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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We describe here the results of experiments on the incorporation of enzymatically active enterokinase into synthetic vesicles and the properties of the bound enzyme.

MATERIALS AND METHODS

Enterokinase was isolated from bovine duodenal mucosal scrapings as described previously (11). Purified enzyme was incorporated into soybean phospholipid vesicles using the procedure described by MacDonald and MacDonald (12). Typically, a mixture of 15 mg of soybean phospholipids and 400 μg of enzyme in 4.0 ml of chloroform:methanol (2:1 vol/vol) was dried on a rotary evaporator at 40°C. The thin film was hydrated at 23°C with buffer at pH 8.1. The synthetic vesicles were separated and purified by ultracentrifugation, gel permeation chromatography, and sucrose density centrifugation. The components were detected by enzyme assay utilizing trypsinogen as substrate and by the determination of protein and phosphorus.

RESULTS

Approximately 35% of the enterokinase activity was associated with synthetic vesicles under optimal conditions. The remaining activity was unbound. 15–30% of the initial enzymatic activity was lost in the organic solvent mixture used in the preparation of the vesicles. When one-half or twice the number of enterokinase units were used in a reconstitution experiment, a corresponding number of units were incorporated into vesicles.

The unbound fraction of enterokinase was collected and reincorporated into vesicles. The incorporation was again only 30% of the total fraction. Both the bound and unbound form are clearly the same species of enzyme, and the observed yield must reflect difficulties in using a protein of such large size (155,000 daltons) and with a high carbohydrate content (35%). The reconstitution procedures of dialysis and sonication (13) were not effective.

We also incorporated another brush border membrane protein, alkaline phosphatase, into synthetic vesicles using the procedure described above and compared its properties with those of enterokinase. The yield of enzyme incorporated into the vesicles was ~ 30% with purified bovine intestinal phosphatase. Washing of the vesicles containing alkaline phosphatase or enterokinase with salt solutions did not release the enzymes. With alkaline phosphatase behaving as a membrane-bound marker protein (14), the similar incorporation of enterokinase and alkaline phosphatase into the synthetic vesicles further demonstrates that enterokinase was associated with the lipid bilayer of the vesicles. Enterokinase was released when the vesicles were disrupted with detergent. It is clear from these studies that alkaline phosphatase and enterokinase are anchored to the bilayer and that approximately half of their active sites face the outside of the vesicles and are accessible to their respective substrates.

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