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THE EFFECT OF THE STRONGLY BOUND PROTEIN FRACTION ON SUGAR TRANSPORT IN HUMAN ERYTHROCYTE GHOSTS

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Summary

The protein fraction released from human erythrocyte membranes with 90% acetic acid enhanced the transport of several sugar species when enclosed in erythrocyte ghosts. Both the influx and the efflux of D-glucose were increased so that permeation rather than sugar binding was involved. The permeation increase was selective, being found with D-glucose, D-galactose and D-xylose but not with L-glucose or lactose. The protein-dependent sugar transport was saturable and the incorporation of proteins into the ghost membrane brought J_{max} to the level corresponding to intact erythrocytes, leaving K_m unchanged.

The modelling of sugar transport with liposomes prepared mostly from plant lipids and enriched with strongly bound proteins from human erythrocyte membranes has been the subject of a number of recent studies [1—3]. Also, vesicles depleted of protein bands 1, 2, 5, and 6 of the erythrocyte membrane have been used for the purpose [4]. The approach of the present investigation consisted of enclosing strongly bound proteins isolated from the erythrocyte membrane into erythrocyte ghosts, since preliminary experiments aimed at modifying the ghost membrane permeability by addition of the proteins to the external medium were not successful.

Erythrocytes from healthy human donors were collected in NaCl-phosphate buffer and erythrocyte ghosts were prepared as described by Beneš, Kolínská and Kotyk [5]. The strongly bound protein fraction was isolated by solubilization with 90% acetic acid according to Schubert, Poensgen and Werner [6]. The proteins were separated on a Sephadex LH-20 column and the fraction characterized by a high peak of absorbance at 280 nm was further used after dialysis; proteins corresponding to other, lower, maxima were found to be without effect on ghost permeability to sugars. Sodium dodecyl sulphate gel electrophoresis of the fraction used in the expriments demonstrated the

presence of bands 3 and 4 with traces of band 2 according to the terminology of Steck [7]. Using the filtration method in concentrated solution of ammonium sulphate [8] uptake of D-glucose by the isolated proteins corresponding to 100—125 pmol/mg protein was found.

The eluate corresponding to the peak was dialyzed against distilled water and the protein precipitate was resuspended in 20 mosM phosphate buffer, pH 7.4. This suspension (5 mg dry solids/ml) was then combined with a suspension of ghosts in the same hypotonic medium, the osmolarity was adjusted to 100 mosM with NaCl-phosphate buffer and the ghosts were separated on a centrifuge, resuspended again in isotonic NaCl-phosphate buffer containing the same protein fraction, left for 1 h, centrifuged and used for the experiments. The uptake of ^{14}C-labelled D-glucose was conducted at 30°C and was terminated at appropriate times by transferring 0.5 ml samples into an equal volume of a hypertonic medium containing 2 mM $HgCl_2$ [9], the final osmolarity being 300 mosM. The efflux of D-glucose from ghosts loaded for 45 min, rinsed with ice-cold medium and transferred to a sugar-free buffer at 30°C was determined either by measuring the radioactivity remaining in the ghosts after terminating the transport as described above, or by measuring the radioactivity which escaped into the incubation medium. The samples were treated with barium hydroxide and zinc sulphate and the activity in the supernatant was assayed using the toluene scintillation solution on a Nuclear Chicago Mark I counter.

Influx measurements. The effect of proteins on sugar uptake was studied with D-glucose, D-galactose and D-xylose; L-glucose and lactose were used for assaying the extent of the nonspecific uptake. The concentration of the sugars in the medium was 20 mM, the radioactivity of the solution being 20 kBq/ml. The amount of dry solids from the ghosts containing the isolated proteins was only slightly higher (by about 5%) than that of control ghosts. The results obtained with D-glucose are shown in Fig. 1; the uptake of D-galactose and D-xylose was found to be quite analogous. It may be seen that the uptake of D-glucose is highly significantly increased in the ghosts containing proteins (according to Student's *t*-test). Phloretin enclosed in ghosts with proteins inhibited the uptake of D-glucose in a similar manner as described previously [4]. This contrasts with the finding of Kasahara and Hinkle [1, 2] who, with their purified transport protein incorporated in liposomes, did not observe inhibition by phloretin. The low inflow of L-glucose and lactose was not enhanced in ghosts containing proteins. When the proteins were added to the external medium only, no effect on D-glucose uptake was found. In some experiments the proteins were sonicated (using Soniprobe, Dawe Instruments, London) prior to their enclosure in ghosts and the D-glucose uptake measurements; after 20 s of sonication the proteins were still able to increase the inflow to some degree but after 3 min their activity disappeared.

Efflux measurements. The radioactivity of D-glucose remaining in ghosts (preincubated for 45 min with 20 mM glucose) after 1 and 4 min of incubation in glucose-free medium is shown in Fig. 2. It is seen that the presence of the proteins reduces significantly the residual radioactivity in ghosts. The same phenomenon was observed by measuring the radioactivity increase in the external medium, as shown in Fig. 3. The initial rate of efflux was determined

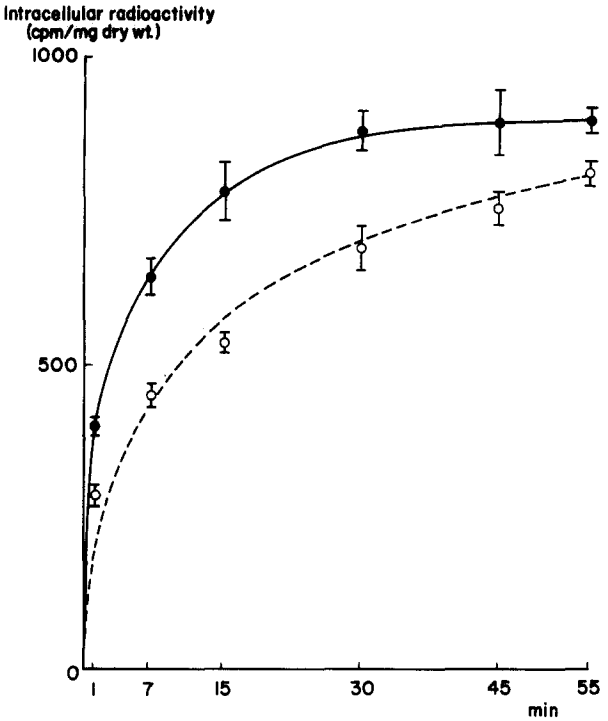

Fig. 1. Influx of D-glucose into ghosts with enclosed proteins and into control ghosts. Final glucose concentration was 20 mM, radioactivity 18 kBq/ml. Suspension contained 9.1 to 9.3 mg dry solids/ml. Empty circles, controls; full circles, ghosts with proteins.

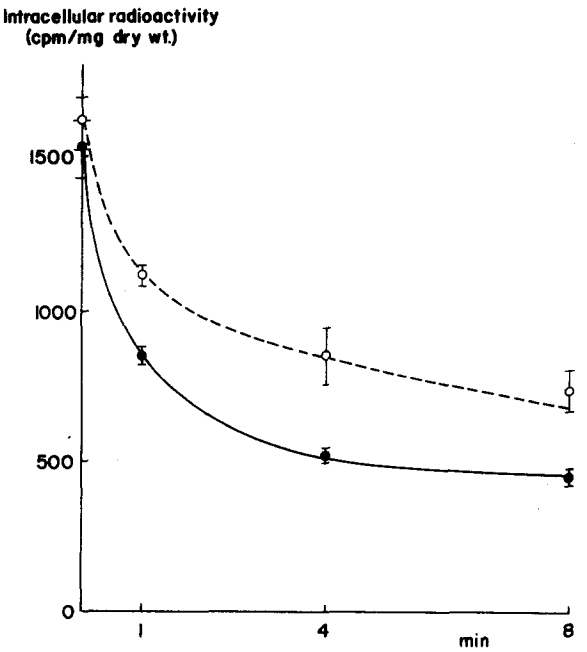

Fig. 2. Efflux of D-glucose from control ghosts and ghosts with enclosed proteins, measured as the radioactivity remaining in ghosts. The ghosts were equilibrated with 20 mM [^{14}C]glucose (40 kBq/ml) prior to the measurement. 10.2—10.3 mg dry solids/ml. Empty circles, controls; full circles, ghosts with proteins.

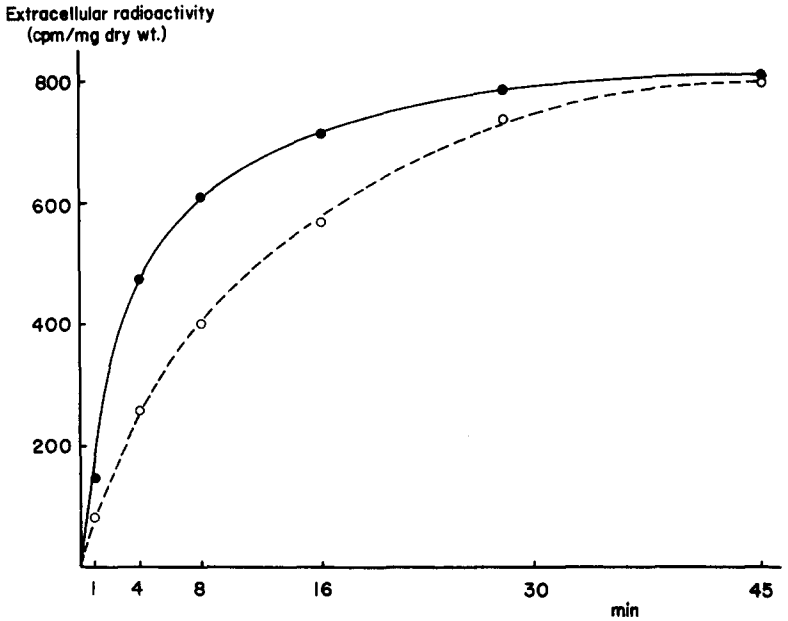

Fig. 3. Efflux of D-glucose from control ghosts and ghosts with enclosed proteins, measured as the radioactivity escaping into the incubation medium. The ghosts were equilibrated with 20 mM [^{14}C]-glucose (40 kBq/ml) prior to the measurement. 11.0—11.3 mg dry solids/ml. Empty circles, controls; full circles, ghosts with proteins.

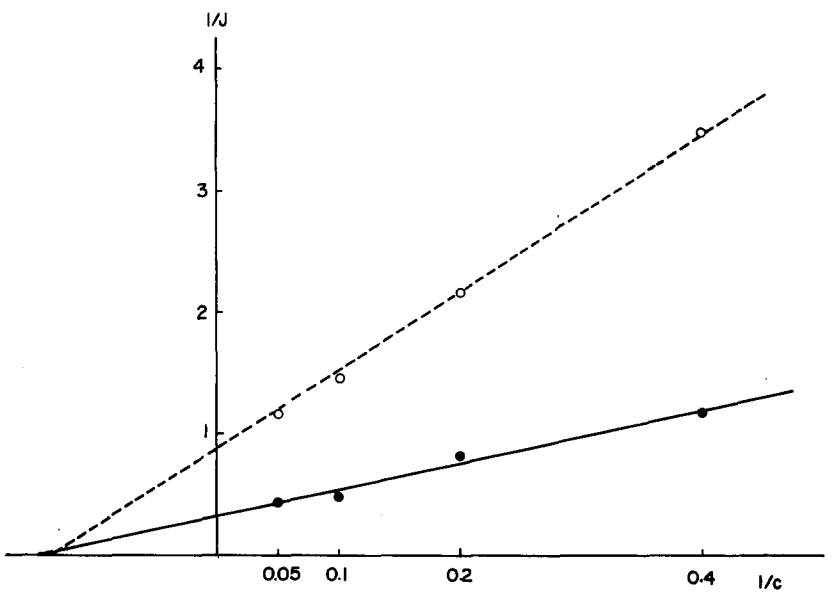

Fig. 4. The Lineweaver-Burk plot of the initial rate of efflux of D-glucose determined with control ghosts (empty circles) and ghosts with proteins (full circles).

with ghosts equilibrated for 45 min with 2.5, 5, 10 and 20 mM D-glucose. The Lineweaver-Burk plot of these data (Fig. 4) gave straight lines indicating that the K_m in control ghosts and ghosts containing the protein fraction is in about the same range (6.2—8.8 mM) as in intact erythrocytes (cf. ref. 4). On the

other hand, the J_{max} for protein-containing ghosts was close to that for intact erythrocytes (all values in the range of 2.9—3.2 μmol/min per g dry solids), whereas in control ghosts lower values were obtained (1.6—2.0 μmol/min per g dry solid). With both the ghosts and the intact erythrocytes the above values were referred to the dry solids of membranes as established by drying at 95°C and after taking corrections for the salt content.

To summarize, the data strongly suggest that the protein fraction prepared as described above is indeed involved in the permeation of certain sugars across the erythrocyte membrane, rather than in, e.g., the binding of these substances inside the ghosts.

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