BBA Report

BBA 71120

Sodium-dependent binding of D-glucose to a filamentous fraction of Tris-disrupted brush borders from hamster jejunum

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(Received October 11th, 1971)

SUMMARY

Tris-disrupted brush borders prepared from hamster jejunum were fractionated by Ficoll density gradient centrifugation. Preferential D-glucose binding to a filamentous, microvillus core fraction was dependent upon the Na^+ concentration in the incubation medium. No binding occurred when K^+, Li^+, NH_4^+, or choline was substituted for Na^+. It has been postulated that microvillus core filaments are intimately involved in the mechanism of active sugar as well as amino acid transport by the small intestine.

Our studies on the initial phase in the mechanism of active sugar transport by the small intestine have been devoted to observing the preferential binding of actively transported D-glucose to isolated intact and Tris-disrupted brush borders prepared from the mucosa of hamster jejunum[1,2]. We have shown that other actively transported sugars, and phlorizin, a competitive inhibitor of active intestinal sugar transport, inhibit preferential D-glucose binding. Furthermore, this preferential binding of an actively transported sugar was temperature dependent, enhanced by Mg^{2+}, and inhibited in the presence of sulfhydryl reacting compounds. Although there is evidence to indicate that a ternary complex which consists of Na^+, the actively transported sugar and a component of the brush border, is formed during the first step in the mechanism of active intestinal sugar transport[3], we did not observe a Na^+ dependency for D-glucose binding in our experiments. It was suggested, however, that sufficient Na^+ was present in our brush border preparation to satisfy a possible Na^+ requirement for this phenomenon.

In a recent study, we demonstrated that a low Na^+ fraction of a disrupted mucosal brush border preparation from hamster jejunum exhibits Na^+-dependent binding of the actively transported amino acid, L-histidine[4]. Consequently, a similar method was employed to obtain a fraction of Tris-disrupted brush borders that would possibly require the presence of Na^+ for preferential D-glucose binding.

Epithelial brush border membranes from the jejunum of six hamsters were isolated according to the method that has been previously reported[1,2]. The isolated intact brush borders were disrupted at 2°C for 45 min with 1 M Tris (hydroxymethyl) aminomethane (pH 8.2) in Krebs bicarbonate saline containing 6 mM dithiothreitol (Cleland's reagent), a protein stabilizer[5]. After this procedure, the disrupted brush borders were washed once with cold 6 mM dithiothreitol by centrifugation at 27 000 × *g* and resuspended in 2 ml of the wash medium. This suspension was placed on a 10, 20, 30, 35, and 40% (w/v) Ficoll density gradient containing 6 mM dithiothreitol and centrifuged in a SW 39 L swinging bucket rotor at 112 000 × *g* for 60 min. Five bands and a precipitate were obtained under these conditions. Each fraction was removed, washed twice with 6 mM dithiothreitol by centrifugation at 112 000 × *g*, and resuspended in 3.3 ml of an incubation medium which contained 6 mM dithiothreitol, 100 mM NaCl, 10 mM $MgCl_2$, 20 mM Tris buffer (pH 7.4), 0.01 μM D-[1-^{3}H] mannose (a sugar which has a low affinity for the active transport system) and 1 μM of D-[U-^{14}C] glucose (a sugar with a high affinity for the active transport system). These experimental, as well as control tubes which did not contain brush border material, were incubated for 30 min at 37°C. Then the experimental tubes containing the fractions of the brush borders were centrifuged, and the $^3H/^{14}C$ dpm ratios were obtained from the supernatant fluid and were compared with the ratios in the control tubes. As previously reported[1,2], an increase in the initial $^3H/^{14}C$ dpm ratio of the supernatant indicated preferential binding of the actively transported D-[^{14}C] glucose to the brush border fraction.

The results of this study demonstrated that only Fraction I, which floated on top of the 10% Ficoll layer, was capable of preferentially binding D-[^{14}C] glucose. The percentage change in the $^3H/^{14}C$ dpm ratios by Fractions I–VI observed in five experiments were 22.6±1.8, 2.7±2.3, 4.5±1.6, 0.8±0.2, −4.0±4.1, and 3.5±7.4, respectively. No binding to Fraction I occurred when Mg^{2+} was omitted from the incubation medium or when dithiothreitol was excluded from any step in the procedure. It is of interest to note that this is the same fraction which exhibited Na^+-dependent preferential binding of L-histidine[4]. The sugar and amino acid binding sites, however, are not the same[2,6].

In order to determine if D-glucose binding to Fraction I was dependent on the presence of Na^+, this cation was substituted in the incubation medium by K^+, Li^+, NH_4^+ and choline. Fig. 1 shows that D-glucose binding is indeed dependent on Na^+ and that no binding occurs in the presence of the other cations. Preferential D-glucose binding to Fraction I varies with the concentration of Na^+ in the incubation medium as is illustrated in Fig. 2. Maximum binding occurs at a Na^+ concentration of 25 mM and there is no increase in this binding even though the Na^+ concentration is elevated to 100 mM.

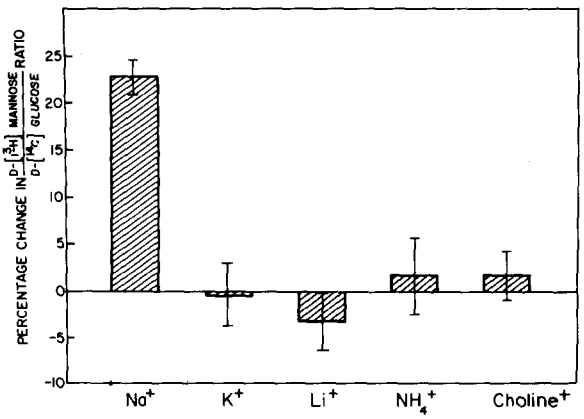

Fig. 1. Preferential binding of D-[U-^{14}C]glucose to brush border Fraction I in 100 mM Na^+ and Na^+-substituted chloride salts, 6 mM dithiothreitol, 10 mM $MgCl_2$ and 20 mM Tris buffer (pH 7.4). Fraction I was incubated for 30 min at 37°C in the presence of 0.01 μM D-[1-^{3}H]mannose, specific activity of 3.8 Ci/mM, and 1 μM D-[U-^{14}C]glucose, specific activity of 288 mCi/mM. Each bar point represents the mean of at least four experiments. The vertical lines represent 1 S.E. above and below the bar points.

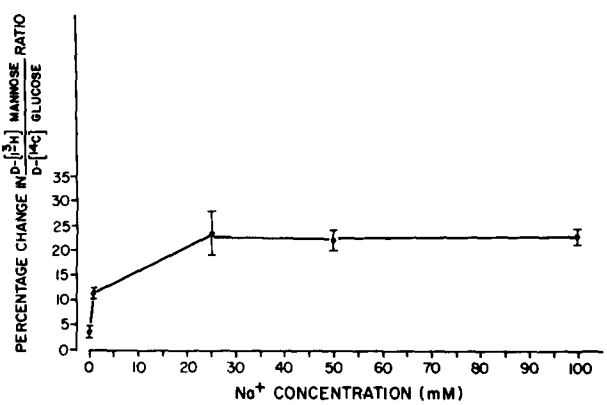

Fig. 2. Effect of various Na^+ concentrations on the preferential binding of D-[U-^{14}C]glucose to brush border Fraction I. NaCl was employed. Each point is the average of at least three determinations and the vertical lines indicate ± 1 S.E. of the mean.

Fig. 3 shows electron photomicrographs, at different magnifications, of Fraction I prepared with glutaraldehyde and osmium tetroxide. The filaments in this homogeneous fraction are subunits of the microvillus core[7–9] which was observed to bind D-glucose in our previous experiments[2]. In addition, this is the same material that was observed to require Na^+ for the binding of L-histidine[4]. In Fig. 4 it can be seen that the microvillus filament contains clumps of diffuse, granular material on its outer surface. The filament has an electron opaque region in the center and the width between the median of the

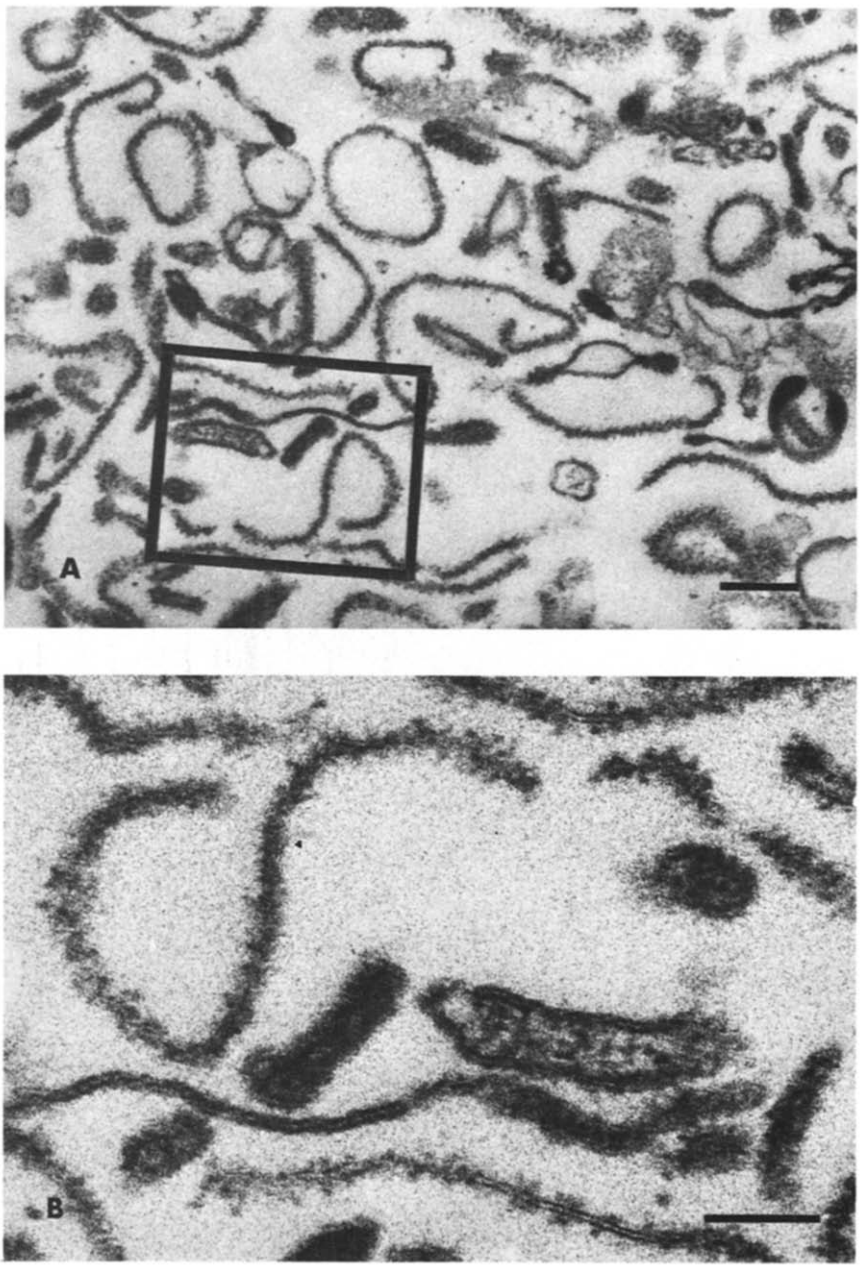

Fig. 3. Electron photomicrographs of Fraction I obtained from the Ficoll density gradient centrifugation of Tris-disrupted brush borders. Glutaraldehyde and osmium. A. Approx. × 50 000; scale is 2000 Å. B. Higher magnification of inset from A. Approx. × 150 000; scale is 1000 Å.

Fig. 4. Electron photomicrograph of a microvillus core filament from Fraction I. Glutaraldehyde and osmium. Approx. × 300 000; scale is 200 Å.

electron dense lines is approximately 35 Å. This diameter corresponds well with the dimension of the filamentous subunits which compose the microvillus core material[7–9]. No sucrase or glycylhistidine peptidase activity was associated with this material (unpublished observation) which further supports the assumption that it is core material and not part of the outer limiting membrane of the microvillus which contains these hydrolytic enzymes[10].

The results of this investigation strengthen the assumption that preferential binding of actively transported D-glucose to a component of the mucosal brush border is related to the initial step in the mechanism of active sugar transport by the small intestine. In addition, it has been indicated that this phenomenon, as well as Na^+-dependent L-histidine binding, occurs within the core of the mucosal brush border. This structure is in close proximity to a major source of these substrates which are normally obtained in the intact intestine from larger molecules by disaccharidase and peptidase activity located on the outer surface or plasma membrane of the brush border[11]. Furthermore, any actively transported sugars and amino acids that may be free in the luminal fluid of the small intestine could diffuse across the plasma membrane and bind directly to the core filaments. It is presumed that this filamentous network within the microvillus would then act as a conduit for the movement of these substances into the interior of the mucosal cell by an energy-requiring process which would result in the intracellular accumulation of sugars and amino acids against their concentration gradients.

The authors wish to thank Mary Jo Burns, Gregory Wilson, and Wilma Hanton for their technical assistance. This work was supported by grant AM 07998 from the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service.

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