digestion with papain (18 EU/27 mg membrane protein) microvilli without surface coat and surface particles (microvilli matrix). The matrix was sedimented at 35 000 *g* for 20 min.

b) by treatment with 0.3% Triton X-100 or 0.1% deoxycholate. Large fragments were sedimented at 35 000 *g* for 20 min.

Subsequently the fragments were suspended in TRA-HCl–EDTA buffer (10 mM triethanolamine-HCl, 5 mM EDTA) pH 7.6 and the phlorizin binding was measured.

In the experiments where Triton X-100 was used, also the phlorizin binding to the small fragments in the supernatants was determined.

2.3. *Binding studies*

The number of the glucose sensitive phlorizin binding sites (n) and the association constant (K_{phl}) of the phlorizin receptor was determined studying the phlorizin binding in the absence and presence of D-glucose [3]. The binding to brushborder membranes and microvilli fragments was achieved in TRA-HCl–EDTA buffer, pH 7.6 containing 0.15 M NaCl. After incubation with [^{3}H]phlorizin the radioacitivity in supernatant and sediment was measured, the amount bound to the sediment was corrected for the dead-space using [^{14}C]mannose. Membrane fragments were sedimented at 35 000 *g* for 20 min, or 100 000 *g* for 2 hr. The data were plotted using the linear transformation of the Langmuir adsorption isotherm by Scatchard [10].

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The specific activity of [^{3}H]phlorizin stock solutions was 150 mCi/mmole, that of [^{14}C]mannose 50 mCi/mmole.

3. Results

If brushborders were disorganized by papain and deoxycholate in the sedimented fragments, neither the binding constants for phlorizin (untreated membranes $K_{phl} = 3.2 \pm 1.1 \times 10^{-6}$ M/l, microvillus fragments $K_{phl} = 2.7 \pm 0.8 \times 10^{-6}$ M/l), nor the number of the glucose sensitive phlorizin binding sites (untreated membranes $n = 3.6 \pm 1.0 \times 10^{-11}$ moles/mg protein, microvillus fragments $n = 3.1 \pm 0.7 \times 10^{-11}$ moles/mg protein) were significantly changed. Under all conditions l-glucose did not inhibit the binding of phlorizin to its receptor.

Treatment with papain removed specifically aminopeptidase from the microvilli but alkaline phosphatase and protein remained bound (table 1). As can be seen in fig. 1, the increase in the ratio of binding sites to aminopeptidase in the sediment indicates that the binding site for phlorizin cannot be removed like the surface-localized aminopeptidase [9]. Instead it behaves like the tightly fixed alkaline phosphatase (% binding sites/% enzyme activity ~ 1) [8].

The degree of the brushborder decomposition with deoxycholate is dependent on the buffer medium in which the membranes are suspended (table 1). In the presence of isotonic sucrose the phlorizin binding sites were removed with membrane proteins to the same degree as aminopeptidase activity (% binding sites/% enzyme activity ~ 1). However deoxycholate in TRA-NaCl–EDTA buffer (II) dissociated nearly all of the aminopeptidase activity, great amounts of protein, and 50% of the alkaline phosphatase activity from the microvilli. By this treatment the ratio binding sites to aminopeptidase activity in the sediment is increased and the ratio binding sites to alkaline phosphatase activity is decreased (fig. 1).

Thus the glucose sensitive phlorizin binding site is not as easily removable as aminopeptidase activity but more easily than alkaline pjosphatase activity. After disorganisation of the brushborder with Triton, neither the sedimentable fragments which contain 80% of the alkaline phosphatase activity but only 30% of the proteins, nor the proteins in the supernatants show glucose sensitive phlorizin binding.

4. Discussion

The aim of this study was to localize the glucose sensitive phlorizin receptor relative to surface located protein, such as aminopeptidase and to a tightly bound protein of the microvillus matrix, such as alkaline phosphatase [8].

The results show that the ease of extraction of

Table 1

The percent recovery of protein, aminopeptidase and alkaline phosphatase in the 35 000 *g* sediments of known amounts of these substances in the untreated brushborder fraction (total).

Solubilizing agent		Protein (% of total)	Enzyme activity (% of total)	
			Aminopeptidase	Alkaline phosphatase
Papain		79 ± 4	12 ± 6	93 ± 7
Sodium deoxycholate	I	66 ± 3	62 ± 7	83 ± 10
	II	29 ± 5	8 ± 4	46 ± 4
Triton X-100		28 ± 6	18 ± 4	79 ± 10

The brushborder fraction was treated 10 min at 4° with the detergents or at 37° with papain. Sodium deoxycholate treatment was done in isotonic sucrose medium of pH 7.6 (I) or in TRA-NaCl–EDTA buffer (II). For correlation of the enzyme activities in untreated brushborders to that of disorganized ones the enzyme activities in the sediments were corrected with factors calculated as described [8]. The means of 8 experiments ±SD are shown.

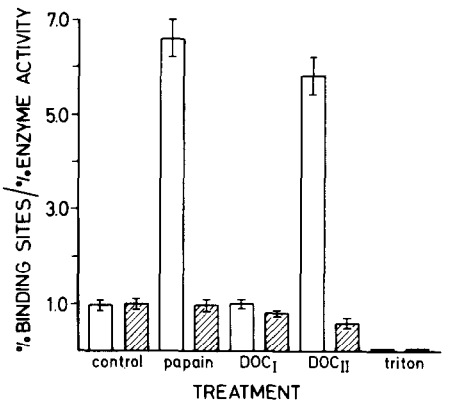

Fig. 1. The percent recovery of glucose sensitive phlorizin binding sites in the 35 000 *g* sediments is correlated to the percent recovery of aminopeptidase and alkaline phosphatase activity in the sediments (% Binding Sites/% Enzyme Activity). The percent values represent the amounts of these substances in the sediments from known amounts of aminopeptidase, alkaline phosphatase and glucose sensitive phlorizin binding sites in the untreated brush border fraction (control). The white bars represent the ratio % binding sites/% aminopeptidase activity, the hatched ones the ratio % binding sites/% alkaline phosphatase activity. An increase of the ratio % binding sites/% aminopeptidase activity indicates a stronger removal of aminopeptidase than of glucose sensitive phlorizin binding sites from the microvillus. A decrease of the ratio % binding sites/% alkaline phosphatase activity indicates a weaker removal of the alkaline phosphatase from the microvillus than of the phlorizin receptor. Deoxycholate = DOC.

the proteins decreases in the following order: aminopeptidase > phlorizin receptor > alkaline phosphatase. This indicates that the receptor is not present in the surface coat nor in the surface particles like aminopeptidase, but is associated with the microvillus matrix. The site of the phlorizin receptor within the membrane differs markedly from that of the sugar transporting sucrase–isomaltase complex which is localized in the surface particles on the microvilli of small intestine [11]. Since the maltase in the brushborder of rat kidney is also removed by papain (unpublished result) the phlorizin receptor cannot be identical with the disacharidase associated sugar transport system described by Semenza. After treatment of the brushborders with Triton neither the sedimented fragments nor the solubilized ones show glucose sensitive phlorizin binding. This suggests that Triton alters the conformation of the phlorizin receptor irreversibly. This could be a change in the receptor itself or a disturbance of the receptor's stoichiometry in relation to other proteins in the membrane. Deoxycholate, on the other hand which solubilizes the brushborders also into large and small fragments, disorganizes only neighbouring structure of the glucose sensitive phlorizin binding site but has no effect on the receptor itself.

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