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The identity of vitamin A esterase activity of rat pancreatic juice

It is well documented that homogenates from the pancreas and other tissues catalyze the hydrolysis of vitamin A esters (see ref. 1). The identity of the enzyme(s) involved has, however, not been established and it has in fact been suggested that there is more than one enzyme involved in this reaction in the different organs. The basis for this suggestion was the finding that sodium taurocholate had different effects on this reaction depending on the enzymatic source[1]. The vitamin A esterase activity of extract from the pancreatic gland was low in the absence of bile salt but strongly stimulated by its presence. As vitamin A esters are insoluble in water and disperse as an emulsion, it was conceivable that they could be substrate for pancreatic lipase (glycerol-ester hydrolase, EC 3.1.1.3). It also seemed possible, however, that vitamin A esters in the presence of bile salt could be substrate for another enzyme (carboxylic ester hydrolase) recently found in this laboratory to split a variety of water-soluble or bile salt-dispersed esters including monoglycerides with long-chain fatty acids[2–4].

The experiments of the present investigation were undertaken to identify the vitamin A esterase activity of rat pancreatic juice. Vitamin A palmitate was a gift of Hofmann-La Roche, Basel, Switzerland, and was freed from vitamin A by thin-layer chromatography.

Emulsified vitamin A palmitate was produced as follows. 10 μmoles vitamin A palmitate dissolved in 2 mg hexadecane were dispersed per ml 0.05 M Tris–HCl buffer (pH 8.6) by sonication using a Branson sonifier. Bile salt dispersed vitamin A palmitate was obtained by insonating 10 μmoles vitamin A palmitate per ml of a solution 6 mM in sodium taurodeoxycholate in 0.15 M phosphate buffer (pH 6.3). The taurodeoxycholate was synthesized according to HOFMANN[5] and at least 97% pure by thin-layer chromatography.

1-ml aliquots of the substrates were mixed with 200 μl of enzyme source and incubated for 1 h at 37°. After the end of the incubation period the emulsified substrate incubation was mixed with 1 ml 1 M acid phosphate (to decrease the pH to enable extraction of the fatty acids). Both incubations were then mixed with 3 times their volume of a mixture of equal parts heptane–diethyl ether and ethanol and shaken. After separation of the phases, aliquots of the upper phases were taken, evaporated to dryness, dissolved in ethanol and titrated with 0.02 M NaOH using Nile blue as indicator.

Lyophilized rat pancreatic juice was separated by gel filtration or by ion-exchange chromatography as earlier described[2,3] and the fractions tested for activity

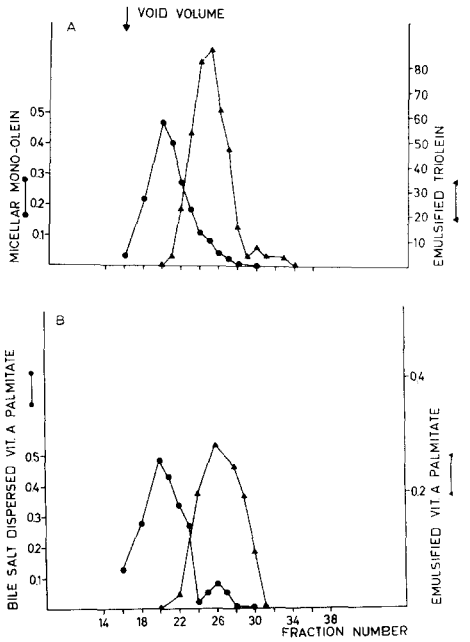

Fig. 1. Chromatography of 10 mg of lyophilized rat pancreatic juice on Sephadex G-100. Temp. 4°. Eluted with 0.15 M NaCl. Flow rate 8–10 ml/h. Total vol. 120 ml. Each fraction 2.5 ml. The ordinates give the enzymatic activities expressed as μequiv fatty acid released per min per fraction. The number of the fractions is identical in the two parts of the figure. A. ●—●, activity against micellar monoolein; ▲—▲, activity against emulsified triolein. B. ●—●, activity against bile salt-dispersed vitamin A palmitate; ▲—▲, activity against emulsified vitamin A palmitate.

against emulsified and micellar mono-olein[2,3]. These fractions were then used as enzyme source for the two vitamin A ester substrates described above.

Representative results for the Sephadex separation are seen in Fig. 1. Activity against micellar mono-olein and emulsified triolein was clearly separated with little cross specificity. The bile salt-dispersed vitamin A palmitate was hydrolyzed at a high rate by the enzyme that splits micellar mono-olein and weakly by the enzyme hydrolysing emulsified triolein (*i.e.* the classical lipase, glycerol-ester hydrolase). The emulsified substrate (with no bile salt) was only split by fractions hydrolyzing emulsified triolein. In the peak fraction against bile salt-dispersed vitamin A palmitate, the hydrolysis of the substrate was around 12% and in the fraction against emulsified substrate, approximately half that value. In all experiments performed, the activity against triolein and emulsified vitamin A palmitate was not, however, completely parallel, the peak of the vitamin A ester activity was displaced one tube to the right of the lipase activity against emulsified triolein (as was the case for the weak activity against bile salt-dispersed vitamin A palmitate). In order to examine if this shift had any relevance for the identification of the activity against emulsified vitamin A palmitate with lipase, the latter activity was first isolated with the cationic fraction of a DEAE-cellulose column[3] (pH 7.4, 0.005 M Tris–HCl buffer). This fraction was then separated on a Sephadex G-100 column. In this case the lipase and activity against emulsified vitamin A palmitate were parallel.

The above results indicate that vitamin A palmitate can be split by two different enzymes of rat pancreatic juice. One of these enzymes is most probably identical with lipase and the other, with a carboxylic ester hydrolase the specificity of which has recently been described[2–4]. Which enzyme is active depends on the composition of the substrate; lipase splitting emulsified substrate, being strongly inhibited by bile salt; carboxylic ester hydrolase only splitting vitamin A ester in the presence of bile salt. It should be pointed out that the bile salt-dispersed substrate used here contained vitamin A palmitate in amounts way above what could be solubilized in micellar solution and therefore contained the substrate both in emulsified and micellar form.

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