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The lipolytic enzymes of rat pancreatic juice

Pancreatic lipase (glycerol-ester hydrolase, EC 3.1.1.3) is usually considered to act on an oil–water interface[1], hydrolyzing only emulsified water-insoluble esters, but it has been shown that rat pancreatic juice will readily hydrolyze mixed monoglyceride–bile salt micelles in isotropic solution[2].

It is possible that an enzyme other than pancreatic lipase is involved in the hydrolysis of micellar monoglyceride. To investigate this, rat pancreatic juice has been subjected to dextran gel filtration on Sephadex G-100 and G-200 and the fractions have been tested for hydrolytic activity against a number of substrates in micellar and emulsified form. At the same time cholesterol esterase activity has been measured.

Sephadex G-100 and G-200 columns of 120-ml vol. were run at 4°. 20–25 mg of lyophilized rat pancreatic juice dissolved in 1 ml of 0.15 M NaCl were applied to the column and eluted with 0.15 M NaCl. Flow rates of 6–10 ml/h were obtained and fractions of approx. 2-ml vol. were collected. Blue dextran (mol. wt. 500 000) was used to indicate the void volume. Initially, hydrolytic activity against emulsified triolein and micellar monoolein and the hydrolytic activity of cholesterol esterase were determined. Emulsified triolein was prepared by sonicating triolein 20 μmoles/ml in buffer (pH 5.8, 0.15 M in Na^+) containing 12 μmoles sodium taurodeoxycholate/ml. Micellar monoolein was made by dissolving 12 μmoles/ml monoolein in the same buffer system containing the same bile salt concentrations to give a clear aqueous solution. Emulsified cholesterol oleate was prepared by sonicating a mixture containing, in a vol. of 1 ml: 16 μmoles of cholesterol oleate, 2 mg of octadecane and 16 μmoles of sodium taurocholate in 0.05 M glycylglycine buffer (pH 9). Free fatty acids were extracted and estimated by titration.

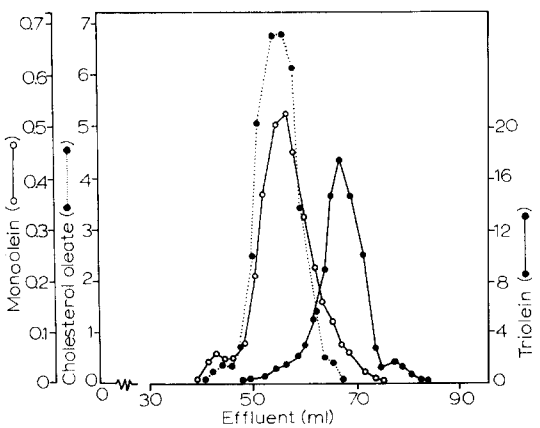

Fig. 1. Chromatography of lyophilized rat pancreatic juice on Sephadex G-100. Temp. 4°; eluted with 0.15 M NaCl. Flow rate approx. 8 ml/h. Void volume 42 ml. ○—○, activity against micellar monoglyceride; ●—●, activity against triglyceride emulsion; ●·····●, activity against cholesterol oleate. Incubation times: monoglyceride, 60 min at room temperature; triglyceride, 10 min at room temperature; cholesterol oleate, 5 min at 25°. Ordinates: μequiv fatty acid released/min per fraction.

5 Samples of juice from 4 rats were subjected to 18 separations on Sephadex G-100 or G-200. In every case hydrolytic activity against emulsified triolein was clearly separated from activity against micellar monoolein with little evidence of cross specificity (Fig. 1). The enzyme active against triolein had an apparent mol. wt. of approx. 40 000 on both G-100 and G-200 and is thus comparable to the lipase separated on Sephadex by others[3–5]. It is almost certainly glycerol ester hydrolase. The enzyme active against micellar monoolein had a mol. wt. of about 70 000 calculated from the K_{av}. Enzymic activity against cholesterol oleate could not be separated from micellar monoglyceride activity.

Glycerides of long-chain fatty acids could thus be hydrolyzed by at least two enzymes present in pancreatic juice. In the present study one of these enzymes attacked monoglyceride while the other (glycerol ester hydrolase) attacked triglyceride, but it was not certain that this difference in the chemical nature of the substrates was the most important factor in determining which enzyme attacked which substrate. As has been pointed out, the monoglyceride substrate was in micellar form and glycerol ester hydrolase probably requires an emulsified substrate for its action. It seemed possible, therefore, that if monoglyceride was presented in an emulsified form, it would be hydrolyzed by glycerol ester hydrolase rather than by the larger enzyme. To test this, an emulsion of monoolein in heptane was used. 1 ml of a heptane solution of monoolein (12 μmoles/ml) was pipetted into an ampoule followed by 0.95 ml phosphate buffer (pH 5.8) and 0.05-ml aliquots from each fraction. The ampoules were then flame-sealed and shaken vigorously at 37° for 1 h, after which released fatty acid was extracted and titrated. Extensive hydrolysis occurred in the fractions showing maximal activity against emulsified triolein with little or no hydrolysis in fractions showing activity against micellar monoolein. These experiments, therefore, supported the hypothesis that the form of the glyceride, rather than its chemical nature, determined whether or not it was hydrolyzed by glycerol ester hydrolase.

The pattern and extent of hydrolysis of monoolein in heptane was markedly altered if bile salts were added to the aqueous phase. In this case considerably less fatty acid was released than in the absence of bile salts, and maximal hydrolytic activity against monoolein shifted to fractions showing activity against micellar monoolein with a secondary peak in the glycerol ester hydrolase area. It seems likely that in these studies monoolein was solubilized in the micellar buffer–bile salt phase, producing micellar monoolein as well as an emulsified monoolein subtrate.

In two experiments, a solution of fatty acid-labeled triolein in mixed lecithin–bile salt micelles was prepared in a manner similar to that of VAHOUNY, WEERSING AND TREADWELL[6] and from this a clear micellar solution was prepared using 50-mμ Millipore filters in filtration chambers of the type described elsewhere[7]. Activity against the material was found in those fractions which showed maximal activity against micellar monoolein. Control micellar solutions containing no triolein showed no hydrolysis of lecithin under these conditions.

When tested against monoolein and triolein it was necessary for the substrate to be in soluble form for hydrolysis by the enzyme with a mol. wt. of 70 000 to occur. To test whether this was a non-specific requirement for a soluble ester substrate, activity against β-naphthyl acetate, which is completely water-soluble at low concentrations, was measured[8]. It was found that strong activity against β-naphthyl

acetate was present in fractions active against micellar monoolein, with little activity elsewhere. Activity was also tested against β-naphthyl laurate, a water-insoluble substrate. In this case hydrolysis only occurred in the region of glycerol ester hydrolase. The enzymic activity against naphthyl esters is thus in keeping with the suggestion that the larger mol. wt. enzyme requires a soluble substrate, while the smaller enzyme is specific for water-insoluble esters.

These preliminary experiments demonstrate that rat pancreatic juice contains more than one lipolytic enzyme and in this respect they confirm recent findings[9]. One of the enzymes is almost certainly glycerol ester hydrolase, and, in agreement with previous work, it has been shown to require an emulsified substrate. It is surprising, however, to find that hydrolytic activity against β-naphthyl laurate is present in the fractions containing glycerol ester hydrolase. β-Naphthyl laurate is an ester of a tertiary alcohol, and if glycerol ester hydrolase does indeed split this substrate then its characterization as an enzyme attacking esters of primary alcohols[9] would seem to be invalid.

The identity of the enzyme of mol. wt. 70 000 is uncertain. Hydrolytic activity towards cholesterol oleate was confined to this region, so cholesterol ester hydrolase (EC 3.1.1.13) was present here. Activity against micellar monoolein and β-naphthyl acetate was also found in this region. However, these activities follow each other quite closely in all tests so far performed and the possibility that a single non-specific esterase attacks all three substrates must be considered. If this is so, then, in view of the earlier comments about the requirement of this enzyme for a soluble substrate, it must be postulated that the cholesterol oleate is present in a micellar as well as an emulsified form. Preliminary studies with filtration chambers of the type used by BORGSTRÖM[7] support this. These experiments, therefore, are in agreement with the suggestion of VAHOUNY, WEERSING AND TREADWELL[6] that "the normal or preferred substrate in the hydrolytic cholesterol esterase system is the micellar or water-soluble form".

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