Biochimica et Biophysica Acta, 771 (1984) 99–102
Elsevier

BBA Report

BBA 70144

AN INTERSPECIES APPROACH TO THE INVESTIGATION OF THE RED CELL MEMBRANE GLUCOSE TRANSPORTER *

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(Received August 16th, 1983)
(Revised manuscript received November 30th, 1983)

Key words: Glucose transporter; Species variation; Polyacrylamide gel electrophoresis; (Erythrocyte membrane)

Glucose transport differs in red cells of various species. The following sequence of transport velocities was found: man > newborn pig > rat, dog > cattle > pig. No correlation was found between the amount of protein in band 3 and glucose transport activity. By contrast, a very clear peak in the band 4.5 region was found for newborn pigs, whereas adult pigs did not exhibit a corresponding peak in the electrophoresis. Thus further evidence is provided by our investigations in favour of band 4.5 region for glucose transport activity in red cells.

Identification of the sugar transporter in red cell membrane has been controversial [1–10]. On the one hand, a broad 90–100 kDa protein (band 3 in the nomenclature of Fairbanks et al. [11]) has been described to be a possible locus of glucose transport [1–4,9] while other groups have claimed this band to constitute the anion transporter (for a recent review, see Ref. 12). By contrast, other papers were published indicating that the region of band 4.5 could be the site involved in glucose transport of the erythrocyte [5–8,10[. Cytochalasin B, however, the compound most interesting for discrimination of binding sites for glucose, was found to exhibit various binding sites with different affinities [13]. Furthermore it was found that band 3 was degraded by proteolysis to proteins in the molecular weight region of band 4.5 [9], while anion [14] and monosaccharide [15] transport were still functional. It was suggested that in the native erythrocyte membrane a component of band 3 is the glucose transport protein, and that during purification of the membrane degradation to the molecular weight of band 4.5 occurs, while glucose transport activity is retained [9]. In this situation, we chose a different methodological approach, which is based on experiments without isolation of the individual membrane proteins. It is known, that some species exist, which do not effectively transport monosaccharides, for example, cattle. Some other species in the course of their individual lives, lose their original transport activity. Newborn pigs, for instance do have a glucose transport system, whereas adult pigs do not. Differences in glucose transport activity may become reflected in the protein pattern of the different membranes separated by SDS gel electrophoresis, and thus allow an insight into the problem.

Fresh blood specimens were obtained and immediately hemolysed in 15 and 10 mM phosphate buffer (pH 7.4) [16]. Washing of the red cells was carried out essentially as described [16]. A final wash was carried out in distilled water, thereafter

* Throughout the paper, the expressions 'band 3' and 'band 4.5' stand for 'the equivalent of human band 3 or band 4.5', respectively.

TABLE I

VELOCITY OF GLUCOSE UPTAKE

0.15 ml of red cells were pipetted into 10 ml of incubation medium (isotonic sodium phosphate buffer, pH 7.4) containing 0.05 ml [^{14}C]glucose (spec. act. 3.1 mCi/mol). The extracellular concentrations of glucose were 6.4 mM [^{12}C]glucose and 0.032 mM [^{14}C]glucose. After incubation for different times the suspensions were poured into 80 ml of fixation medium (310 mM NaCl/2 mM $HgCl_2$/1.25 mM KI), centrifuged and washed with 15 ml of fixation medium at 0°C. Subsequently the red cells were hemolyzed with 6 ml of distilled water. Then 0.5 ml of 0.3 M $Ba(OH)_2$ was added, and after 10 min 0.5 ml of 0.3 M $ZnSO_4$. After vigorous shaking and filtration 200 μl of the filtrate were counted for radioactivity.

Species	Glucose content inside red cells (μmol/ml)	Temp. (°C)	nmol glucose/ml red cells; incubation time					
			10 s	30 s	60 s	3 min	5 min	1 h
Pig	0.41	37	–	–	–	–	–	2.9
	5.5	37	–	2.9	3.2	3.2	3.2	3.0
Cattle	0.06	37	–	–	–	–	–	8.3
	8.3	37	–	2.9	2.9	2.9	3.0	8.9
Dog		37						30 min
	0.3	37	–	–	–	–	2.3	2.8
	10.9	37	–	–	–	–	4.4	12.9
Rat	2.9	37	–	–	–	–	2.6	3.3
	7.9	37	–	–	–	–	7.8	10.7
Newborn	0.56	0	3.4	8.2	14.8	–	37.4	–
pig	45.00	0	14.9	44.4	74.6	–	186.7	–
Man	0.41	0	5.0	9.4	12.1	–	–	–
	7.9	0	47.7	123.4	200.0	–	–	–

the membranes were freeze-dried and stored at −80°C. Polyacrylamide slab gel electrophoresis in SDS was carried out essentially as described previously [17], except for the use of 5–10% gels. Glucose transport velocities in freshly obtained red cells were measured according to Lacko et al. [18].

In Table I, we find clearly expressed the differences in glucose transport velocity in the various species, depending on the amount of preloading the red cells with unlabeled glucose. Due to the very low transport activity in red cells of pigs, cattle, dogs and rats the temperature in experiments with these species was raised to 37°C. Conversely in the experiments with newborn pigs and man, the temperature was lowered to 0°C; at 37°C uptake of glucose is diminished by its instantaneous exit. Therefore the glucose uptake velocity cannot be estimated within 10s at 37°C. It is evident that the only two species which do effectively transport glucose after preloading is newborn pig and man. The obtained kinetics revealed that the following sequence exists for glucose transport velocities: man > newborn pig > rat, dog > cattle > pig. In the latter two species, velocity of

TABLE II

QUANTITATIVE ANALYSIS OF THE ELECTROPHORESIS

Percentages of band 3 compared with band 4.5 region after SDS gel electrophoresis (densitometric measurement with a 'Quick Scan' (Desaga, Heidelberg).

Species	Percentage of all membrane proteins	
	Band 3	Band 4.5
Pig	34.3	3.0
Cattle	25.3	4.9
Dog	5.1	4.8
Rat	3.9	12.6 [a]
Newborn pig	27.5	9.5
Man	29.6	7.0

[a] Probably also containing protein not belonging to the 'original' band 4.5 region.

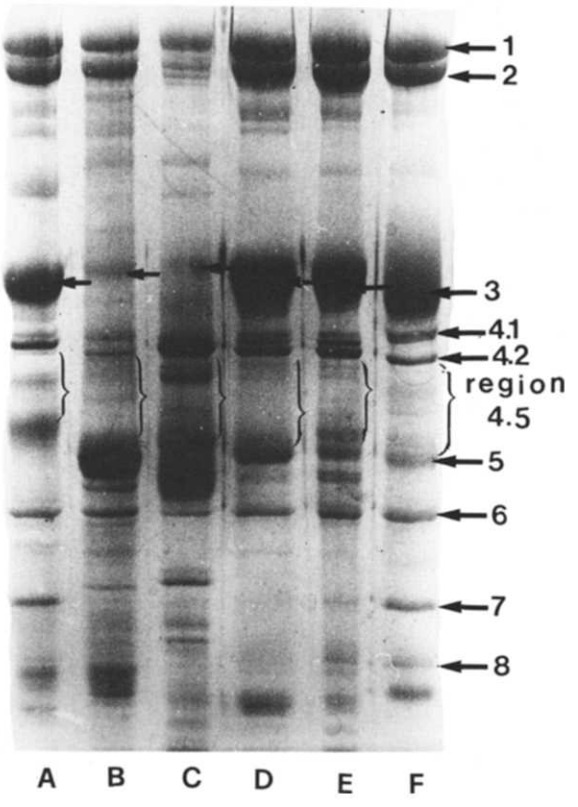

Fig. 1. Red cell membranes were solubilized by the method of Fairbanks et al. [11] in 1% SDS/7.5% sucrose/1 mM EDTA/40 mM dithiothreitol/10 mM Tris-HCl (pH 8) at 25°C and for 3 h. The gradient SDS gel electrophoresis was carried out with 5–10% gel. Electrode buffer: 50 mM Tris/0.38 mM glycine/0.1% SDS/2 mM EDTA (pH 8.8). About 100 μg of protein was applied to the gels. A, cattle; B, dog; C, rat; D, adult pig; E, newborn pig; F, man. The band numbers presented are those according to Fairbanks et al. [11].

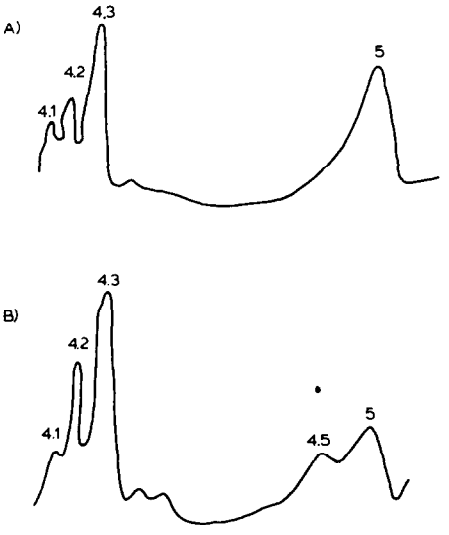

Fig. 2. Densitometric determination of proteins in the region of band 4.5. (A) Adult pig; (B) newborn pig.

glucose uptake is not enhanced by preloading the cells with a higher amount of unlabeled glucose. This indicates either non existence of carrier mediated transport or (conventionally) that the loaded carrier does not move faster through the membrane than the unloaded one.

Electrophoretic results on membrane proteins are shown in Fig. 1. Most prominent changes are seen in the band 3 region. A quantitative analysis of the electrophoresis is shown for the band 3 and band 4.5 region in Table II. Evidently, no correlation exists with the amount of protein found in band 3 and glucose transport velocity. A correlation, however, is found for the band 4.5 region. All band intensities in this region for non-transporting species is below 5% (except for rats, see Table II). By contrast, in newborn pigs and man the value for band 4.5 is 9.5% and 7%, respectively. The densitometric trace for adult and newborn pigs further underlines the striking difference between the band 4.5 region in these differently aged animals (Fig. 2), whereas the amount of band 3 proteins is similar.

Thus, independent physiological evidence in favour of band 4.5 region in glucose transport activity is obtained by our investigations. It should be noted, moreover, that the probability that band 4.5 represents a degradation product of band 3 protein [9] has been very much reduced recently, since, while this study was finished, differences were found between peptide maps of bands 3 and 4.5 [10]. Also, the N-terminus lysine has been found in band 4.5, while the N-terminus of band 3 was reported to be blocked [19]. It is remarkable that the position of the band in the 4.5 region (Fig. 2B) completely corresponds with the position of the peak of the iodinated transporter fraction found by Sase et al. [10] (Fig. 5 of their paper).

We thank Mrs. Luise Mainka, Erika Krüger and Barbara Wittke for expert technical assistance.

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