Zinc inhibition of glucose uptake in brush border membrane vesicles from pig small intestine

D. W. Watkins¹, C. Chenu², and P. Ripoche²

Abstract. The effect of zinc on sodium coupled glucose uptake was studied in pig intestinal brush border membrane vesicles. In this system zinc inhibited glucose uptake and appeared to have a Ki of 0.25 mM. When tested by spectrophotometry, electron microscopy and protein determination following centrifugation, no evidence of significant vesicle aggregation was found with 0.5 mM zinc treatment. Zinc inhibition of glucose uptake persisted when the vesicle membrane potential was clamped with identical KCl concentrations inside and outside the vesicles in the presence of valinomycin. Variation of the glucose and sodium concentrations gave results indicating that zinc reduces glucose affinity for the carrier but not sodium binding to the transporter. The glucose inhibitory effect was not due to a rapid dissipation of the sodium gradient as zinc failed to affect sodium uptake in the absence of glucose. Zinc also failed to inhibit glucose efflux from vesicles under isotopic exchange conditions, when glucose and sodium concentrations were identical inside and outside vesicles. The t½ of glucose inhibition by zinc was relatively long, i.e. 6 min. We conclude that zinc acts as an inhibitor of glucose transport by interacting with the sodium-glucose co-transporter. The long zinc incubation time required to achieve maximal inhibition of glucose transport suggests that this interaction takes place within vesicles.

Key words: Brush border membrane - Pig small intestine - Glucose uptake - Zinc

Introduction

Zinc is known to be contained in a large number of metalloenzymes [7]. However, the symptoms associated with zinc deficiency, such as growth retardation [10], incompetence of immune function [1], and impairment of taste and smell [31] have not yet been described in terms of specific changes in zinc metalloenzymes. Instead Bettger [4] has proposed, that pharmacologic doses of zinc and zinc deficiency may exert some of their actions directly upon cellular membranes, either by altering permeability or by modulating the activity of membrane-bound enzymes some of which do not contain zinc. For example, zinc inhibition of the transport enzyme system Na⁺/K⁺ ATPase [23] has been reported. Similarly uptake of glucose by intestinal segments in vitro [26] and saline solution absorption in human volunteers [33] were also inhibited by zinc.

This study was undertaken to examine the effect of zinc on glucose uptake by brush border membrane vesicles, and to elucidate the mechanism of any interactions. The results indicate that zinc inhibits glucose uptake by brush border vesicles in the range of concentrations 0.1 - 1 mM. The observed zinc inhibition may result from interaction with the sodium-dependent glucose transport mechanism at a site which decreased the sugar affinity for its carrier and thereby affected glucose transfer.

Materials and methods

Materials. Pig intestines were obtained at the INRA (National Institute of Agronomic Research) breeding center at Jouyen-En-Josas, France, from animals sacrificed for other purposes. The intestines were immediately rinsed in ice-cold 0.9% NaCl containing 0.1 mM PMSF (phenyl methyl sulfonyl fluoride) and 0.01% lithium azide, and stored in the same solution for transport to the laboratory.

Reagents were of analytical grade and the radioactive glucose, D-6-3H glucose and ²²Na were obtained from the Service des Molécules Marquées, C.E.N. de Saclay, France.

Vesicle preparation. Vesicles were prepared according to the technique described by Kessler [19] with the substitution of MgCl₂ for CaCl₂ precipitation. The intestine was cut into 10 cm segments, everted, and scraped. Scraping were introduced, at a concentration of 1 g scraping/50 ml buffer, into a solution containing either MTH (mannitol 50 mM, HEPES 10 mM, adjusted to pH 7.1 with Tris, LiN₃ 0.01%) or MHK (mannitol 100 mM, HEPES 10 mM, adjusted to pH 7.4 with 1 M KOH). This suspension was then homogenized for 30 s in a blender. The homogenate was treated by MgCl₂ addition to 10 mM final concentration, incubated 20 min, then centrifuged twice at 2900 x g for 15 min and the pellet discarded. The supernatant was then centrifuged at 28,000 x g for 30 min. The high speed pellet was then resuspended in MHK buffer at a dilution of 7 ml buffer/g original scraping, and homogenized by 10 strokes in a Potter teflon homogenizer at 800 RPM. The suspension was centrifuged again at 800 x g for 15 min (pellet discarded) and then at 28,000 x g for 30 min. This final pellet was resuspended in MHK by passing through a 21 gauge needle ten times to give an approximate concentration of 15 - 25 mg protein/ml buffer (Bradford [5] assay with bovine serum albumin as standard).
The resulting suspension of vesicles was divided into 0.3 ml aliquots, rapidly frozen, and stored in liquid nitrogen.

**Enzyme assays.** The purity of the vesicle preparation was determined from brush border marker enzyme assays. Leucine aminopeptidase activity [24] and alkaline phosphatase activity [16] were both enriched in the final vesicles by a factor of 9.9 ± 1.7 (n = 8) and 9.01 ± 0.7 (n = 4) (range: 8–12) respectively compared to the original homogenate. The absence of significant enrichment for K⁺-stimulated phosphatase [29], NADH-cytochrome c reductase [32] and glucuronidase [9] activities indicates a very small contamination of the preparation by basolateral plasma membrane, endoplasmic reticulum and lysosomal membranes.

**Vesicle aggregation.** Possible aggregation of vesicles was measured by three techniques: spectrophotometry of vesicle suspensions at 400 nm [21], determination of pellet protein [25] after centrifugation at 3000 × g for 15 min [8] and electron microscopic examination of freeze fracture preparations [17]. For spectrometry, 20µl of vesicles (20mg/ml) were incubated in 2 ml of the MHK buffer solution containing 0.1 M KCl. The dilution of vesicles in the medium corresponds to the condition used in the uptake measurements. Samples of vesicles for the freeze fracture process were quick frozen with a propane jet technique [12], with a cryojet QFD 101 apparatus (Balzers, Lichtenstein). After two hours of incubation at 22°C in the presence or absence of 0.5 mM Zn²⁺, vesicles were freeze fractured (Balzers 300 cryo-pump freeze etch unit, Balzers, Lichtenstein) under high vacuum and processed for electron microscopy (Philips 301). Statistical treatment of photographs of freeze fractured vesicles consisted of counting the number of vesicles and aggregates, which were defined to be more than four vesicles in contact.

**Glucose uptake experiments.** Glucose uptake was measured at room temperature using the classical rapid filtration technique described by Hopfer et al. [15]. Frozen vesicles were rapidly thawed (in water at 37°C) and then equilibrated for two hours in 100 mM KCl MTH with 11 µM valinomycin (the optimal concentration tested). Glucose uptake was initiated by injecting 20 µl of vesicles, containing 300 µg protein, into 200 µl of 100 mM NaCl MTH incubation medium to which 0.1 mM glucose and 4–8 µCi of D-(³H) glucose have been added. Uptake was stopped by addition of 2.5 ml ice cold NaCl-MTH. Diluted vesicles were then rapidly vacuum filtered onto 0.65 µm pore size cellulose nitrate filters, size 25 mm diameter (Sartorius GmbH, Göttingen, FRG). Filters were placed in plastic counting vials and dissolved in 4 ml of scintillation fluid (Pico-fluor, Packard, Zürich, Switzerland). The ³H-glucose retention on filters was counted in a scintillation spectrometer (model A4430, Packard Instruments). Data shown in figures are from one of three or four experiments, each showing similar results. Each point represents the mean of triplicate determinations. Results have been expressed as the picomoles of glucose uptake/mg protein [25] retained on the filters. Medium compositions and procedures different from the above have been indicated in the figure legends.