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Relation between adsorption at an oil/water interface and membrane permeability

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SUMMARY

Lipid permeation through a cell membrane involves adsorption onto the membrane, diffusion through the membrane and desorption into the interior solution. In order to determine the rate-limiting step, we have compared lipid permeability coefficients of human red cells with the free energies of adsorption, $\Delta G_{O/W}$, of the same molecule, from water onto an oil/water interface. Sha'afi, Gary-Bobo and Solomon (*J. Gen. Physiol.*, 58 (1971) 238) had measured the permeability coefficients of human red cells to a series of homologous lipophilic amides and found a smooth increase from propionamide through butyramide to valeramide, as expected from lipid solubility. However, the permeability coefficients of the two branched isomers, isobutyramide and isovaleramide were each about 1/3 of that of the straight chain isomer. We have measured $\Delta G_{O/W}$ for the branched and straight chain isomers at a decane/water interface, and found only a very small difference between the members of each isomer pair. The differences in red cell permeability coefficients are therefore attributed to different rates of diffusion within the membrane as a result of the increased steric hindrance in the branched chains. Thus diffusion within the membrane plays an important role as a determinant of membrane permeability.

Permeation of lipophilic solutes through a cell membrane requires at least three steps: adsorption from the aqueous medium onto the membrane, diffusion through the membrane, and desorption into the internal aqueous medium. The question arises as to which of these is the rate-limiting process. It seemed likely that an answer to this question might be found by comparing the permeability coefficients of a series of homologous lipophilic molecules with their free energies of adsorption, $\Delta G_{O/W}$, from water to oil/

water interface. Since Sha'afi *et al.*[1] had measured red cell membrane permeability coefficients for a series of lipophilic amides including propionamide, butyramide, isobutyramide, valeramide and isovaleramide, $\Delta G_{O/W}$ for these solutes was measured at a decane/water interface.

Surface and interfacial tensions were measured by the drop-volume method[2] employing the Harkins and Brown[3] correction curve. A stainless steel tip was used together with a micrometer syringe and a micrometer screw. The effective tip radius (0.252 cm) was determined using the known interfacial tensions for the *n*-decane/water and air/water interfaces. The entire apparatus was contained in a constant-temperature chamber which was maintained at 25 ± 0.2°C.

Decane (Eastman 'Practical Grade') was purified by passing through an alumina column until it gave a constant interfacial tension against water. Butyramide and isobutyramide were obtained from Fluka ('Purum Grade'). Valeramide and isovaleramide (both obtained from Eastman) were purified by recrystallization from ethanol–ether. The paraffin wax used for sealing the tip onto the syringe was purified by dissolving it in xylene and passing the solution down an alumina column. Part of the xylene was then evaporated off and the wax was recovered by filtration and washed with ether. All water used in the experiments was glass distilled twice. All glassware was treated with sodium dichromate-sulfuric acid cleaning solution and thoroughly rinsed with twice distilled water before use.

Nine sets of measurements were made in the concentration range 0.001 to 0.1 M, one on propionamide and two each on the other homologous amides. It was first necessary to determine A_o, the close packed area of the adsorbed molecule at the decane/water interface. This may be done by making use of the equation of state:

$$\pi (A - A_o) = kT \tag{1}$$

in which $\pi = (\gamma_o - \gamma)$. γ is the interfacial tension in the presence of solute and γ_o in the absence of solute, k is the Boltzmann constant, A is the area per molecule of adsorbed solute and T is temperature. Eqn. 1 requires a plot of $1/\pi$ against A to have a slope of $1/kT$, a requirement which was satisfied in four of the experiments. The value of A_o in these four experiments was found to be 24 Å^2, which may be compared with a value of 18.5 Å^2 for short-chain alcohols and 21.5 Å^2 for butyric acid as given by Haydon and Taylor[4]. The precision of our measurements was not sufficient to detect any difference in the value of A_o between the straight chains and the isomers.

$\Delta G_{O/W}$ may be determined from the adsorption isotherm[4]:

$$c_2 e^{-\Delta G_{O/W} RT} = [A_o/(A - A_o)] e^{[A_o/(A - A_o)]} \tag{2}$$

in which c_2 is the solute concentration expressed as a mole fraction and R, the gas constant. Eqn. 2 is based on ideal solute behavior in both the bulk aqueous phase and adsorbed

interfacial phase (standard states: unit mole fraction in bulk phase; $A = 2A_o$ in surface). The values* obtained for $\Delta G_{O/W}$ are given in Table I, in which the experiments in which A_o could not be determined from Eqn. 1 are denoted with a star. The average $\Delta G_{O/W}$ for

TABLE I

FREE ENERGY OF ADSORPTION FOR HOMOLOGOUS AMIDES COMPARED WITH PERMEABILITY COEFFICIENTS

Amide	$-\Delta G_{O/W}$ *(cal/mole)*	*Average* $-\Delta G_{O/W}$ *(cal/mole)*	*Isomer difference (cal/mole)*	*Permeability coefficient, ω (moles·dyne^{-1}·sec^{-1} × 10^{15})*
Propionamide	2821	2820		4.0 ± 0.5
Butyramide	3514*			
	3761*	3640		14 ± 1
Isobutyramide	3465			
	3642*	3550	90	5 ± 1
Valeramide	4480			
	4547	4510		27 ± 2
Isovaleramide	4188*			
	4442*	4320	190	7.2 ± 0.5

*See text.

increasing the chain length by one $-CH_2-$ group is −820 cal/mole in good agreement with the value of −820 cal/mole given by Haydon and Taylor[4] for a petroleum ether/water interface. The effect of branching the chain is very small since it decreases $-\Delta G_{O/W}$ by 90 cal/mole for the butyramide pair and 190 cal/mole for the valeramides, making an average of 140 cal/mole. The data of Haydon and Taylor[4] also suggest that introduction of a single branch at the end of a carbon chain has relatively little effect. Their value of $-\Delta G_{O/W}$ for isoamyl alcohol is 5000 cal/mole which does not differ from the value of 4995 cal/mole obtained by interpolation between their figures for the straight-chain butanol and hexanol.

Fig. 1 compares our data on all the amides with those of Haydon and Taylor[4] for short-chain alcohols. Extrapolation to zero chain lenght leads to a figure of −400 cal/mole as the free energy of adsorption of a $CONH_2$ group, which may be compared with the value of −800 cal/mole given by Haydon and Taylor[4] for the hydroxyl group and −1630 cal/mole for the COOH group.

The last column in Table I gives the permeability coefficients determined by Sha'afi *et al.*[1]. There is a clear correlation between the adsorption free energies and the

*The value for $\Delta G_{O/W}$ differs from the 'true' value by RT, so that $-(\Delta G_{O/W})_{true} = -\Delta G_{O/W} + RT$ as pointed out by Rich[5].

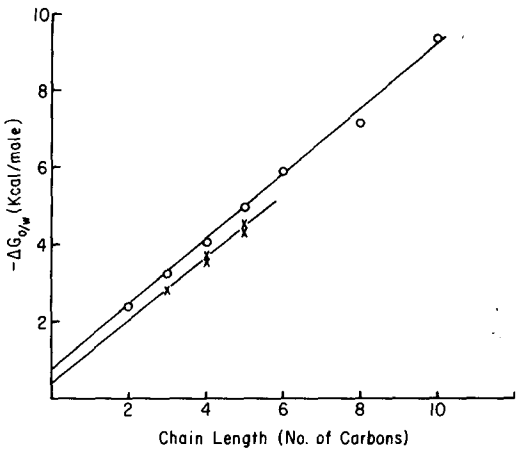

Fig. 1. Free energy of adsorption at decane/water interface. ○, data of Haydon and Taylor[4] on short-chain alcohols; x, present results.

permeability coefficients of the straight-chain amides. This is not unexpected because these permeability coefficients are directly proportional to the ether/water partition coefficient, k_{ether}, in conformity with classical expectation. Table I shows about a 3-fold difference between the permeability coefficients for each straight chain and that for its branched isomer. Sha'afi *et al.*[1] have suggested that this effect may be ascribed to steric hindrance within the membrane because the logarithm of the ratio (permeability coefficient/k_{ether}) is linearly related to the cylindrical radius of the permeating solute for these homologous amides. The present experiments were specifically designed to determine the feasibility of an alternative explanation in terms of a difference in adsorption at an oil/water interface.

The similarities between the free energies of adsorption of the two pairs of isomers suggest either that the difference in the permeability properties may not be ascribed to the process of adsorption or that the decane/water interface is not a satisfactory model for a cell membrane. For example, the mobility of the hydrocarbon chains in phospholipids might be quite different from that of decane molecules close to the interface. A similar problem has been studied by Haydon[6] who compared the surface tension of a black film made from glycerol monooleate and *n*-decane with that of the decane/water interface of the bulk phase in equilibrium with the black film. The surface tension of the black film was found to be equal to that of the bulk phase. Haydon extended the argument to black films made from decane solutions of phospholipids such as stearoyl oleyl phosphatidyl choline and concluded that, in this case also, the surface tension of film and bulk phase were essentially equal. These results indicate that surface tension measurements at decane/water interfaces are indicative of those in the far more ordered structure of black lipid films.

The comparison of the present results with permeability coefficients does not depend on the absolute magnitude of $\Delta G_{O/W}$ for the various amides, but rather on the relative magnitudes of these energies for two isomers with the same polar head group.

Thus, though the free energies of adsorption onto the red cell membrane will include contributions from the interactions with the specific phosphatides, cholesterol and protein that form the membrane, Haydon's findings make it seem unlikely that the relative free energy of adsorption of these pairs of isomers would differ appreciably from the relative free energies of adsorption at a decane/water interface, provided the membrane protein does not significantly restrict adsorption. It appears, therefore, that the observed differences in the permeability coefficients of the two pairs of isomers may indeed be attributed to different rates of diffusion within the membrane rather than to differences in adsorption from the aqueous medium onto the membrane. Such steric hindrance would fit with present views of a quasi-ordered structure in the hydrocarbon chains of phosphatide lamellae. Clearly diffusion within the membrane is an important determinant of the permeability of lipophilic molecules and plays a role comparable with that of lipid solubility.

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