Sodium-induced conformation changes in membrane transport proteins

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Received 10 July 1985

In the presence of KCl, tryptic digestion of vesicles derived from pigeon erythrocyte membranes inactivates sodium-dependent uptake of alanine by the vesicles, whereas digestion in the presence of NaCl does not. Extensive degradation of vesicle proteins occurs under both conditions. Similarly, the extent of inhibition by *N*-ethylmaleimide of the sodium-dependent influxes of both glycine and alanine into human erythrocytes is greater when the cells are exposed to the thiol reagent in the presence of KCl than when NaCl is used. These observations are interpreted as providing evidence for sodium-induced conformation changes in these transport proteins.

Membrane protein *Sodium dependence* *Amino acid transport*

1. INTRODUCTION

It is generally accepted that the membrane NaK-ATPase can adopt two different conformations, one being stabilised by Na^+ and the other by K^+ [1]. Perhaps the most convincing evidence for this view was the demonstration that the course of proteolytic digestion of the enzyme is determined by the nature of the monovalent cation present [2], that finding providing the basis for many subsequent studies of this transport system. For example, the way in which the thiol reagent *N*-ethylmaleimide (NEM) inhibits the NaK-ATPase under various conditions has also been interpreted in terms of the conformation changes induced by Na^+ and K^+ [3]. Here we present similar evidence which indicates that Na^+-induced conformation changes occur in three different membrane systems responsible for the Na^+-dependent transport of amino acids.

2. MATERIALS AND METHODS

Human erythrocytes (fresh to 7-days-old) were incubated at 37°C for 15–60 min in either NaCl-medium or KCl-medium (145 mM NaCl or KCl, 2 mM $MgSO_4$, 20 mM Tris/HCl, pH 7.8, at 20°C) containing either 0.2 mM *N*-ethylmaleimide (NEM) (for subsequent incubation with glycine) or 1 mM NEM (for subsequent incubation with L-alanine) or with no addition (controls). Then 2-mercaptoethanol was added to give a final concentration in excess of the NEM concentration and the cells were sedimented by centrifugation and washed twice by resuspension and centrifugation in 10 vols of isotonic NaCl or KCl. Na^+-dependent uptake of glycine (from a concentration of 0.05 mM) and alanine (from a concentration of 0.1 mM) was then measured as described by Al-Saleh and Wheeler [4].

Vesicles prepared from pigeon erythrocyte membranes by a slight modification of the method of Watts and Wheeler [5] were suspended in mannitol buffer (300 mM mannitol, 1 mM $MgSO_4$, 5 mM L-alanine, 1 mM [^{3}H]sucrose and 5 mM Tris/HCl, pH 7.6, at 20°C) and samples incubated at 20°C in either 100 mM NaCl or 100 mM KCl containing (a) trypsin (1 mg/ml) or (b) trypsin plus trypsin inhibitor (5 mg/ml) and phenylmethylsulphonyl fluoride (1 mM). After 30 min the two inhibitors were also added to the samples that initially contained only trypsin, to stop further action of tryp-

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00145793/85/$3.30

sin, and the vesicles were sedimented by centrifugation for 10 min at about 10000 × *g*. They were washed 3 times by resuspension and centrifugation with about 20 vols of mannitol medium, without the [^{3}H]sucrose, and finally suspended to their original volumes in the same buffer solution. Samples of each vesicle suspension were used for assay of phospholipid content [5] and Na^+-dependent uptake of alanine [6], and analysis of residual proteins by polyacrylamide gel electrophoresis (PAGE) [7]. Because of the difficulty of maintaining strictly Na^+-free conditions after exposure to 100 mM NaCl, Na^+-dependent uptake of alanine from a 0.1 mM concentration of [^{14}C]alanine was taken as the difference in uptake measured in the absence or presence of 10 mM L-serine in Na^+-medium [6]. Intravesicle volumes were monitored by measurement of the trapped [^{3}H]sucrose. Trypsin (Type IX), trypsin inhibitor (Type II-O), *N*-ethylmaleimide and phenylmethylsulphonyl fluoride were obtained from Sigma. All other materials and methods were as described previously [4–6].

3. RESULTS AND DISCUSSION

In a previous study of Na^+-dependent uptake of amino acids by human erythrocytes we found that the extent of inhibition caused by preliminary incubation of the cells with NEM in NaCl was the same as that produced during incubation with NEM in KCl [4]. However, a subsequent brief report that differential inhibition had been detected with the use of slightly different conditions [8] prompted us to examine these experimental conditions more closely. The results in table 1 show that the extent of inhibition by NEM of the Na^+-dependent fluxes of both glycine and alanine can be affected by the nature of the monovalent cation present during exposure to NEM. In both cases there is less inhibition when the cells are incubated with NEM in the presence of Na^+ than there is with NEM and K^+. For glycine uptake, which is particularly sensitive to inhibition by NEM [4], the differential inhibition was clearly apparent in our experiments only with NEM concentration in the range 0.15 to 0.2 mM. For alanine uptake, however, the effect was most pronounced with 1 to 2 mM NEM. Other experiments showed

Table 1

Differential effects of Na^+ and K^+ on the inhibitory action of *N*-ethylmaleimide

Incubation with *N*-ethylmaleimide in	Inhibition (%) of Na^+-dependent influx of	
	Glycine	Alanine
Sodium medium	46 ± 3 (10)	49 ± 3 (5)
Potassium medium	68 ± 3 (10)	69 ± 1 (5)
Choline medium	67 (2)	62 (1)

Human erythrocytes were incubated in the indicated media in the presence or absence of NEM and then the Na^+-dependent influxes of glycine or alanine into the washed cells were measured as described in the text. Mean values (± SE) are given for the results from the number of experiments indicated in parentheses

that KCl, but not NaCl, could be replaced with choline chloride, suggesting that Na^+ decreases, rather than K^+ promotes, the action of NEM (table 1). Since the Na^+-dependent influxes of glycine and alanine into these cells are mediated by distinct transport systems, with little overlap [4], it appears that each of these systems must contain a thiol group (or class of groups) that is less accessible to NEM in the presence of Na^+.

Fig.1A shows how Na^+ protects another Na^+-dependent transport system from inactivation by proteolytic digestion with trypsin. Under the conditions given in fig.1, exposure of vesicles derived from pigeon erythrocyte membranes to trypsin in the presence of KCl largely inactivates Na^+-dependent uptake of alanine by the vesicles. In contrast, when the KCl is replaced with NaCl the vesicles retain about 85% of their Na^+-dependent uptake of alanine. Choline chloride again mimicks the action of KCl rather than NaCl (83% inactivation in choline chloride). Fig.1B shows that the tryptic digestion degraded a significant proportion of the vesicle proteins under both conditions, there being no detectable difference in the profiles of the proteins separated by PAGE after digestion in the presence of NaCl or KCl. In general the larger proteins, constituting bands 1, 2 and 3 (see [9] for nomenclature), were extensively degraded and two main degradation products were produced with M_r values of around 60000 and 40000

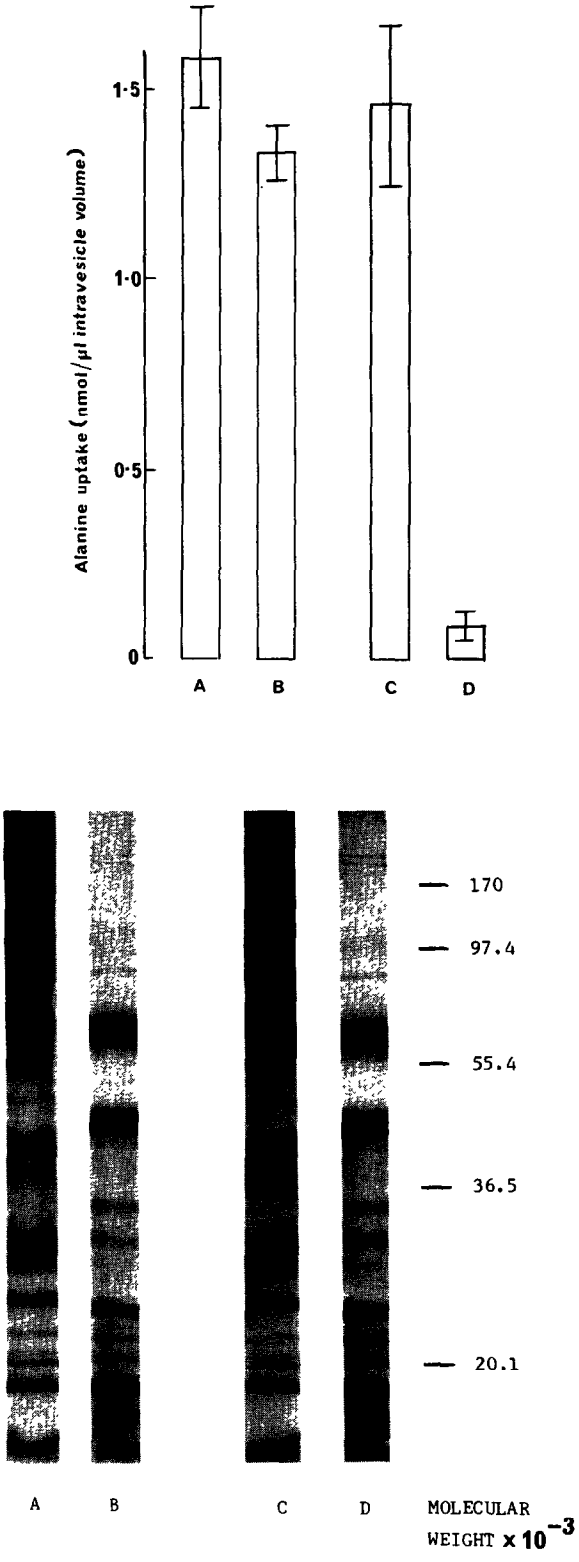

Fig.1. Effect of Na^+ on trypsin inactivation of Na^+-dependent uptake of alanine by membrane vesicles from pigeon erythrocytes. Vesicles prepared from pigeon erythrocyte membranes were incubated in either NaCl or KCl with trypsin, or trypsin plus trypsin inhibitors, as described in the text. Samples of the vesicles were then used for assay of Na^+-dependent alanine uptake (A). Other samples of the vesicles were analysed by PAGE and B shows photographs of the gels after they had been stained with Coomassie blue. (A) After incubation in NaCl with trypsin plus trypsin inhibitors; (B) after incubation in NaCl with trypsin; (C) after incubation in KCl with trypsin plus trypsin inhibitors; (D) after incubation in KCl with trypsin. The values in A are means (± SE) from 4 experiments.

(fig.1B). However, the complete pattern of degradation tends to be obscured by the appearance of several smaller proteins. These results show that the activity of trypsin itself is not generally influenced by Na^+, overall proteolysis being the same in the presence of both NaCl and KCl, and the obvious interpretation is that a Na^+-induced conformation change must protect just a few proteins from tryptic digestion.

A brief survey of the literature reveals other reports of evidence for Na^+-induced conformation changes in membrane transport proteins, in addition to the NaK-ATPase. For example, Becker and Duhm [10] showed that the rate of inhibition by NEM of the Na^+/Li^+ exchanger in the membranes of both human and ox erythrocytes is markedly accelerated by Na^+ and concluded that Na^+ induces a conformation change in this transport protein. Those findings have recently been confirmed by an independent study [11]. More recently, Pearce and Wright [12] reported that the effects of isothiocyanates on the Na^+-dependent transport of glucose by intestinal brush border membranes are consistent with a Na^+-induced conformation change in the transport protein. Hence it appears that such conformation changes may be a common feature of Na^+-dependent transport proteins, which are widespread in mammalian membranes. Also, it seems possible that this property may be open to further exploitation. First, results such as those shown in fig.1, or differential labelling with radiolabelled NEM under conditions similar to

those in table 1, might provide an indication of the molecular size of the transport protein. Second, the ability to degrade and remove a considerable proportion of the total membrane proteins without significant loss of the transport protein should greatly enhance the possibility of being able to extract the latter for purification and reconstitution into liposomes. Finally, with a purified transport protein, the differential sensitivity to proteolysis and inhibitors in the presence and absence of Na^+ should permit more detailed analysis of structure and function relationships, along the lines already applied to the NaK-ATPase [1].

ACKNOWLEDGEMENTS

We thank Susan J. Nicklin for technical help with this work, which was supported by a project grant from the Medical Research Council.

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