GLUTATHIONE XI. SPECIES VARIATION OF MEMBRANE DIFFUSION COEFFICIENTS DERIVED FROM INTRACELLULAR THIOL OXIDATION WITH DIP HOMOLOGS

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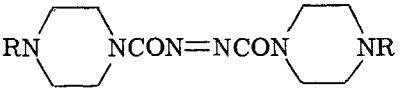
The membrane diffusion coefficients of DIP homologs (diazenedicarboxylic acid bis (N'-alkylpiperazides, e.g., N'-alkyl = ethyl) (I) vary substantially in red cells of different species. The coefficients range from 2.0 to 23.0 $cm^2/sec \times 10^9$ at 1°C (sheep r.b.c., chimpanzee r.b.c.) and the increase is correlated with increasing fluidity of the membrane, as indicated by a "fluidity factor" based on the double bond content of the fatty acids of the membrane. High variability of the coefficient in rabbits might be related to cholesterol content.

We have described a new method for the determination of membrane diffusion coefficients based on the initial rate of oxidation of intracellular glutathione(GSH) by homologs of diazenedicarboxylic acid bis(N'-alkylpiperazide) (DIP, I) (1). A logical extension of the measurements for human red blood cells was to red blood cells of other species, especially in view of the large variation in glycerol permeability reported by Wessels and Veerkamp for different mammalian species by the method of osmotic lysis (2). Relatively little systematic work has been carried out on the comparative permeability of red blood cells. The comparative permeability of amides in human and canine red blood cells was studied by Sha'afi, Gary-Bobo and Solomon (3). Sha'afi and Gary-Bobo cited very old qualitative work on the permeability of r.b.c. of fishes, birds, reptiles and mammals to different hydrophilic solutes (4a); more detailed work on

such permeability has been reported by Hays and coworkers (4b).

We now report the application of the GSH oxidation method to the measurement of the membrane diffusion coefficients of nine mammalian species. Many of the coefficients were measured with more than one DIP homolog, but we have not emphasized this aspect of the work. Naccache and Sha'afi (5) have looked at the variation in the permeability of hydrophilic solutes as a function of the structure of the solute, using human red blood cells.



I ((DIP) R= CH_3)

Homologs: EDIP, R= CH_3CH_2 iPrDIP, R= $(CH_3)_2CH$ PrDIP, R= $CH_3CH_2CH_2$

Materials and Methods

DIP derivatives were available from our previous work (1). Additions of DIP derivatives, GSH analyses and diffusion rate calculations were carried out as previously described (1). Blood was freshly drawn from animals of the Animal Institute of the School of Medicine, either in Ramat-Aviv or in Abu Kabir, except for the camel blood which was obtained by Doctor Kariv in Be'ersheva. Blood was protected against coagulation by heparin. Red cells were washed and suspended in NaCl- phosphate buffer, pH 7.3.

Results and Discussion

The membrane diffusion coefficients for the red cells of sheep, goat, cow, cat, rabbit, camel, dog, human and chimpanzee are listed in Table 1 for ethylDIP(I, R= CH_3CH_2-) and other DIP homologs. The coefficients for the sheep red cell are the lowest and that for the chimpanzee, the highest. In general, the results parallel those for glycerol, although the scale is smaller for DIP (factor of 11) than that for glycerol (factor of 60). The factors would possibly differ to a greater extent if the comparisons were

Table 1

Membrane Diffusion Coefficients for DIP Derivatives[a] through Erythrocyte Membranes of Different Species at 1°C.

Species		DIP homolog used[a]			
Mammal name (cell radius)[b] (species)		"True" Membrane Diffusion Coefficients[c] in $cm^2/sec \times 10^9$			
		DIP	EDIP	iPrDIP	PrDIP
Sheep (1.95 μ) (Ovis aries)		1.9	2.0	0.42	1.8
Calf (2.29 μ) (Bos taurus)			2.5		
Goat (1.66 μ) (Capra hircus)			2.7		
Rabbit (2.44 μ) (Oryctologus cuniculus)	German brown	3.0	2.9		2.4
	grey	4.1	5.5		
	white				2.3
	brown		6.6		
	brown[d]	14	20	3.7	7.6
Cat (2.39 μ) (Felis domesticus)			6.6		3.9
Camel (1.88 μ) (Camelus dromedarius)			8.4	2.3	2.7
Dog (2.51 μ) (Canis familiaris)			8.8		
Man (2.75 μ) (Homo sapiens)		12	12	2.1	5.5
Chimpanzee (2.69 μ) (Pan troglodytes)			23		

[a] DIP homolog, formula I

[b] Cell radius derived from cell volume as reported in Blood and Other Body Fluids", Fed.Amer.Soc.Exp.Biol., Washington, D.C., 1961, pp. 116-120, except for the data on the camel which is derived from ref. 11.

[c] Apparent membrane diffusion coefficient divided by the 2-octanol/water partition coefficient as explained in ref. 1.

[d] A single rabbit of uncertain parentage. Results shown were repeated a few times with this particular individual rabbit.

made under the same conditions. The glycerol measurements were made at 37°C with the membranes in a largely fluid state and the DIP measurements were made at 1°C, a temperature at which there is certainly considerable order in the lipid.

Although Wessels and Veerkamp state that no clear correlation could be found between the glycerol permeability and the lipid composition of the membrane (on which they carried out an extensive series of analyses), we believe that a relationship can be shown by deriving (from their data) a "fluidity factor". The "fluidity factor" is defined as the moles of unsaturated methyl ester per mole of total methyl estersof membrane fatty acids. The "FF" may be readily calculated from lipid analyses, and must not be construed to represent anything more than the potential disorder in the membrane. Phase transitions, lateral phase separations, cholesterol content and the contribution of the protein are ignored but not forgotten.

The variation in the size of the membrane diffusion coefficient at 1°C for different species and its parallelism to the "fluidity factor" suggest a substantial difference in the number of permeable domains present in the membranes of the red cells. The high temperature coefficient for the membrane diffusion process in human red cell membranes over the temperature range 1-17°C (results to be published) is in accord with a cooperative effect. One might expect that the diffusion itself in a constant medium would not exhibit a high temperature coefficient, so that the great increase in the rate of diffusion with temperature must be due to the creation of pathways for diffusion, i.e., permeable domains, presumably created through lateral phase separation.

Wessels and Veerkamp noted an unusually large variation in permeabilities measured for rabbit red cells and we have also noted a similar phenomenon. Wessels and Veerkamp reported that no variation in fatty acid composition could be responsible for the variation. It may be noted, however, that rabbit r.b.c. have the highest cholesterol/lipid ratio of the

species described by Wessels and Veerkamp if one counts glycolipid with phospholipid. It is tempting to suggest that an unusually high permeability might be due to an unusual (low) cholesterol content.

Harvey and Portman (6) found a wide range of hemolysis times for 23 different primate species and no variation in cholesterol/phospholipid ratio. Unfortunately, they did not report the lipid analyses which they made and it was thus not possible to estimate an "FF" for the cells they used.

It is, of course, well known that cholesterol decreases the permeability of membranes (7,8). Masiak and LeFevre (9) have demonstrated that glucose transport is inhibited by excess cholesterol, increased by a small partial removal of cholesterol and inhibited by a large partial removal of cholesterol from the human red blood cell membrane.

Membrane diffusion coefficients in human r.b.c. as a function of cholesterol composition and physiological state might provide an interesting index for prognosis and treatment. Small differences in membrane permeability might not show up unless searched for carefully. Insofar as these small changes in membrane permeability are related to other membrane properties (transport, protein motion, enzymatic activity, response to hormones), we might expect the coefficients to reflect the general functional capacity of the membrane, as for example, in the inhibition of glucose efflux by hashish components (10).

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