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A spin label study on fluidization of human red cell membrane by esters of p-hydroxybenzoic acid: structure-functional aspects on membrane glucose transport

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In studies of the influence of esters of *p*-hydroxybenzoic acid on glucose transport of human red cells, the hydrophobicity of these substances was found to play a primary role in inhibitory potency, judging from the octanol/water partition coefficients [1]. The interaction of these drugs with the red cell membrane is subject of the present paper. For investigation of the influence of the compounds on the membrane components, the method of spin labeling the membrane using two types of stearic acid labels was chosen.

Ortho- and *para*-hydroxybenzoic acid, and from the latter derived methyl-, ethyl-, propyl-, and butyl-esters were purchased from Sigma (München, West Germany). Spin labels No. 618, the 2-(3-carboxypropyl)-4.4-dimethyl-2-tridecyl-3-oxazolidinyloxyl, and No. 616, the 2-(14-carboxytetradecyl)-2-ethyl-4.4-dimethyl-3-oxazolidinyloxyl were purchased from Syva (Palo Alto, CA). The ESR instrument was a Bruker, B-MN, 155-45 S 16 spectrometer. Individual experiments were carried out as follows: Red cell membrane (1 mg/ml) was suspended in sodium phosphate buffer 0.01 mol/l pH 7.0 in 0.9 per cent NaCl. After centrifugation with an Eppendorf 3200 centrifuge 100 μl of buffer was added to the membrane pellet and, after homogenization, mixed vigorously with 1 μl of spin label (5 mmol/l in ethanol). The investigated substances in appropriate dilution were added 1 min before the spin label. Spectra were recorded immediately thereafter.

The fatty acid spin labels provide information on the amplitude of anisotropic molecular motion, which is measured as order parameter. Mean angular deviation of the molecular axis from the system axis is described.

In red cell membrane, the type of signal obtained with spin label 616 does show much less anisotropy compared with that of spin label 618 (see Fig. 1). Estimation of order parameters with spin label 616 thus goes to the border of the method. $T_{\parallel}$ and $T_{\perp}$ are, in case of spin label 616, only functions of the measured distances, and not the distances themselves. We anticipate, however, that the functions are monotonous and thus do not reveal much interference. Since our statements make use of the qualitative character of order parameters, and since we do not need absolute terms of these parameters, it is justified to calculate with the relative values obtained. Correlation times, which were also calculated, exhibit the same tendency as do order parameters. These values are, however, not shown.

The distances measured were used in the way described by Gaffney *et al.* [2] to obtain the order parameter S

$$S = \frac{T'_{\parallel/2} - (T'_{\perp/2} + C)}{T'_{\parallel/2} + 2(T'_{\perp/2} + C)} \cdot 1.723,$$

$$C = 1.4 - 0.053 \cdot (T'_{\parallel/2} - T'_{\perp/2}),$$

$T'_{\parallel}$ = separation of outer extrema in spectra; $T'_{\perp}$ = separation of inner extrema; C = correction factor.

The order parameter S may be changed by addition of drugs to the membrane suspension [3]. A value of 1 for the order parameter means negligible motion, at 0 mobility of spin label molecules is unhindered. The spin labels used in this study bear their nitroxides either at the polar (618) or apolar (616) end of the stearic acid molecule. In this way, interaction of investigated compounds with the different parts of the membrane can be judged by order parameter changes.

Preparation of red cell membrane was carried out according to Dodge *et al.* [4] as described previously [5].

The results presented in Tables 1 and 2 reveal that the order parameter S practically decreases with increasing hydrophobicity of the drugs (with the exception of 1 mM concentration, Table 1). The maximal decrease of order parameter obtained with spin label 618, which reports on the polar/apolar interface of the membrane, is found with the *p*-hydroxybenzoic acid butyl-ester at 5 mM concentration to reach −8 per cent of the control value (Table 1). In comparison, the more systematic decrease of order parameters for spin label 616, reporting on the hydrophobic membrane interior, attains −21 and −25% for the propyl and butyl esters at 5 mM concentration (Table 2). In presence of the drugs, the spin labels thus report a quantitatively considerably increased change in the apolar membrane phase as compared to the membrane interface. In Fig. 1, order parameters for spin label 616 have been plotted against the partition coefficients (P) of the compounds. It can be seen that (though there is a correlation between P and order parameter S) P is not - in contrast to K_i values,

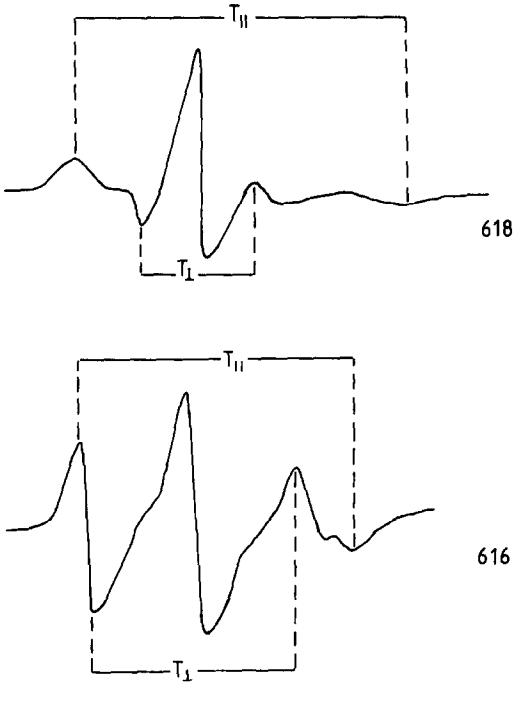

Fig. 1. (a) Type of signal obtained with spin label 618. (b) Type of signal obtained with spin label 616.

Table 1. Change of order parameters of spin label 618 in red cell membrane by *para*-hydroxybenzoic acid and by esters derived therefrom

	0	1	2	3	4	5	Concentration of compound mmole/l
Control	(21)* 0.744 ±0.008†						Per cent change control compared with last column
phb		(2)‡ 0.718	(4) 0.723 ±0.008	(2)‡ 0.742	(4) 0.696 ±0.005	(3) 0.719 ±0.006	−3
phb-methyl		(6) 0.714 ±0.004	(8) 0.754 ±0.005	(4) 0.734 ±0.005	(7) 0.755 ±0.007	(5) 0.708 ±0.005	−5
phb-ethyl		(6) 0.725 ±0.003	(7) 0.714 ±0.004	(7) 0.718 ±0.003	(8) 0.719 ±0.009	(7) 0.707 ±0.008	−5
phb-propyl		(1) 0.720‡	(4) 0.711 ±0.001	(4) 0.729 ±0.001	(6) 0.693 ±0.007	(2) 0.690	−7
phb-butyl		(3) 0.720 ±0.006	(5) 0.706 ±0.0007	(4) 0.689 ±0.01	(6) 0.697 ±0.004	(5) 0.687 ±0.009	−8

* Numbers of experiments in parentheses.
† Values from single scans of different samples.
‡ P indeterminable; P of all other values <0.001 as compared to control.

Fig. 2 — directly proportional to order parameter S. The hydrophobicity as expressed by the partition coefficients (P) decisively influences the order parameter S. Considering the results of our previous paper [1] it can be concluded that both (P and S) are important for the K_i values (affinity of the inhibitors to the glucose transporter).

The more polar *p*-hydroxybenzoic acid perturbs the membrane in another way as compared to the esters of our series (Table 2). Small concentrations increase, while only higher concentrations decrease the order parameter S of spin label 616. This biphasic behaviour of *p*-hydroxybenzoic acid is no longer observed beginning with the methyl ester,

Table 2. Change of order parameters of spin label 616 in red cell membrane by *para*-(*ortho*-)hydroxybenzoic acid and by esters derived therefrom

	0	1	2	3	4	5	Concentration of compound mmole/l
Control	(15)* 0.270 ±0.005†						Per cent change control compared with last column
phb		(6) 0.281 ±0.004	(4) 0.275 ±0.002	(7) 0.271 ±0.005	(4) 0.267‡ ±0.004	(5) 0.254 ±0.008	−6
phb-methyl		(6) 0.268‡ ±0.003	(4) 0.266‡ ±0.007	(7) 0.266‡ ±0.01	(4) 0.266‡ ±0.004	(6) 0.266 ±0.004	−1,5
phb-ethyl		(5) 0.262 ±0.002	(4) 0.257 ±0.007	(5) 0.244 ±0.004	(5) 0.234 ±0.002	(7) 0.233 ±0.005	−11
phb-propyl		(6) 0.260‡ ±0.01	(4) 0.248 ±0.009	(5) 0.238 ±0.008	(7) 0.224 ±0.009	(12) 0.214 ±0.009	−21
phb-butyl		(3) 0.259 ±0.003	(3) 0.239 ±0.003	(3) 0.225 ±0.002	(6) 0.219 ±0.005	(7) 0.203 ±0.01	−25
ortho-hb		(3) 0.271‡ ±0.006	(4) 0.268‡ ±0.006	(3) 0.265‡ ±0.005	(3) 0.263 ±0.003	(4) 0.249‡ ±0.002	−8

* Numbers of experiments in parentheses.
† Values from single scans of different samples.
‡ P n.s.; P of all other values <0.025 or better as compared to control.

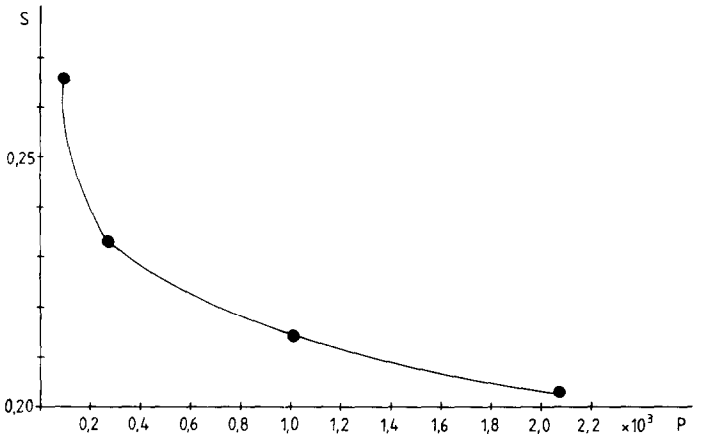

Fig. 2. Plot of K_i values in glucose transport *vs order parameters S (spin label 616) at 5 mM concentrations of p-hydroxybenzoic acid esters: * from reference [1].

which produces no significant changes of the parameter S. With longer hydrophobic chains, all esters significantly decrease the order parameter. Increase in order will prevent the inhibitory molecules of p-hydroxybenzoic acid to reach the hydrophobic binding site of the transporter, resulting in an exceptionally high K_i value [1].

Polarity of non-esterified hydroxybenzoic acid is furthermore dependent on the position of the hydroxyl group. In *ortho*-position the order parameter was lower at all concentrations as compared to the *para*-compound (Table 2). This also agrees with the K_i values previously found [1].

The above findings are an indication of increasing fluidization of the membrane lipid alkyl chains by increasing number of CH_2 residues of esterified compounds. Perturbation of the lipid annulus around the integral membrane proteins [6] may thereby change protein structure. Thus, fluidization of membrane lipids by esters of p-hydroxybenzoic acid and inhibition of glucose transport in human red cells are correlated phenomena.

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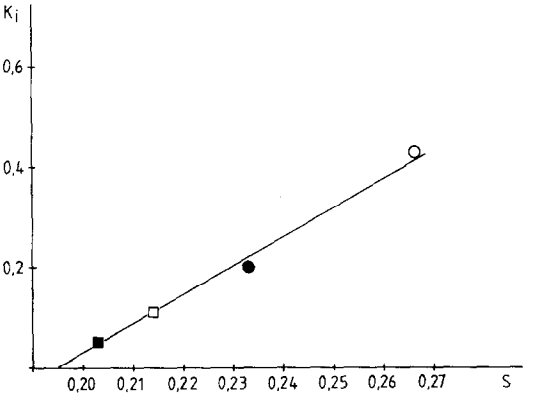

Fig. 3. Plot of K_i values in glucose transport *vs order parameters S (spin label 616) at 5 mM concentrations of p-hydroxybenzoic acid esters: * from reference [1].
○ Methyl; ● ethyl; □ propyl; ■ butyl.

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