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INTRINSIC PERTURBING ABILITY OF ALKANOLS IN LIPID BILAYERS

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Summary

The proportionality constant between the equipotency concentrations of a series of solutes and the fraction of a solute in the membrane phase is directly related to the solute to lipid mol ratio. Experimental measurements of partition coefficient and of several alkanol-induced effects show that the solute/lipid mol ratios for a series of alkanols are not constant at their equipotency concentrations. The deviations in the solute/lipid ratios are similar in the various systems, and these deviations seem to depend primarily upon the chain length and branching in alkanols. It is suggested that such intrinsic differences in the perturbing ability of alcohols arise from a specificity of interaction between alkanols and lipid bilayer. We have correlated partition coefficients (in *n*-octanol, in egg phosphatidylcholine liposomes, and in dipalmitoyl phosphatidylcholine liposomes) for thirteen alkanols to the equipotency concentrations for their ability to modify the order-disorder thermotropic transition in dipalmitoyl phosphatidylcholine, ability to stimulate the hydrolysis of phosphatidylcholine in a bilayer by bee venom phospholipase A_2, and for the activation of the galactoside transport system in *Escherichia coli*. Significant correlation is found between equipotency concentrations for perturbing the order-disorder transition, the activation of phospholipase A_2-catalyzed hydrolysis and the activation of galactoside transport system.

Introduction

Alkanols induce a variety of effects in lipid bilayer of biomembranes [1—6]. In such studies, the concentration dependence of the response induced by a series of alcohols is accounted for by the assumption that an equal membrane concentration of the various alcohols induces an equal response. For most studies it has also been assumed that the membrane/water partition coefficients

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of alkanols can be approximated to those of the bulk organic solvent/water partition coefficients [7]. It is also implicit in the above hypothesis that the membrane-perturbing ability of the various alcohols (or of any 'lipid-soluble' solute for that matter) is the same. A direct test of the above assumptions has not been made for the membrane systems. In this communication we wish to report observations which show that the solute/lipid mol ratio for a series of alkanols at the equipotency concentrations can be quite different, thus suggesting that the intrinsic perturbing ability of the various solutes can be quite different.

Methods and Calculations

According to the partitioning theory of drug action, the equipotency concentration (EPC) of a solute is inversely related to the fraction of the solute (F_1) incorporated into the membrane phase.

$$\text{EPC} \cdot F_1 = d \quad \text{where } F_1 = \frac{\text{EPC} - S_w}{\text{EPC}}$$

Thus, S_w is the solute concentrations in the aqueous phase. In this terminology the partition coefficient $P = F_1/(1 - F_1)$.

A molecular significance may be attributed to the proportionality constant, d, if the total concentration of lipid phase is known. If one assumes that M mol of membrane phase is dispersed in 1 l of the aqueous phase, the mol ratio of a solute to lipid (S/L) in the membrane phase is given by

$$\frac{S}{L} = \frac{\text{EPC} \cdot F_1}{M}$$

Three sets of values for partition coefficients (P_{oct}, P_{DPPC}, and P_{EL}) are presented in Table I. All these values refer to the equilibrium concentrations of the solute per g of the organic phase (or the lipid) divided by the solute concentration per g of the aqueous phase. Partition coefficient for the *n*-octanol/water system (P_{oct}) were obtained from the published literature [7]. Partition coefficients in phosphatidylcholine (P_{EL}) and dipalmitoyl phosphatidylcholine liposomes (P_{DPPC}) at 24°C were measured by determining the total (without) and the residual (with sedimented liposomes) aqueous phase concentrations of alkanols by gas chromatography (Hewlett-Packard model 5750 equipped with a flame ionization detector and a 6 × 3/16 inch glass column packed with Porapak Q obtained from Supelco, Inc.). A standard deviation of up to ± 10% is expected in the P_{DPPC} and P_{EL} values [16].

Three sets of equipotency concentration values for the alkanols are also given in the next three columns of Table I. HHW_{100}, the concentration for doubling the half-height width of the phase transition profile [8], PL_M, the concentration required for the maximal activation of the phospholipase A_2 (bee venom)-catalyzed hydrolysis [9], and TM_{20}, the concentration required for a 20% activation of the galactoside transport system in *Escherichia coli* [10], were taken from the published literature. The principles, procedure, and the limitations of the data is described in these publications. The HHW_{100} data is

TABLE I

PARTITION COEFFICIENT VALUES, EQUIPOTENCY CONCENTRATIONS AND SOLUTE/LIPID MOL RATIOS FOR ALKANOLS

Alcohol number	Alkanols	Partition coefficients			Equipotency concentrations			Solute/lipid ratios X 10^3	
		P_{oct}	P_{EL}	P_{DPPC}	HHW_{100}	PL_M***	TM_{20}	at HHW_{100}	at PL_M
1	*n*-Propan-1-ol	3.0	2.1 **	0.371 **	150	1200		40	1882
2	*n*-Butan-1-ol	8	6.13 **	1.34 **	33.4	560	90.9 *	31	2545
3	*n*-Pentan-1-ol	25.2	18.1	4.8	11.5	195	50 *	33	2560
4	*n*-Hexan-1-ol	101	50	25.2	2	46	6.66	16	1577
5	*n*-Heptan-1-ol	340	170	50.4	1.4	19	1.73	14	1840
6	*n*-Octan-1-ol	1 010	378	201	0.73	8.8	0.910	9.5	1460
7	*n*-Nonan-1-ol	3 400	1 400	1 020	2.1	5.72	0.625	29	1657
8	*n*-Decan-1-ol	10 100	4 500 **	4 400 **	2.1	5.4	0.910	30	1931
9	*n*-Undecan-1-ol	34 000	12 000 **	16 000 **	2.8	6.0		40	2298
10	*n*-Dodecan-1-ol	101 000	40 000 **	59 000 **	3.3	9.9	1.60	47	3910
11	*n*-Octan-2-ol	680	196	54	0.833	11.5		9	1233
12	*n*-Octan-3-ol	680	162	41	3.0	13.5		29	1258
13	*n*-Octan-4-ol	680	131	26	3.8	15.2		31	1198

* Values obtained by extrapolation of the activation profile since less than 20% activation was observed.

** Extrapolation values obtained from a plot of chain length vs. log *P*.

*** These measurements were made at 1 mM phospholipid for C_3—C_8 alcohols, and at 2 mM for C_9—C_{12} alcohols.

for the dipalmitoyl phosphatidylcholine liposomes. The PL_M values are the phospholipase-catalyzed hydrolysis of egg phosphatidylcholine liposomes. Some additional data was also obtained by the procedures described earlier. The statistical calculations were performed on a DEC-PDP/11 computer using a standard algorithm for linear regression analysis.

Results and Discussion

Alkanols modify a variety of biomembrane and lipid bilayer characteristics which are ultimately related to their ability to get into and to perturb the lipid bilayer. Partition coefficient is a measure of the ability of a solute to get into a medium from the aqueous phase. This would mean that if a series of alcohols could bring about an equal perturbation in a bilayer, their equipotency concentrations would be related to their membrane/water partition coefficient. In Table II we have presented results of such correlations between the three types of partition coefficients and the three sets of equipotency concentration data from Table I. The values of the slope, the intercept, the correlation coefficient, and the F ratio (Fisher's variance ratio distribution for a one tailed confidence test) obtained by a linear regression analysis on the log-log plots of the x and y values given in column 2 are presented in the columns 4—7 of Table II.

TABLE II

LINEAR REGRESSION ANALYSIS CORRELATION DATA

Row number	Alkanols (number from Table I)	Plot		Slope ± S.D.	Y intercept	Correlation coefficient	F distribution ratio (exponent)
		log x	log y				
1	1—13	P_{oct}	HHW_{100}	—0.356 ± 0.113	1.5	—0.688	9.3E — 03
2	1—6	P_{oct}	HHW_{100}	—0.906 ± 0.094	2.40 ± 0.183	—0.979	6.58E — 04
3	6—13	P_{oct}	HHW_{100}	0.128 ± 0.118	0.23 ± 0.116	+0.403	0.2
4	1—13	P_{oct}	PL_M	—0.522 ± 0.079	2.88 ± 0.24	—0.893	5.3E — 06
5	1—6	P_{oct}	PL_M	—0.864 ± 0.026	3.49 ± 0.05	—0.998	3.9E — 05
6	6—13	P_{oct}	PL_M	—0.111 ± 0.07	1.0 ± 0.69	—0.543	1.6E — 01
7	1—8	P_{oct}	TM_{20}	—0.50 ± 0.1417	1.97 ± 0.436	—0.821	1.2E — 02
8	1—6	P_{oct}	TM_{20}	—0.904 ± 0.094	2.76 ± 0.23	—0.98	6.5E — 04
9	1—13	P_{EL}	HHW_{100}	—0.322 ± 0.123	1.357 ± 0.33	—0.620	2.3E — 02
10	1—6	P_{EL}	HHW_{100}	—1.03 ± 0.10	2.36 ± 0.17	—0.981	5.2E — 04
11	1—13	P_{EL}	PL_M	—0.54 ± 0.098	2.71 ± 0.26	—0.855	1.9E — 04
12	1—6	P_{EL}	PL_M	—0.974 ± 0.045	3.44 ± 0.75	—0.995	2.67E — 05
13	6—13	P_{EL}	PL_M	—0.062 ± 0.11	1.04 ± 0.173	—0.224	0.56
14	2—10	P_{EL}	TM_{20}	—0.52 ± 0.160	+0.88 ± 0.45	—0.796	1.8E — 02
15	2—8	P_{EL}	TM_{20}	—1.017 ± 0.124	2.73 ± 0.263	—0.971	1.24E — 03
16	1—13	P_{DPPC}	HHW_{100}	—0.25 ± 0.102	1.093 ± 0.255	—0.595	3.2E — 02
	1—10	P_{DPPC}	HHW_{100}	—0.269 ± 0.106	1.24 ± 0.29	—0.667	3.5E — 02
17	1—6	P_{DPPC}	HHW_{100}	—0.867 ± 0.062	1.68 ± 0.083	—0.990	1.5E — 04
18	6—13	P_{DPPC}	HHW_{100}	0.06 ± 0.08	0.256 ± 0.12	0.283	0.5
19	1—13	P_{DPPC}	PL_M	—0.42 ± 0.086	2.28 ± 0.215	—0.827	4.9E — 04
20	1—6	P_{DPPC}	PL_M	—0.816 ± 0.03	2.79 ± 0.044	—0.997	1.5E — 05
21	1—8	P_{DPPC}	TM_{20}	—0.44 ± 0.139	1.51 ± 0.355	—0.79	1.9E — 03
22	1—6	P_{DPPC}	TM_{20}	—0.84 ± 0.116	2.033 ± 0.21	—0.964	1.9E — 02
23	1—6	HHW_{100}	PL_M	0.923 ± 0.078	1.22 ± 0.09	+0.986	2.2E — 05
24	1—13	HHW_{100}	PL_M	1.06 ± 0.17	0.809 ± 0.147	+0.882	6.7E — 05
25	1—10	HHW_{100}	PL_M	1.10 ± 0.20	0.785 ± 0.19	+0.889	5.7E — 04
26	1—8	HHW_{100}	TM_{20}	1.38 ± 0.30	—0.06 ± 0.22	+0.910	5.7E — 03

Results of a correlation of log P_{oct} with log HHW_{100}, log PL_M, and log TM_{20} are presented in the first eight rows of Table II. A poor to fair correlation (correlation coefficient 0.5—0.85 with $P > 0.0002$) is observed when on considers all the thirteen alcohols (rows 1, 4 and 7). By contrast the plots of the first six alcohols (*n*-propanol to *n*-octanol) show excellent correlation (rows 2, 5 and 8). However, the last eight alcohols show no noticeable correlations. The analysis suggests that the P_{oct} values may provide a good approximation to the membrane environment only for the short (C_3—C_8) and straight chain alcohols. The slopes of nearly —0.9 for these also suggests that the hydrophobicity of *n*-octanol may be slightly higher than the hydrophobicity of the bilayer and the membrane phases under comparison.

As shown in rows 3, 6, and 7 such correlations with partition coefficients break down both for the branched and for the long chain alcohols. To ascertain whether such a lack of correlations is due to the anisotropy of organization of the acyl chains in the bilayer, a comparison was made between the partition coefficients in the bilayer (P_{DPPC} and P_{EL}) and the HHW_{100}, PL_M, and TM_{20} values (rows 9—22). Here once again one observes excellent correlation (correlation coefficient >0.97) for the lower alcohols (<C_8) with slopes of almost —1 (rows 10, 12, 15, 17, 20 and 22). The correlation for the branched and the longer chain alcohols (>C_8) is however, still poor. Data in rows 11—13 of Table II, however, shows that the trends for the branched chain alcohols are in the right direction, that is the equipotency concentrations increase monotonically as the partition coefficients (P_{DPPC} and P_{EL}) decrease. Isotropic solvents like *n*-octanol are not expected to be sensitive to such structural features.

The correlations elaborated in rows 1—22 of Table II thus demonstrate that the equipotency concentrations correlate well with partition coefficients only for the straight chain lower (C_3—C_8) alkanols. In this regard the P_{oct} values offer a fair approximation to the actual P_{DPPC} and P_{EL} values. Significant differences between the bilayers and the isotropic bulk solvent partition coefficients may be noted both in terms of the absolute values of the partition coefficients and the incremental free energies. Such differences could not, however, account for the differences in the effects of the branched and long chain alcohols; these differences are observed only in the effects induced by the alcohols but not by their partition coefficients. We wish to suggest that such effects could be due to an intrinsically lower perturbability of the long chain alcohols. This would imply that a good relationship should exist between the equipotency concentrations of these alcohols. Indeed as shown in rows 23—26 a correlation of HHW_{100} values with PL_M and TM_{20} values gives correlation coefficients of better than 0.9 for the straight chain alcohols. The correlation for the higher and the branched chain alcohols is relatively poor. However, the data presented in Table I (columns 6 and 7, last three rows) shows a qualitative trend for the branched chain alcohols, that is, the HHW_{100} values are much more sensitive to the branching than the PL_M values even though both of these values increase. This would imply that the branched chain alcohols have intrinsically higher perturbability than the straight chain analogs.

To test both of these possibilities we calculated the alcohol to lipid mol ratios for the broadening of the phase transition profile at HHW_{100} and for the

maximal activation of the phospholipase A_2-catalyzed hydrolysis at PL_M. These values are presented in the last two columns of Table I. This data shows that for every 1000 lipid molecules in the bilayer the number of alchol molecules in the bilayer required to induce an equal effect varies over a 2-fold range for the phospholipase system and over a 4-fold range for the phase transition system. There are other significant differences between these two systems. The phospholipase A_2 acts on the lipid bilayer presumably in the interfacial region, whereas the thermotropic change arise primarily from an order-disorder transition in the C_1—C_8 region of the acyl chains [11,12]. The substrate for the phospholipase is egg lecithin (in this case), wheras the thermotropic transitions were measured on dipalmitoyl phosphatidylcholine liposomes. The phospholipase effects are those for the maximal activation, whereas the HHW_{100} concentration values correspond to an 100% increase in the half-height width (at higher concentrations the half-heights widths increase linearly till the transition peak flattened out). Thus, the absolute values of solute/lipid mol ratios for the phospholipase system is about 10 times higher than the corresponding solute/lipid ratios at HHW_{100}.

In spite of these differences some obvious trends may be noted in these two sets of solute/lipid ratios. Within each series the solute/lipid ratios are not constant, but they decrease with increasing chain length up to C_8. For the higher alcohols ($>C_9$) and solute/lipid ratio increases with the chain length. This difference is quite significant since a maximum scatter of ±20% is expected in the solute/lipid ratios. Within this homologous series there is a tendency for the odd length alcohols to have slightly higher solute/lipid ratios for either HHW_{100} or for PL_M. This could reflect a difference in the intrinsic perturbability of the odd and even alkyl chains (see ref. 8 for a detailed discussion). This effect is, however, barely above the maximum for the scatter in the data.

The solute/lipid ratio for 2-octanol in both the systems is smaller than for 1-octanol. However, this similarity is not observed for 3- and 4-octanols. The 3- and 4-isomers are more effective than 1- and 2-octanols for activating the phospholipase A_2-catalyzed hydrolysis. By contrast the 3- and 4-octanols are noticeably less effective than the other two isomers in modifying the phase transition profile. Although these differences are small, the opposite trends in the solute/lipid ratios of 1- and 2- compared with 3- and 4-octanols is probably real. The anomalous behavior of propanol is within experimental uncertainty especially since a small partition coefficient value is multiplied by a large equipotency concentration term to obtain the solute/lipid ratios.

The data on the solute/lipid ratios is thus consistent with our earlier conclusions that the intrinsic perturbing ability of the various alcohols is different. A lower perturbing ability of long chain alcohols as reflected in anomalously higher equipotency concentrations has been observed for a variety of membrane processes [4,5,8—10,12]. The origin of such a 'cut-off' effect is in all likelihood in the microheterogeneities in the bilayer phase [17]. Before we attempt to elaborate on such an explanation let us recapitulate our original hypothesis and some of the properties of the system under consideration.

According to the classical ideas, an equal mol proportion of different solutes in the membrane should induce equal response. This is an extension of Roult's law which predicts the concentration vs. response relationships for the

colligative properties of an ideal solution. In such a system, the response is a function of only the number of molecules independent of the nature of the molecules. "Ideality in a solution is defined by complete uniformity of cohesive forces" [14]. This assumption would require that a bilayer be approximated as an isotropic bulk solvent. This condition cannot be satisfied for even the simplest of a lipid bilayer system [15] let alone the biomembrane [12]. Along the thickness of a bilayer there is a gradient of polarity and motional freedom ('fluidity') of chains. Similarly, in the plane of the membrane there are clusters of lipid in different states and of different compositions. These will be interspersed by the regions of mismatch. Such microheterogeneities in the membrane phase would tend to determine the localization, oreientation, and motional freedom of a solute in the membrane phase. Alkanols which we have examined are intrinsically amphipathic. Such molecules are, therefore, expected to localize in a bilayer such that the hydroxyl group lies near the interface and the hydrocarbon chain would align parallel to the acyl chains of phospholipid. Such a localization of the solute would perturb the hydrophobic interactions that stabilize the lipid bilayer. Disruption of the interactions in the C_1—C_8 region of the acyl chains is expected to modify not only the thermotropic order-disorder transitions but also the average intermolecular separation and possibly the orientation of the polar group. Such factors are obviously important in understanding the changes induced by alkanols in the properties of a bilayer. Thus, increasing the chain length of an alkanol would disrupt interactions in the C_1—C_8 region. Longer chain alcohols would be less effective in perturbing a bilayer since their alkyl chains can effectively replace the lipid acyl chains in the C_1—C_8 regions, where a maximum interchain overlap occurs. The branched chain octanols would similarly induce a greater perturbation then their straight chain analogs.

Such aspects of a solute-bilayer interaction are elaborated in details elsewhere [8]. A knowledge of the average lipid bilayer/water partition coefficients allows one to calculate the solute/lipid mol ratio at the equipotency concentrations. The solute/lipid ratios for a series of solutes provide an indication of the relative ability of these solutes to perturb the lipid bilayer. The most significant finding elaborated in this paper is that the intrinsic bilayer perturbing ability is qualitatively similar in some very diverse membrane systems. The effects of branching and chain length cannot only be qualitatively appreciated, but the solute/lipid ratios offer a possibility for quantitating the relative perturbing ability of different solutes. The solute/lipid ratios are expected to be constant only if the solutes do not interact 'specificallly' with the bilayer, a situation that may be encountered, for example, if a solute is localized in the middle of a bilayer where the motional and polarity gradients are absent.

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