STRUCTURAL CHANGE OF HUMAN RED CELL MEMBRANES IN THE GLUCOSE-PRELOADED STATE

G. ZIMMER and L. LACKO

Institut für vegetative Physiologie, Chemisch-Physiologisches Institut der Universität, 6 Frankfurt a.M.-Süd, Ludwig-Rehn-Strasse 14, Germany.

Received 13 December 1970

1. Introduction

It was found previously, that changes of the pH of the incubation medium do not seriously influence the uptake of glucose into the non-preloaded red cells [1]. On the other hand, preloading with glucose has a remarkable effect on glucose or galactose [2] transport into erythrocytes.

In the present paper, it is reported that, in glucose-preloaded red cells, there is similarly a distinct change in the glucose transport rate at different pH values, a maximum transport rate is found at pH 7.5, which decreases towards higher and lower pH values to reach a lowest value near pH 10 and pH 4.5.

Studies on erythrocyte membranes on the binding of ^{14}C-glucose in glucose-preloaded and non-preloaded states revealed differences. A similar binding pattern to that in preloaded red cell membranes was found without preloading at pH 7.5 in phosphate buffer. Furthermore, the ANS* and light scattering responses of sonicated erythrocyte membranes of glucose-preloaded and non-preloaded states disclosed differences, which are interpreted as a structural change of the red cell membrane in the preloaded state.

2. Materials and methods

2.1. *Experiments with intact cells*

See legend to fig. 1.

2.2. *Experiments with erythrocyte membranes*

We used recently outdated blood from the bank or concentrated erythrocytes. The erythrocytes were lysed with 10 vol. of H_2O/NH_3 pH 8 and subsequently washed at 5° by the method of Dodge et al. [3] with 10 mM phosphate buffer pH 7.5, so that the water soluble proteins of the membranes [4] were discarded during the procedure. Afterwards the membranes were lyophilized.

^{14}C-D(+) Glucose (specific activity: 2.9 mCi/mmole) was purchased from the Radiochemical Centre, Amersham. Sodium dodecylsulfate cryst. puriss. and ANS puriss. were obtained from Serva Heidelberg. Aquacide was purchased from Calbiochem, Lucerne.

2.3. *Incubation of non-preloaded membranes in dodecylsulfate and phosphate buffer with 0.07 mM ^{14}C-glucose*

20 mg of the membrane preparation was dissolved with 1 ml of 1% dodecylsulfate within 30 min. The pH was adjusted to the required value with 2.5 ml of NaH_2PO_4/Na_2HPO_4 (each 0.154 M). Incubation was carried out for 30 min with 70 μM ^{14}C-glucose at 23°. Immediately afterwards, gel filtration on Sephadex G-100 was performed.

2.4. *Incubation of glucose-preloaded and non-preloaded membranes respectively with 6.3 mM ^{14}C-glucose*

20 mg of membrane preparation was incubated in 2 ml 0.9% NaCl with 1.5% of cold glucose (preloaded membranes) or in 2 ml 0.9% NaCl (non-preloaded) for 30 min at 23° with stirring. The membranes were then centrifuged (Servall RC 2b) for 10 min at

* 1-anilino-8-naphthalene sulfonate

15,000 *g* at 5°. The supernatants were carefully decanted and the residues dispersed equally at the wall of the centrifuge tube. The membranes were then suspended in 2 ml of 6.3 mM ^{14}C-glucose for 20 sec, 36 ml of chilled (5°) fixation medium [5] (2 mM $HgCl_2$, 310 mM NaCl, 125 mM KI) added, and the suspension was stirred vigorously. The membranes were centrifuged (rotor 30) for 10 min at 20,000 *g*, the supernatants poured off and the pellets dissolved in 3 ml of 1% dodecylsulfate. Gel filtration on Sephadex G-100 was then performed.

2.5. *Sephadex G-100 gel filtration*

A column of Sephadex G-100 (450 × 12 mm) was equilibrated with 0.01 M K_2HPO_4, 0.5 M NaCl pH 8.4. The dissolved membranes were layered onto the column and gel filtration was carried out at 23°. Equal run of the columns was achieved. Proteins in the effluent were estimated continuously by an LKB Uvichord at 280 nm. Fractions of about 2 ml were collected and 0.2 ml samples were tested for radioactivity.

2.6. *Cellulose acetate electrophoresis*

The radioactive fractions after gel filtration were pooled and dialysed over night against H_2O/NH_3 pH 8–9. The fractions were concentrated with aquacide to about 0.2 ml and a few µl were applicated to a strip of cellulose acetate and run in the buffer system: 4.56 g sodium barbital, 3.22 g sodium acetate, 30 ml 0.1 N HCl, 180 g urea and 500 ml aq. bidest. pH 8.6 for 30 min at 2 mA. The strips were then cut into sections of 6 mm and the cuttings were counted in the Tricarb scintillation counter in dioxane scintillation fluid.

2.7. *ANS and light scattering experiments*

See legend to table

3. Results and discussion

As shown in fig 1, transport rates of the glucose-preloaded red cells are highly sensitive to pH changes. Since transport in non-preloaded cells is much less sensitive to pH, one can suppose that preloading causes a parallel increase in the reactivity of ionizable groups in the membrane.

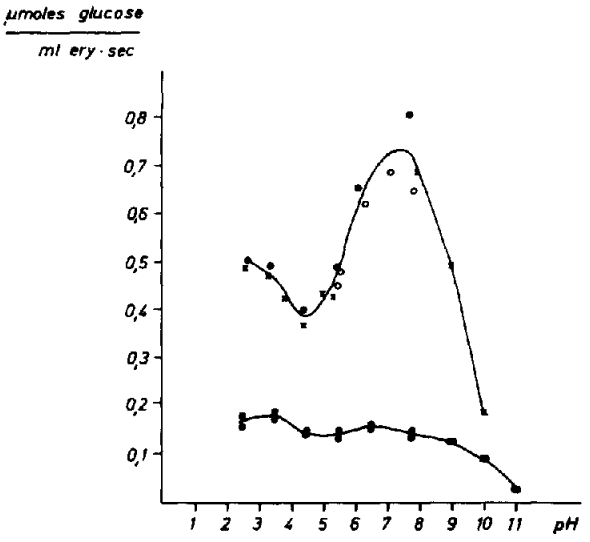


Fig. 1. About 50 ml of blood from healthy donors was drawn and pipetted into ¼ vol of a solution of ACD (11 g sodium citrate, 35 g glucose, 4 g citric acid with aq. bidest. ad 1000 ml). The erythrocytes were centrifuged at 3000 *g* and washed at 30–35° 5 times either with 4 vol. of 0.9% NaCl with 1.5% glucose (preloaded) or with 4 vol. of 0.9% NaCl (non-preloaded). 0.2 ml of the concentrated erythrocyte suspension (about 20 ml) were pipetted into 10 ml of the incubation medium (isotonic sodium phosphate buffer at the different pHs as shown, with 1.7 mM ^{14}C-glucose) at 20°. After 5 sec incubation time glucose uptake was stopped by pouring the suspension into 80 ml of 2 mM $HgCl_2$, 310 mM NaCl, 1.25 mM KI at 0°. After centrifugation at 3000 *g* at 0° and an additional wash with the stopping solution the proteins were precipitated with 0.3 N $Ba(OH)_2$ and 5% $ZnSO_4$. Glucose content/ml erythrocyte was calculated from the radioactivity in 0.2 ml of the supernatant, counted in dioxane scintillation fluid in the Tricarb. *Upper curve:* preloading experiments (3 experiments). *Lower curve:* non-preloading experiments.

In fig 2 glucose-preloaded (a) and non-preloaded (b) red cell membranes are compared after a short incubation with 6.3 mM ^{14}C-glucose. Evidently, the glucose-preloaded membranes have a much broader radioactive peak (about 3-fold in 5 experiments, calculated according to ml effluent) than the non-preloaded membranes. Similarly, in cellulose acetate electrophoresis, more radioactivity is found in the Coomassie-stained protein fractions in the preloaded, than in non-preloaded membranes (fig. 3 a and b). When non-preloaded erythrocyte membranes were

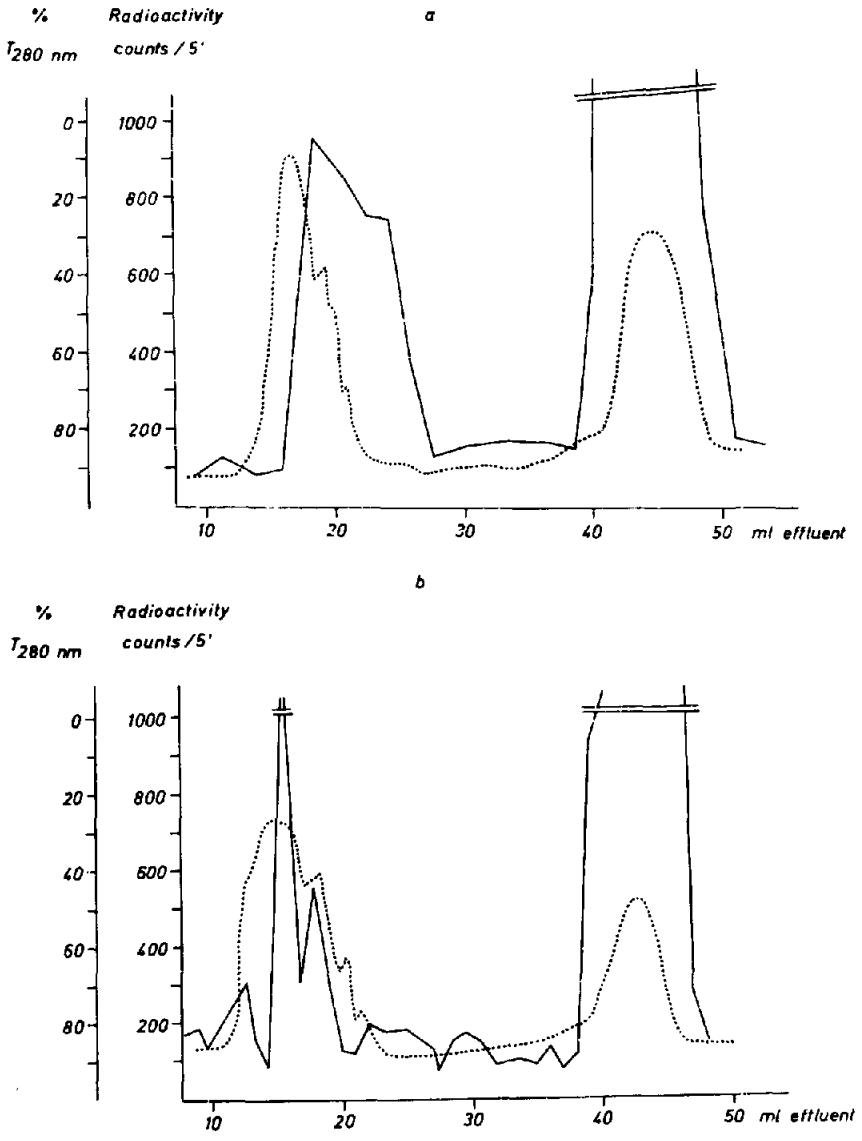


Fig. 2. Sephadex G-100 gel filtration of a) preloaded and b) non-preloaded erythrocyte membranes, incubated for 20 sec with 6.3 mM ^{14}C-glucose..... %transmission at 280 nm; ——— radioactivity. For further experimental details see Materials and methods.

incubated in phosphate buffer at pH 7.5 with 0.07 mM ^{14}C-glucose for 30 min, the electrophoretic pattern was similar to that of the glucose-preloaded membranes (fig. 3c and a). Therefore, the two additive effects on glucose uptake in intact erythrocytes (preloading, pH 7.5) seem to be reflected in these binding studies of the isolated red cell membranes. The pH 5 pattern of non-preloaded membranes in phosphate buffer resembled that of the non-preloaded membranes, especially in the case of cutting 5 (fig. 3 b), not shown.

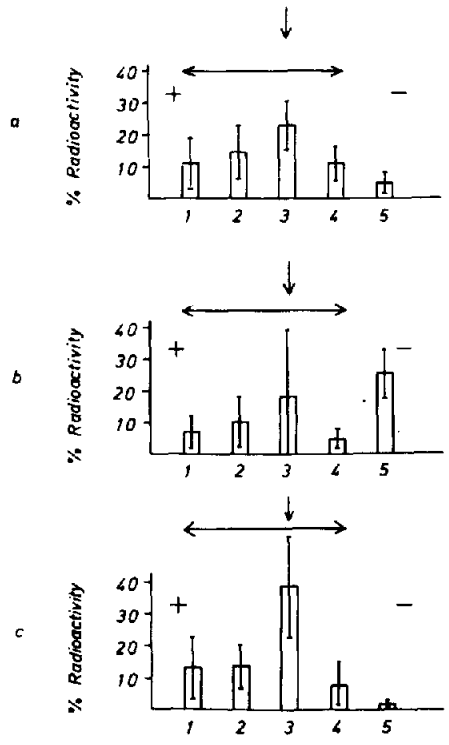
In order to substantiate these findings further, ANS fluorescence measurements [6] and 90° light scattering determinations [7] were performed in

Table 1
ANS and light scattering response of preloaded and non-preloaded erythrocyte membranes.

Conditions	preloaded/or not	ANS concn. (μM)	Protein concn. (mg/ml)	% increase when compared with contr.*
		a) Relative fluorescence increases in % of maximum response		
Glucose	preloaded	5–50	0.2–0.55	11.25 ± 2.2 **
Sucrose	non-preloaded	2.5–12	0.25–0.6	6.4 ± 1 **
		b) Light scattering increases in % of maximum response		
			(μg/ml)	
Glucose	preloaded		28–61	7.26 ± 4 **
Sucrose	non-preloaded		12–37	9.2 ± 2.75 **

* 'control' means when conditions show 'glucose preloaded' = glucose non-preloaded and under conditions 'sucrose non-preloaded' = sucrose preloaded.
** number of experiments: with glucose = 5 (means ± SD); with sucrose = 3 (means ± SD).

20 mg of the lyophilized membrane preparation (about 12–15 mg protein) was suspended in 5 ml of 0.9% NaCl with 1.5% glucose (preloaded) or with 0.9% NaCl (non-preloaded). Incubation was carried out for 120 min at 23° with stirring. The membrane suspension was then sonified with a Branson S-75 sonifier for 4 min at 0.5 A. Fluorescence measurements were performed at 380 nm excitation and 480 nm emission [6] in an Aminco-Bowman spectrofluorometer in 0.154 M NaH_2PO_4. Light scattering at 90° was determined at 420 nm in 0.9% NaCl. Protein was estimated by the method of Lowry et al. [9].



preloaded and non-preloaded membranes. It was found that light scattering and ANS-fluorescence responses run parallel, both exhibiting higher values after 120 min incubation in the glucose-preloaded membranes (see table 1). Sucrose preloading, on the other hand, revealed higher values in the non-preloaded membranes after 120 min incubation (sucrose has no effect on transport) [8].

It is concluded that there is a structural change in the erythrocyte membrane caused by preloading with glucose, probably involving a conformational

Fig. 3. Cellulose acetate electrophoresis of radioactive membrane fractions after Sephadex G-100 gel filtration. The results are expressed as the percent of radioactivity of the whole cellulose acetate strip that were found in the 6 mm cutting ± SD. Only the cuttings at or near the Coomassie-stained protein bands ⟷ are shown. Therefore, the radioactivity in the cuttings shown does not add up to 100%. The origin is indicated by a vertical arrow. (a) preloaded and (b) non-preloaded erythrocyte membranes, incubated for 20 sec with 6.3 mM ^{14}C-glucose; (c) non-preloaded erythrocyte membranes incubated for 30 min with 0.07 mM ^{14}C-glucose in phosphate buffer, pH 7.5.

change of the membrane proteins. Alternatively, the preloading procedure with glucose may preserve the red cell membrane in a pH sensitive structure, in comparison to the non-preloaded control.

Acknowledgements

We thank Professor Erich Heinz for suggestions and facilities during this work, Dr. G.K. Radda for valuable discussion of the ANS and light scattering experiments. Miss Karin Krieger and Miss Barbara Wittke provided expert technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft.

References

[1] A.K. Sen and W.F. Widdas, J. Physiol. 160 (1962) 392.
[2] L. Lacko and M. Burger, J. Biol. Chem. 238 (1963) 3478.
[3] J.T. Dodge, C. Mitchell and D.J. Hanahan, Arch. Biochem. Biophys. 100 (1963) 119.
[4] J.Th Hogeveen, R. Juliano, J. Coleman and A. Rothstein, J. Membrane Biol. 3 (1970) 156.
[5] M. Levine and W.D. Stein, Biochim. Biophys. Acta 127 (1966) 179.
[6] R.B. Freedman and G.K. Radda, FEBS Letters 3 (1969) 150.
[7] B. Chance and Ch.P. Lee, FEBS Letters 4 (1969) 181.
[8] L. Lacko and M. Burger, Biochem. J. 83 (1962) 622.
[9] O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. Biol. Chem. 193 (1951) 265.