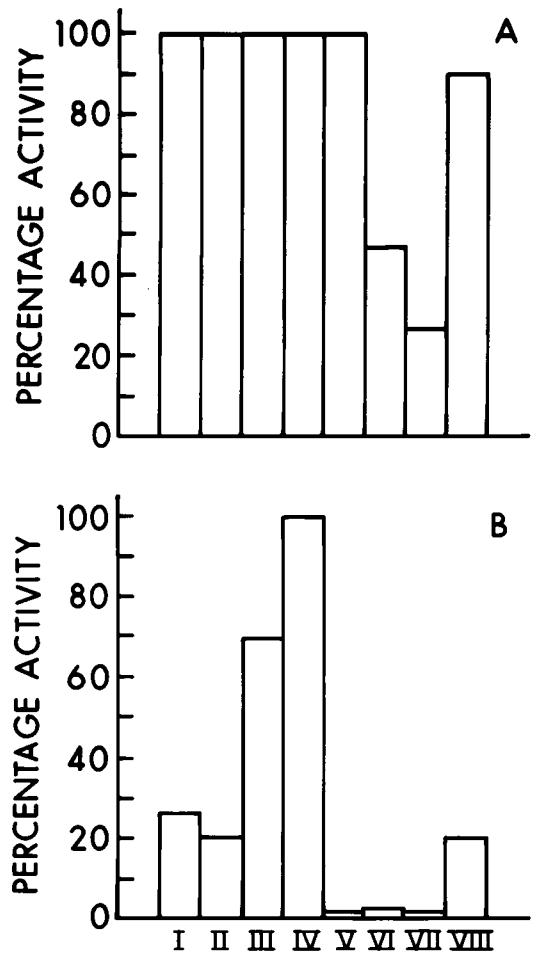

FIGURE 1 Phospholipid specificity of adenylate cyclase activation before and after delipidation. Solubilized adenylate cyclase before (*A*) or after (*B*) delipidation was assayed with phospholipid (10 mg ml^{-1} in assay) or Triton X-100.

In conclusion, these results suggest adenylate cyclase has a specific phospholipid requirement for catalytic activity. Changing the lipid environment of the enzyme might well be a mechanism whereby activity is normally modulated by hormones, by changes in the metabolic status of the cell, or during differentiation. Many possible mechanisms exist for effecting changes in the lipid environment of the enzyme. For example, exchanging an inhibitory for a stimulatory lipid by a phospholipid exchange protein or hydrolyzing phosphatidylcholine to produce lysophosphatidylcholine can both be predicted to activate adenylate cyclase. These and other possibilities are currently under investigation.

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REGULATION OF MICROSOMAL HMG-CoA REDUCTASE BY ENZYME-LIPID INTERACTIONS

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The enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) catalyzes the rate-limiting reaction of hepatic cholesterol synthesis under most physiological conditions (Rodwell et al., 1976). The enzyme is unique among the early enzymes of the pathway in that it is bound to the endoplasmic reticulum in vivo. This gives rise to the potential regulation of the enzyme through interactions with the membrane lipids. Interactions between membrane bound proteins and lipid components has been the subject of several recent reviews (Lenaz

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et al., 1975; Kimmelberg, 1977). The physiological relevance of the protein-lipid associations will depend upon the extent to which membrane lipids can affect the function of membrane bound proteins. Enzyme-lipid interactions can arise from bulk lipid effects (usually interpreted as fluidity) or from interactions between specific lipids and the enzyme.

Preliminary studies from several laboratories (Mitropoulos, et al., 1977; 1978 *a, b*) and our laboratory have shown that diet alters the temperature-dependent kinetics of the HMG-CoA reductase catalyzed reaction concomitantly with alterations in the specific activity of the enzyme at 37°C. This implies that diet-induced changes in HMG-CoA reductase activity could be mediated by enzyme-lipid interactions. This report is on studies designed to characterize the mechanism by which endoplasmic reticulum lipids interact with HMG-CoA reductase.

THEORY

Recent literature generally supports the hypothesis that a relationship exists between discontinuities in Arrhenius plots of membrane bound enzyme activity and membrane lipid phase transitions (Fourcans and Jain, 1974). This temperature-induced change in the environment of the enzyme may be accompanied by localized conformational changes leading to the observed kinetic modifications (Kimmelberg, 1977).

For the analysis of temperature-dependent kinetic data we assume that HMG-CoA reductase exists in two conformations, and propose that the conformation of the enzyme is dependent upon association with either the gel or liquid-crystalline phase of the membrane lipids. Thus, at each temperature the total enzyme is partitioned between the fraction associated with the gel phase (low temperature, low specific activity conformer) and that associated with the liquid-crystal phase (high temperature, high specific activity conformer). The reaction rate at each temperature is a weighted sum of the velocities of both conformers (each of which can be described by an equation for the absolute reaction rate), as the ratio of gel to liquid-crystal phases varies throughout the temperature range studied. We have sought to apply a thermodynamic characterization of the solubility of a solute in two immiscible phases as a means of evaluating the equilibrium partitioning of the enzyme between the two phases as a function of temperature. The model predicts the temperature dependence of enzyme activity by assuming the enzyme is distributed in the two-phase system according to the ideal solubility equation

$$\ln \frac{f_{Hi}}{f_{Lo}} = \frac{\Delta H}{RT}(1 - T/T_m)$$

In the equation, ΔH, T_m, and f represent the enthalpy change for the phase change, the melting temperature, and the fraction of enzyme in each conformation, respectively. So the reaction rate can be evaluated at each temperature during nonlinear regression analysis using the ideal solubility equations and the fitted parameters ΔG_{L0}, ΔG_{Hi}, ΔH, and T_m.

RESULTS

Rats fed a control chow diet display a triphasic Arrhenius plot. The data are well described by the model. The value for the Gibbs free energy of activation (15.2 kcal/mol) is within the 10–20 kcal/mol range usually seen for enzyme catalyzed reactions. The transition temperature of lipid associated with the change in enzyme conformation (21.99°C) is characteristic of hydrated phospholipids (Hinz and Sturtevant, 1972) as well as hepatic microsomes (Mabrey et al., 1977). The enthalpy for the conformational transition (92.58 kcal/mol) is in the same range as that seen for the changes in conformation associated with the ionization of amino acid side chains of Apo-Al (Rosseneu et al., 1977).

Because cholesterol is a common component of cell membranes, it is possible that part of its inhibitory effect on the enzyme is mediated through enzyme-lipid interactions. Cholesterol generally appears to induce an intermediate degree of fluidity in the bilayer. Rats fed a 2% cholesterol diet for three days exhibited a slightly lower specific activity (723 pmol/min-mg compared to 754 pmol/min-mg for the control) consistent with a modest increase in the energy of activation of the high temperature conformer to 15.23 kcal/mol. The enthalpy change for the transition decreased to 65.07 kcal/mol. After 14 d on the cholesterol diet, the trend continued. The specific activity is substantially less than the control animals (436 pmol/min-mg); the free energy of activation for the high temperature conformer continued to increase to 15.48 kcal/mol, while that of the low temperature conformer continued to decrease to 15.70 kcal/mol from 16.16 kcal/mol for the control.

This is consistent with a substantial decrease in the enthalpy change (84 kcal/mol less than for control animals). So with prolonged cholesterol feeding, the conformational change requires more energy which implies the conformational change has been physically influenced, which is consistent with the regulatory mechanism exerting its effect through enzyme-lipid interactions. Cholesterol alters a temperature-dependent conformational change in the enzyme so that the equilibrium between a high temperature conformer and a low temperature conformer at 37°C is changed according to the ideal solubility equation. Indeed it was shown that after 14 days on the diet, the high temperature conformer was diluted by the low temperature conformer by 30%. So the kinetic behavior of the enzyme has been related to a physiologically relevant experimental condition mediated by protein-lipid interactions within the membrane.

The model is supported by its ability to describe the temperature dependence of the reaction velocity as diet compositon (including cholesterol, cholestyramine, triglyceride, and carbohydrate) alters the membrane lipid compositon and by the results of circular dichroism (CD) studies in which soluble enzyme-lipid complex is shown to undergo a conformational change, the T_m and ΔH of which are dependent upon the lipid composition of the complex. In order to study the enzyme-lipid interactions further, HMG-CoA reductase has been purified to homogeniety and shown to be a protein-lipid complex. We have characterized the pure enzyme-lipid complex with respect to lipid composition and its influence on temperature-dependent activity. Different enzyme preparations contain different lipid compositions. This is consistent with variable thermodynamic parameters and CD spectra associated with different enzyme-lipid preparations. Both far-UV and near-UV CD spectra show a variation in secondary or tertiary structure, respectively, depending upon the lipid compositon and phase state of the lipids.

CONCLUSION

It is probably unreasonable to suppose that a metabolic pathway as important as cholesterol biosynthesis will be regulated by a single mechanism. However, regulation by enzyme-lipid interactions is a convenient mechanism of regulation because of the association of the enzyme with the membrane. Furthermore, temperature-dependent kinetic data as described above and physical evidence of a conformational change as provided by the circular dichroism studies support the hypothesis.

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INCORPORATION OF BOVINE ENTEROKINASE INTO SYNTHETIC PHOSPHOLIPID VESICLES

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The initiation of mammalian protein digestion takes place in the intestine with the selective proteolysis of pancreatic trypsinogen to produce active trypsin. This highly specific activation is catalyzed by intestinal enterokinase (enteropeptidase, E.C. 3.4.21.9). The enzyme has been localized in the microvilli of the duodenal epithelial cells as determined from microdissection (1, 2), histochemical (3), and immunofluorescence (4) studies. The membrane-bound nature of the protein has been deduced from the copurification of enterokinase with the brush border membrane marker proteins sucrase, aminopeptidase, and alkaline phosphatase (2, 5–7). Treatment of brush border membranes with proteases also releases the enzyme from the membrane (6, 8, 9). Additionally, solubilization of the mucosal cells with detergent releases the protein (10, 11).

The goal of our research is to investigate the manner in which enterokinase is anchored to the membrane and the influence of the membrane on the properties and function of the enzyme. The use of synthetic phospholipid vesicles as a membrane model will prove useful in such a study.

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