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THE EFFECTS OF LONG-CHAIN ALCOHOLS ON MEMBRANE LIPIDS AND THE $(Na^+ + K^+)$-ATPase

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SUMMARY

The $(Na^+ + K^+)$-ATPase obtained from sheep kidney outer medulla is irreversibly denatured by long-chain aliphatic alcohols. The denaturation proceeds by causing a change in the structure of the membrane lipids rather than by binding directly to the protein. The alcohols decrease the ability of the membrane lipid bilayer to orient the spin label 3-(4′,4′-dimethyloxazolidinyl)-5α-androstan-17β-ol. For the low molecular weight alcohols the ability of the membrane to orient the label is completely lost while for alcohols with more than five carbons only partial loss of the orienting ability of the membrane occurs. The alcohol concentrations necessary to denature the enzyme correspond to the concentrations that produce the maximal change in the ability of the membrane to orient the label, and correlate well with the hydrophobicity of the alcohols as measured by their water–octanol partition coefficients.

INTRODUCTION

The role of lipids in the functioning of the $(Na^+ + K^+)$-ATPase is not well understood. The ATPase is found in plasma membranes of considerably different lipid composition[1] although the properties of the ATPase obtained from various tissues are quite similar[2,3]. We have previously shown that the ATPase can be denatured by disruption of the protein structure or the lipid structure independently[4]. In this paper we present further data on the role of membrane lipids in maintaining the function of the $(Na^+ + K^+)$-ATPase.

MATERIALS AND METHODS

Frozen, unwashed sheep kidneys and rabbit muscle were obtained from Pel-Freeze Biologicals and stored at −20 °C until used. Rabbit muscle lactic dehydrogenase was obtained from Sigma Chemical Co. Rabbit muscle pyruvate kinase was prepared by the method of Tietz and Ochoa[5]. Synthesis of 3-(4′,4′-dimethyloxazolidinyl-*N*-oxyl)-5α-androstan-17β-ol was according to the procedure of Keana *et al.*[6]. Lamb kidney $(Na^+ + K^+)$-ATPase was purified as previously described[4] and was

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assayed by the continuous method by coupling the ATPase to pyruvate kinase and lactic dehydrogenase[7]. The ATPase had a specific activity of 10–14 μmoles ATP hydrolyzed per mg protein per minute with 99% of the ATPase activity being inhibitable by ouabain. The inhibition studies and the spin labeling studies were carried out as previously described[4].

RESULTS

Data for the denaturation of the $(Na^+ + K^+)$-ATPase by ethanol and octanol is shown in Fig. 1. The concentrations necessary to give 50% irreversible inhibition by six aliphatic alcohols are given in Table I. The concentration range in which denaturation occurs is quite narrow. Reversible inhibition occurs at much lower alcohol concentrations. In assaying for enzyme activity after incubation with a given alcohol the enzyme was always diluted to a noninhibitory alcohol concentration.

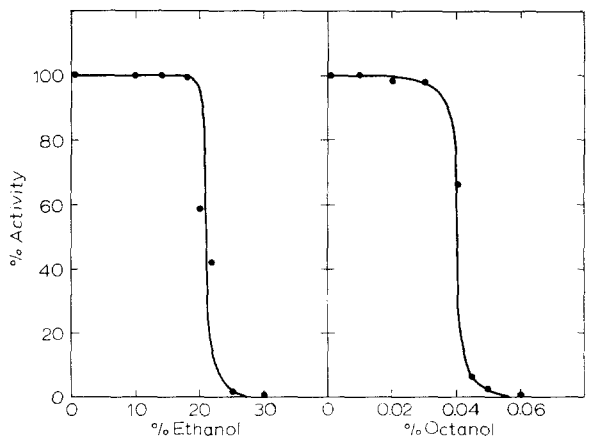

Fig. 1. Effect of ethanol and octanol on lamb kidney $(Na^+ + K^+)$-ATPase activity. The enzyme was incubated for 30 min in 50 mM imidazole (pH 7.0) at 25 °C with the alcohols and then diluted 3000-fold into the assay mixture.

TABLE I

EFFECTS OF ALCOHOLS ON MEMBRANE LIPIDS AND THE $(Na^+ + K^+)$-ATPase

Alcohol	*Concentration for 50% inhibition of ATPase (weight %)*	*Concentration for maximal change in ΔH for lipid spin label (weight %)*
Ethanol	22.5	20
Butanol	3.0	3
tert-Butanol	10.0	9
Pentanol	1.02	1
Heptanol	0.12	0.1
Octanol	0.042	0.04

Spin labeling studies

A typical EPR spectrum of 3-(4′,4′-dimethyloxazolidinyl-*N*-oxyl)-5α-androstan-17β-ol is shown in Fig. 2 for the label incorporated into the ATPase membrane. The splitting designated as ΔH in Fig. 2 is the parameter which was examined. A plot of ΔH *vs* ethanol and octanol concentrations is shown in Fig. 3. The concentration at which denaturation occurs is indicated by an arrow. One of the characteristics of the androstanol label in natural membranes and phospholipid bilayers is rapid rotation about an axis roughly parallel to the long axis of the steroid with restricted motion of the axis itself[8]. It has been shown that the label intercalates into natural membranes with the long axis of the steroid roughly perpendicular to the surface of the membrane. In the ATPase preparation studied here the average angular deviation of the long axis of the steroid from the perpendicular is about 22 °.

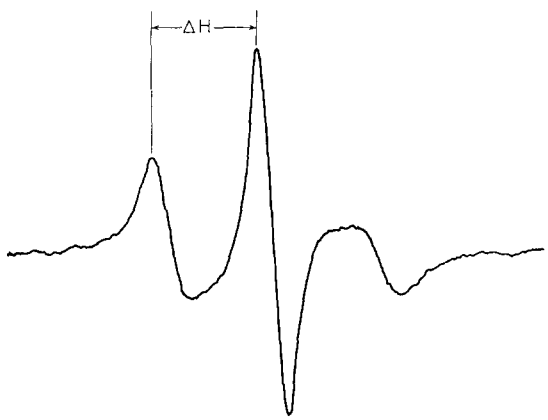

Fig. 2. EPR spectrum of the lamb kidney $(Na^+ + K^+)$-ATPase membrane labeled with 3-(4′,4′-dimethyloxazolidinyl-*N*-oxyl)-5α-androstan-17β-ol.

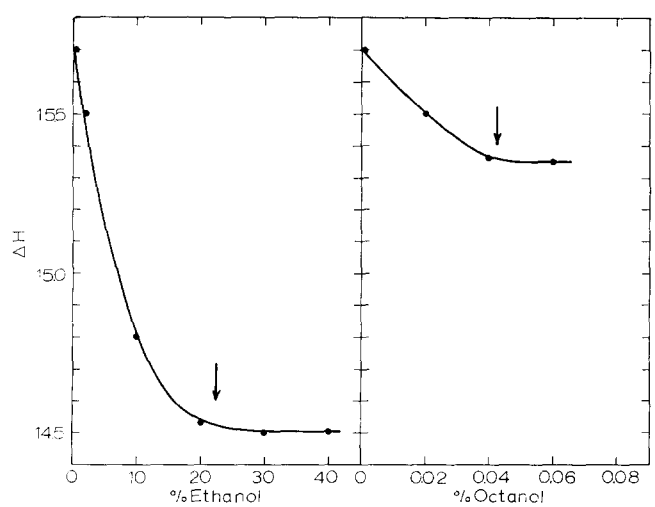

Fig. 3. The effect of ethanol and octanol on ΔH for 3-(4′,4′-dimethyloxazolidinyl-*N*-oxyl)-5α-androstan-17β-ol in the lamb kidney $(Na^+ + K^+)$-ATPase membrane.

The decrease in ΔH with increasing alcohol concentrations is indicative of a loss of the ability of the membrane lipids to orient the label. As can be seen from Fig. 3 the alcohol concentration producing the maximal effect on ΔH corresponds to the point at which enzyme activity is irreversibly lost. The data for six alcohols is given in Table I. It can be seen that in all cases the alcohol concentration giving denaturation corresponds to the concentration producing the maximal effect on ΔH.

As the chain length of the alcohol is increased the extent of the effect on ΔH decreases. For straight chain alcohols smaller than pentanol ΔH decreases to 14.5 G, the value expected for a relatively freely tumbling molecule in a hydrophobic environment, and so the membrane lipids have virtually lost their ability to orient the steroid label. For longer chain alcohol only partial loss of the ability of the membrane to orient the steroid label occurs. The minimum value of ΔH to be expected for any type of molecular motion of the label is 14.5 G, and so the change from 15.7 G to 14.5 G represents the maximal change in ΔH, $\Delta\Delta H_{max}$. This is illustrated in Fig. 4 in which $\%\ \Delta\Delta H_{max}$ is plotted *vs* the chain length of the alcohol.

Drugs which presumably have their site of action on membranes or which must pass through a membrane to reach their target site usually show a linear correlation between their water–octanol partition coefficients and their biological activity for a series of similar compounds[9]. This is also observed in the action of alcohols on the ATPase and the androstanol label (Fig. 5). It is of interest that *tert*-butanol

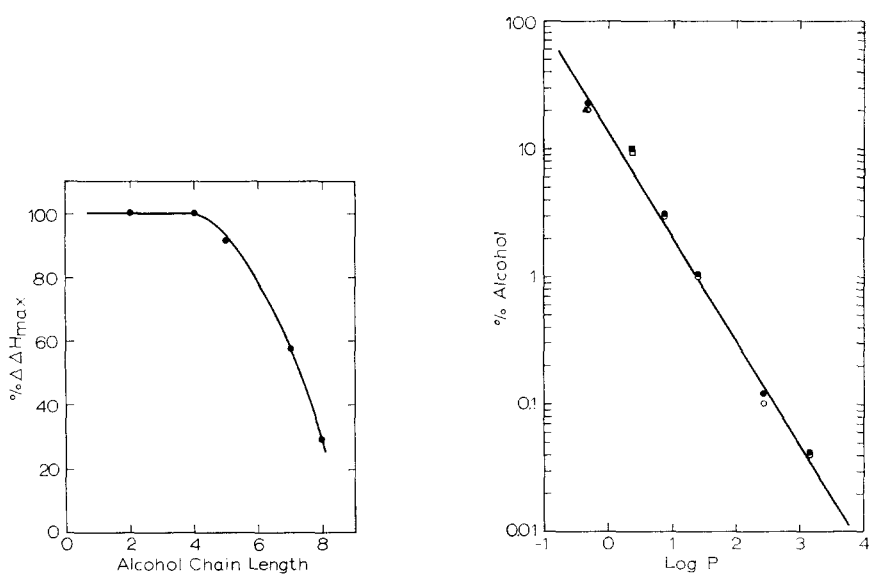

Fig. 4. The effect of alcohol chain length on the maximal change in ΔH, $\%\ \Delta\Delta H_{max}$, for a series of straight-chain alcohols.

Fig. 5. Correlation of water–octanol partition coefficients, P, with the concentration of alcohol to give the maximal change in $\Delta\Delta H$ (○) and to give 50% inhibition of the lamb kidney ($Na^+ + K^+$)-ATPase (●) for straight chain alcohols. The effect of *tert*-butanol on ΔH (□) and on ATPase activity (■) and of dioxane on ATPase activity are also included (▲).

which is a much less effective denaturant than *n*-butanol, also has a smaller water–octanol partition coefficient and so it falls on the plot with the straight chain alcohols. Even dioxane fits fairly well.

DISCUSSION

We have previously shown that denaturation of the $(Na^+ + K^+)$-ATPase by ethanol and *tert*-butanol occurs by substantially altering the lipid structure with little effect on protein structure[4]. This has been extended to show that for the series of straight chain alcohols a close correlation between the ability of the alcohol to disrupt lipid structure and denature the ATPase exists. The crucial factor in the effectiveness of the alcohols to disrupt lipid structure and denature ATPase is the hydrophobicity of the alcohol. The greater the hydrophobicity of the alcohol, and therefore the greater its lipid solubility, the more effective it is (Fig. 5).

Although the alcohols increase the fluidity of the membrane as evidenced by greater mobility of the steroid label, it is almost certainly not fluidity *per se* that is responsible for denaturation. We have shown, for example, that the dramatic change in the activation energy of the ATPase that occurs near 20 °C is due to a phase transition in the membrane, with the lipids being more fluid when the enzyme activity is the greatest[10]. A possible explanation of the nature of the alcohol inhibition is suggested by the work of Metcalfe *et al.*[11]. In a study of the interaction of benzyl alcohol with erythrocyte membranes they found that lytic concentrations of the alcohol produced irreversible changes in the protein–lipid interactions and that hydrophobic sites on the membrane proteins became exposed to the alcohol. If the inhibition is due to the binding of the alcohols to hydrophobic regions of the ATPase protein and thereby disrupting critical protein–lipid interactions, irreversible inhibition would not occur until sufficient alcohol had dissolved in the membrane lipids. The striking correlation between the effectiveness of an alcohol and its octanol–water partition coefficient (Fig. 5) is consistent with this interpretation. It is significant that a branched chain alcohol and dioxane also fit the correlation. It is likely that the octanol–water partition coefficients reflect the ability of the alcohol to partition between the membrane and water. This would mean that the more lipid-soluble the alcohol is, the more potent it is as an inhibitor, because the lower its aqueous concentration would have to be to produce a disruptive concentration in the membrane itself.

It is likely that the steroid label is reporting changes occurring throughout the membrane lipids and not just in isolated pockets. We have found that the steroid label and some other lipid labels can undergo facile lateral diffusion in natural membranes and must be fairly uniformly distributed[12]. This is also probably true of the alcohols dissolved in the membrane. The changes in the motion of the steroid spin label due to the added alcohols quite likely reflect changes occurring throughout the membrane lipids.

ACKNOWLEDGEMENTS

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