Studies in bile salt solutions

Deoxycholate stimulation of human milk lipase

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Stimulation of human milk lipase by deoxycholate and its taurine and glycine conjugates was demonstrated by measuring the esterolysis reaction of 4-nitrophenylacetate. The steroidal surfactants did not bind strongly to the polar substrate but they did bind effectively to a hydrophobic site on the enzyme and these bile salt-enzyme complexes were effective catalysts. These results are compared with those for stimulation of the enzyme by cholate surfactants and it has been demonstrated that the absence of a 7α-OH substituent on the steriod nucleus does not prevent stimulation of either the esterolytic or lipolytic activity of the enzyme.

Bile salt-stimulated lipase *Human milk lipase* *Esterase activity*

1. INTRODUCTION

Authors in [1] have demonstrated that there are at least two lipases (glycerol ester hydrolase, EC. 3.1.1.3) in human milk. One lipase is stimulated by serum and inhibited by bile salts while the other is inhibited by serum but stimulated by bile salts. Later studies [2,3] showed that in the absence of bile salts this latter enzyme has no activity against milk fat or against emulsified trioleylglycerol, and that the primary bile salts, sodium cholate and sodium chenodeoxycholate, and their taurine and glycine conjugates, but not the secondary bile salt, sodium deoxycholate, or its taurine or glycine conjugates, caused a pronounced activation of the enzyme against emulsified trioleylglycerol. The authors in [4] interpreted this result as meaning that there was a specific structural requirement by the enzyme for a 7α-hydroxyl group on the bile salt and designed an affinity chromatography purification procedure of the enzyme which depended on this property. A recent review of gastrointestinal lipid digestion [5] states the taurodeoxycholate will not stimulate the enzyme. Similar statements have become increasingly common in the literature; e.g., [6,7].

These observations and statements suggest that there is no binding of the steroidal surfactants, the 3α,12α-dihydroxycholanoic acids, to bile salt stimulated human milk lipase (HML) which leads to stimulation of this enzyme's activity. We have calculated [8] that about 21% of the bile salt pool, upon entering the intestine, consists of these secondary bile salts. Thus, such an inactive component would restrict the effectiveness of the enzyme in the digestive process if the absence of stimulation were also to be true for all the enzyme's known esterolytic activity. (This proportion of bile salt composition was calculated for adults; that for infants will be somewhat lower [9].) We now show that there is no such restriction on the activity of the deoxycholates.

2. EXPERIMENTAL AND RESULTS

Human milk lipase was extracted and purified by a modification [11] of the method in [10]. Stock solutions of 2.6 g · dm^{-3} in 5 mM bis–Tris propane

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buffer containing 0.154 mol · dm^{-3} NaCl at pH 7.4 were stored in the refrigerator. Enzyme activity was determined using 50 μl of the stock enzyme solution in 3 ml of a solution of the bile salt, at pH 6.5, and 0.5–5 μl PNPA (0.06 mol · dm^{-3} in acetone). The bile salts were from Sigma and the breast milk was a kind donation from the National Women's Hospital, Auckland.

In keeping with our studies on esterolytic activity in bile salt solutions [11] all activities are expressed as pseudo first-order rate constants of hydrolysis, k_ψ, at 37°C, and the esterolytic reactions were run to completion. The values of k_ψ were calculated from absorbances at 400 nm by substituting them in eq. 1:

$$k_\psi = [\ln(A_\infty - A_A) - \ln(A_\infty - A_B)]/(t_{B-A}) \quad (1)$$

where A_A, A_B and A_∞ are absorbances at times (t) A, B and infinity, respectively.

Stimulation by glycodeoxycholate (GDC) taurodeoxycholate (TDC) and deoxycholate (DC) was demonstrated by an increase in HML activity as the concentration of bile salts was increased (fig.1).

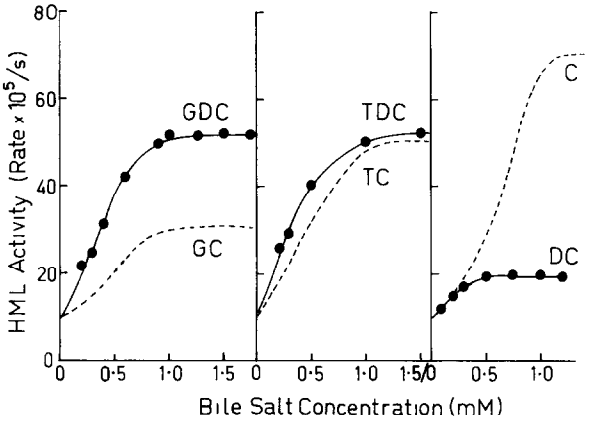

Fig.1. Variation of the activity of human milk lipase stimulated by glycodeoxycholate (GDC), taurodeoxycholate (TDC) and deoxycholate (DC) (—●—). Activity of the enzyme stimulated by glycocholate (GC), taurocholate (TC) and cholate, (C) (– – –). Enzyme activity is expressed as pseudo first-order rate constants of hydrolysis, k_ψ in s^{-1}, of 4-nitrophenylacetate in solutions of 0.01 mol · dm^{-3} bis–Tris propane buffer (pH 6.5), 37°C. All data have been corrected for background contributions of the buffer solution and the wavelength of measurement was 275 nm.

The dotted lines in fig.1 represent the activity of the corresponding cholate salts, glycocholate (GC), taurocholate (TC), and cholate (C) measured under identical experimental conditions.

We have also confirmed that the bile salt solutions were free of esterase activity. In some cases the values of k_ψ for hydrolysis of PNPA decreased in the presence of the added bile salt. All measurements were made in buffered solutions at 37.5°C, and the pH, the values of k_ψ and range of bile salt concentrations used for studies in the absence of HML are summarized below: pH 6.5, 0–5 mM C, k_ψ $5.5 \times 10^{-6}\,s^{-1}$, with a minimum value of $4.4 \times 10^{-6}\,s^{-1}$ at 0.6 mM; pH 5.0, 0 mM bile salt, $2.9 \times 10^{-6}\,s^{-1}$, 1 mM GC, $2.5 \times 10^{-6}\,s^{-1}$, 1 mM GDC, $2.5 \times 10^{-6}\,s^{-1}$; pH 7.0, 0 mM DC, $11.5 \times 10^{-6}\,s^{-1}$, 0.008–1.5 mM DC, $8.8 \times 10^{-6}\,s^{-1}$, 0–5 mM TDC, $11–12 \times 10^{-6}\,s^{-1}$. These values are at least one order of magnitude less than those obtained in the presence of the same concentrations of bile salts used to stimulate the enzyme.

3. DISCUSSION

The activity-concentration profiles shown in fig.1 provide tangible evidence that the deoxycholates, TDC, GDC and DC, can interact with HML and stimulate the enzyme to esterase activity, and that the maximum esterolytic activity arising from stimulation by the taurine and glycine conjugates, TDC and GDC, is equal to or greater than those of their cholate analogs; that of DC, while of smaller magnitude, is still significant. The absence of a 7α-OH substituent on the steroid nucleus does not prevent bile salt interaction with the enzyme. Thus, the statement frequently made [2–7] that the activation of human milk lipase is specific for primary bile salts (i.e., only cholates and chenodeoxycholates) is too restrictive. Careful examination of the literature reveals that there is also evidence of lipolytic activity for HML and that DC can activate the enzyme to hydrolyse the fats of human milk [12–14], and that even if one used trioleylglycerol and cholesteryl oleate as substrates there is some activation of hydrolysis [4,6] by DC. GDC and TDC, as well as glycochenodeoxycholate and TC protect HML activity from chymotrypsin attack [2] and these results provide additional evidence that both primary and secondary bile salts can undergo similar interactions.

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