TAUROCHOLATE PROTECTION OF CHOLESTEROL ESTERASE AGAINST PROTEOLYTIC INACTIVATION[1]

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Received February 17, 1964

A "cofactor" role of certain bile salts in cholesterol esterase activity *in vitro* has been proposed by several groups (Swell *et al.*, 1953; Korzenovsky *et al.*, 1960; Murthy and Ganguly, 1962). Murthy and Ganguly (1962) also reported that the cholesterol esterase of pancreas is protected by cholic acid against pH inactivation during incubation at 37°C. After incubation of an extract of pancreas with taurocholate, and removal of excess bile salt by exhaustive dialysis, the cholesterol esterase was still resistant to pH inactivation. The authors suggested that proteolysis was probably not responsible for the loss in enzymatic activity in the absence of bile salt, since the addition of trypsin inhibitor did not prevent this inactivation. However, we have found that addition of crystalline trypsin inhibitor to bile-free pancreatic juice does provide protection to cholesterol esterase from proteolytic inactivation (unpublished observations).

Since a free or conjugated trihydroxy bile salt is an absolute and specific requirement for synthetic and hydrolytic cholesterol esterase activity (Vahouny, Weersing and Treadwell, in preparation), and since the bile salt may complex with the enzyme, it seemed possible that both the "cofactor" and protective activities of the trihydroxy bile salts were due to the formation of a specific enzyme-bile salt complex. The

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[1] Supported by grants from the National Heart Institute (HE-02033-09) and the Life Insurance Medical Research Fund.

nature and extent of this protective activity was tested by determining tryptic and chymotryptic action on total proteins, and on synthetic cholesterol esterase activity of pancreatic juice in the presence and absence of taurocholate.

Two ml of fresh rat pancreatic juice, free of bile, and 2 ml of 0.154 M phosphate buffer, pH 6.2, were incubated at 37°C with and without sodium taurocholate (2.5 mg/ml). Crystalline trypsin or chymotrypsin (1 mg) was added and samples were removed initially and at 10 and 30 minutes for determination of trichloroacetic acid-precipitable protein and for cholesterol esterase assay. In a second experiment, an additional mg of trypsin was added after 30 min. incubation, and samples were withdrawn at 10 and 30 minutes thereafter. Protein was precipitated in 10 volumes of 10% trichloroacetic acid (TCA), the precipitates were washed with TCA, and then dissolved in 1 ml of 10% $NaHCO_3$ in 0.1 N NaOH. After dilution, protein was determined with Folin phenol reagent (Lowry et al., 1951).

For assay of cholesterol esterase, 0.5 ml of the incubated sample was added to 2.5 ml of an homogenized substrate mixture containing 15.5 μmoles cholesterol-4-C^{14}, 46.5 μmoles oleic acid, 31.0 μmoles sodium taurocholate, 26.4 mg ammonium sulfate, and 6 mg albumin in 0.154 M phosphate buffer, pH 6.2 This was incubated at 37°C, and 50 μl samples were withdrawn initially and at 5 and 20 minutes and placed in 50 μl of acetone-ethanol (1:1). Free and esterified cholesterol-4-C^{14} were determined after separation by micro-thin layer silicic acid chromatography by the method of Vahouny, Borja and Weersing (1963).

Figure 1 shows the rapid loss of cholesterol esterase activity in pancreatic juice during incubation at 37°C for 30 minutes. Addition of 2.5 mg/ml sodium taurocholate to the juice prior to incubation Resulted in essentially complete retention of cholesterol esterase activity. These results confirmed the findings of Murthy and Ganguly (1962), and suggested the possibility that the bile salt combines with

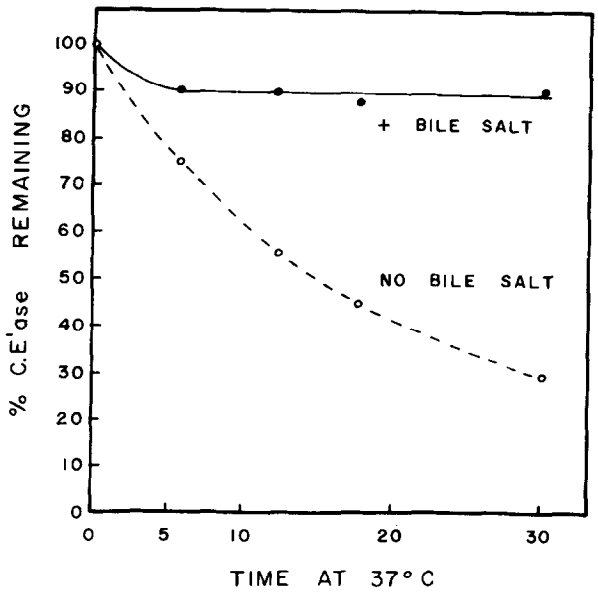

Figure 1. Taurocholate protection of pancreatic juice cholesterol esterase inactivation at 37°C. Pancreatic juice was incubated at 37°C with or without 2.5 mg/ml taurocholate.

the enzyme and protects it against inactivation during incubation at 37°C. This protection could be due to a) protection of the native enzyme from denaturation; b) a general inhibition of proteolysis of pancreatic juice proteins by endogenous proteolytic enzymes; or c) a specific inhibition against proteolysis of cholesterol esterase.

To test the possibility that the bile salt was inhibiting protein digestion by proteolytic enzymes, crystalline trypsin was added to buffered pancreatic juice with and without added sodium taurocholate. Addition of trypsin to fresh pancreatic juice would activate endogenous tryptic, chymotryptic, and carboxypeptidase action on pancreatic proteins. Figure 2 shows that the rate and extent of loss of TCA-precipitable protein was the same whether or not taurocholate was included in the incubation. Thus, the bile salt is not acting as a general inhibitor of proteolytic activity. However, in the absence of the bile salt, synthetic cholesterol esterase was completely inacti-

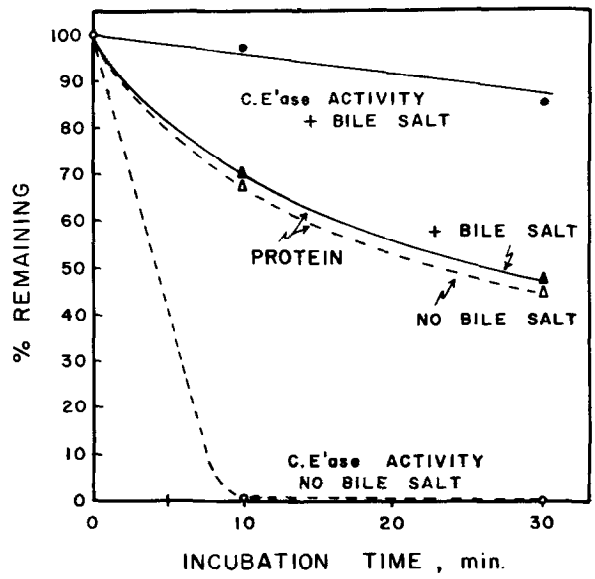

Figure 2. Effect of taurocholate on trypsin action on pancreatic juice proteins and cholesterol esterase. One mg trypsin was added to 4 ml of pancreatic juice-phosphate buffer, pH 6.2 (1:1) and incubated at 37°C.

vated within 10 minutes, while in the presence of taurocholate, there was almost complete protection of this activity. Repeated trials have shown 92-100% protection of cholesterol esterase activity by bile salts toward tryptic inactivation, while complete loss of enzyme activity always occurred within 10 minutes in the absence of the bile salt (Table 1). These data show that the bile salts specifically protect the cholesterol esterase protein, and suggest the possibility that a specific cholesterol esterase-bile salt complex is formed which is highly resistant to proteolytic action.

Addition of chymotrypsin to pancreatic juice, and incubation at 37°C without taurocholate resulted in 19% loss of protein and 27% loss in cholesterol esterase activity in 10 minutes (Table 1). However, when taurocholate was included, there was again complete protection of cholesterol esterase activity but no effect on chymotryptic digestion of protein.

Table 1

EFFECT OF TAUROCHOLATE ON
TRYPTIC AND CHYMOTRYPTIC INACTIVATION
OF PANCREATIC CHOLESTEROL ESTERASE

Additions[1]	% Remaining in 10 min.	
	Protein	Cholesterol Esterase
Trypsin (1 mg)	67	0
Trypsin + taurocholate (2.5 mg/ml)	61	92
Trypsin + taurocholate (30 min) + additional trypsin	38	67
Chymotrypsin (1 mg)	81	73
Chymotrypsin + taurocholate	84	100
Chymotrypsin + taurocholate (30 min) + trypsin	64	90

[1] The additions were to 2 ml rat pancreatic juice + 2 ml 0.154 M phosphate buffer, pH 6.2. This was incubated at 37°C and samples (0.5 ml) were withdrawn for precipitation of proteins in TCA, and for cholesterol esterase assay.

In a third experiment after incubation of pancreatic juice and taurocholate with crystalline trypsin for 30 minutes, 57% of the pancreatic proteins were no longer TCA-precipitable, while almost 90% of the cholesterol esterase activity still remained. An additional mg of trypsin reduced the TCA-precipitable protein to only 38% of its original level in 10 minutes. However, 67% of the original cholesterol esterase activity still remained (Table 1). With chymotrypsin, 80% of the protein and 100% of the cholesterol esterase activity remained after 30 minutes. Subsequent addition of trypsin resulted in an additional loss of 16% of the protein in 10 minutes but no appreciable loss in enzyme activity (Table 1).

It appears that taurocholate specifically protects the active site of pancreatic cholesterol esterase against tryptic and chymotryptic inactivation. At present, no information is available concerning the mechanism of this effect. The bile salt may combine with the enzyme

and alter its tertiary configuration so that the entire protein is resistant to proteolysis; or, it may combine and protect only the active site of the protein.

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