PRELIMINARY NOTES

PN 51036

Positional specific hydrolysis of phospholipids by pancreatic lipase

Recently, VAN DEN BOSCH *et al.*[1] demonstrated that pancreatic tissues of some mammalian species contain two phospholipases each attacking different fatty acid ester positions in lecithin, to give two isomeric lysolecithins. One of these enzymes was obtained in a purified form; on account of its heat stability and specific action on the 2-acyl ester bond only, it is identical to phospholipase A (EC 3.1.1.4) (refs. 2, 3). The other lipolytic enzyme, which catalyses the hydrolysis of the fatty acid ester linkage at the 1-position, turned out to be very heat labile. At the same time we observed that highly purified[4] pancreatic lipase (EC 3.1.1.3) can, under certain conditions, catalyse the conversion of egg lecithin into lysolecithin with a concomitant release of mainly saturated fatty acids. As these fatty acids are known to occupy predominantly position 1 in egg lecithin, we had to conclude either that the lipase preparation was still contaminated with a 1-specific phospholipase or that lipase itself catalyses the hydrolysis of 1-linked fatty acids of phospholipids. In this paper we wish to present evidence that the observed degradation of phospholipids is an intrinsic property of lipase itself and not due to the presence of other lipolytic enzymes.

Pig pancreatic lipase, purified according to SARDA *et al.*[4], and having a specific activity of about 7000 (μequiv. fatty acids released per min by 1 mg of protein) has been shown by ultrasedimentation studies to be a protein more than 95% homogeneous sedimenting with a value of $s_{20} = 4.1$. These preparations were free of phospholipase A and of all other enzymic activities known to be present in pancreatic tissues and appeared to be homogeneous upon DEAE-cellulose and sulphoethyl-Sephadex chromatography.

Although the specific action of the enzyme on egg lecithin suggests a hydrolysis of only 1-linked fatty acid ester bonds, it remained to be proved that the positional attack of the enzyme is not governed by the position in the molecule of the saturated fatty acids. For that reason, apart from egg lecithin, a synthetic mixed-acid lecithin, 1-oleoyl-2-stearoyl-glycero-3-phosphoryl choline, was used as substrate.

As shown in Fig. 1, this compound, upon incubation with lipase, is completely converted into a lysolecithin containing only stearic acid, indicating that the site of attack of the enzyme is the same for triglycerides and lecithins, independent of the degree of saturation of the fatty acid constituents.

As lipase is known[5,6] to catalyse non-stereospecifically the hydrolysis of both C_1- and C_3-esterified fatty acids of enantiomeric triglycerides, it was of interest to compare the lipase breakdown of stereoisomeric phosphatides also. It appeared that 3-oleoyl-2-stearoyl-glycero-1-phosphorylcholine was hydrolysed in the same way and at a comparable rate, indicating that the enzymic action on phospholipids also proceeds non-stereospecifically.

Finally it was found that the optimal reaction conditions for hydrolysis of lecithin by lipase were comparable with those used for the degradation of triglycerides

(pH, temperature, requirement of sodium deoxycholate and Ca^{2+} ions). Any treatment which lowers the specific activity of the enzyme with respect to triglycerides, such as prolonged storage, dialysis, lyophilization or inhibition by diethyl-*p*-nitrophenyl phosphate, causes a comparable loss of activity in the hydrolysis of phospholipids. Heating a neutral aqueous solution of lipase for 1 min at 60° destroys all the activity of the enzyme towards triglycerides. Moreover, after this treatment, no activity with respect to phospholipids could be detected.

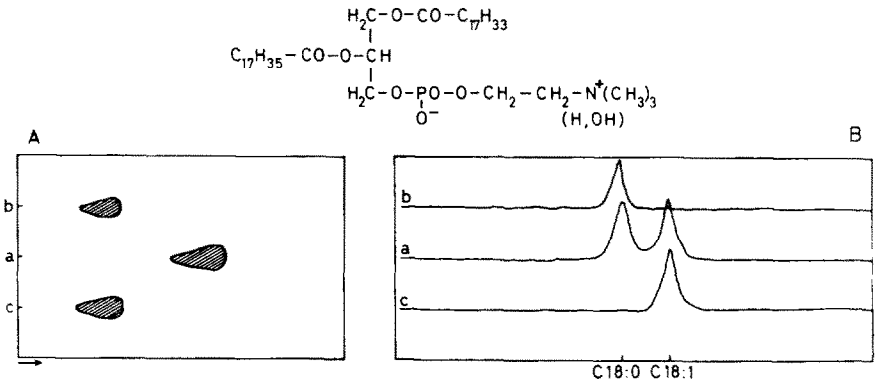

Fig. 1. Enzymic degradation of 1-oleoyl-2-stearoyl-glycero-3-phosphorylcholine by pancreatic lipase and phospholipase A from snake venom. A. Thin-layer chromatogram in chloroform–methanol–water (65:25:4, v/v/v). (a), 1-oleoyl-2-stearoyl-glycero-3-phosphorylcholine. (b), Incubation mixture containing 10 mg of the lecithin (a), 7 mg of sodium deoxycholate, 1 ml of borate buffer (pH 8; $[Ca^{2+}]$, $5 \cdot 10^{-3}$ M), 4.5 mg of bovine serum albumin, 400 I.E.U. of lipase (0.070 mg). Temp. 30°; time of incubation, 2 h. (c), Incubation mixture containing 10 mg of the lecithin (a), 1 ml of borate buffer (pH 8); $[Ca^{2+}]$, $5 \cdot 10^{-3}$ M), 2 mg of crude *Crotalus adamanteus* phospholipase A. Temp. 30°; time of incubation, 3 h. Detection of the spots by phosphate spray according to HANES AND ISHERWOOD[9]. B. Gas–liquid chromatograms of fatty acid methyl esters of the lecithins and lysolecithins shown in A. Analyses were carried out as described previously[12].

Although the presence in crude pancreatic extracts of other phospholipases with a specificity different from the well known phospholipase A cannot be precluded, several reports[7,8] in the literature concerning phospholipase activity specific for the 1-ester position might be explained, at least partially, by the presence of the rather ubiquitously occurring lipase. In this respect it is worth mentioning that a highly purified lipase preparation from the mold Rhizopus species No. 12426 (ref. 6) was found to catalyse the hydrolysis of the 1-linked fatty acids of egg lecithin at a rate comparable with that for the pancreatic enzyme.

Quite recently, VOGEL *et al.*[10] reported the action of a post-heparin phospholipase in human plasma. This enzyme appears to catalyse the hydrolysis of C-1 linked fatty acid esters of phosphatidyl ethanolamine with the formation of lysophosphatidyl ethanolamine. In the presence of methanol, ethanol or glycerol, the conversion of phosphatidyl ethanolamine into lysophosphatidyl ethanolamine was accompanied by the formation of fatty acid esters of these alcohols, indicating a direct transesterification activity in the post-heparin plasma. Although this transesterification reaction could not be demonstrated with lecithin as substrate, in our opinion it cannot be precluded that the reactions observed by VOGEL *et al.*, are caused by heparin-induced "lipoprotein" lipase. In this respect it has to be mentioned that VOGEL AND

BIERMAN[11] failed to separate post-heparin lipase and phospholipase activity of human plasma.

Preliminary experiments have already shown that not only lecithin, but also all other types of naturally occurring phospholipids are 1-specifically degraded by pancreatic lipase. An interesting biochemical application might be the preparation of 2-acyl lysophospholipids containing polyunsaturated fatty acids.

This investigation was carried out during a recent stay at the Institute of Biochemistry of Marseille of one of the authors, G. H. De Haas, who was a Centre National du Recherche Scientifique (C.N.R.S.) fellow sponsored by the Netherlands Organization for the Advancement of Pure Research (Z.W.O.). We thank Prof. P. DESNUELLE and Prof. L. L. M. VAN DEENEN for their interest and support.

Laboratory of Organic Chemistry, Biochem. Dept., Utrecht (The Netherlands) G. H. DE HAAS
L. SARDA
Institut de Chimie Biologique, Marseille (France) J. ROGER

1 H. VAN DEN BOSCH, N. M. POSTEMA, G. H. DE HAAS AND L. L. M. VAN DEENEN, *Biochim. Biophys. Acta*, 98 (1965) 657.
2 W. L. MAGEE, J. GALLAI-HATCHARD, H. SANDERS AND R. H. S. THOMPSON, *Biochem. J.*, 83 (1962) 17.
3 L. L. M. VAN DEENEN, G. H. DE HAAS AND C. H. TH. HEEMSKERK, *Biochim. Biophys. Acta*, 67 (1963) 295.
4 L. SARDA, M. F. MAYLIÉ, J. ROGER AND P. DESNUELLE, *Biochim. Biophys. Acta*, 89 (1964) 183.
5 N. H. TATTRIE, R. A. BAILEY AND M. KATES, *Arch. Biochem. Biophys.*, 78 (1958) 319.
6 M. L. KARNOVSKY AND D. WOLF, *Proc. Intern. Congr. Biochem. 4th, Vienna, 1958*, Abstr. Commun., (1958) 208.
7 J. LLOVERAS, L. DOUSTE-BLAZY AND P. VALDIGUIÉ, *Compt. Rend.*, 256 (1963) 1861.
8 H. VAN DEN BOSCH AND L. L. M. VAN DEENEN, *Biochim. Biophys. Acta*, 84 (1964) 234.
9 P. LABOUREUR AND M. LABROUSSE, *Compt. Rend.*, 259 (1964) 4394.
10 W. C. VOGEL, W. G. RYAN, J. L. KOPPEL AND J. H. OLWIN, *J. Lipid Res.*, 6 (1965) 335.
11 W. C. VOGEL AND E. L. BIERMAN, *Federation Proc.*, 24 (1965) 439.
12 C. S. HANES AND F. A. ISHERWOOD, *Nature*, 164 (1949) 1107.

Received September 20th, 1965

Biochim. Biophys. Acta, 106 (1965) 638–640

PN 51035

Effect of polyunsaturated fatty acids on the desaturation *in vitro* of linoleic to γ-linolenic acid

In weanling rats reared on a fat-deficient diet the content of eicosa-5,8,11-trienoic acid synthesized from oleic acid increases[1]. The addition of acids of the linoleic family (linoleic or arachidonic acid) or the linolenic family[2] (linolenic, eicosa-5,8,11, 14,17-pentaenoic or docosa-4,7,10,13,16,19-hexaenoic acid) to the diet decreases the synthesis of eicosatrienoic acid. This effect has been considered by DHOPESHWARKAR AND MEAD[3] and HOLMAN[4] as the result of a competition between oleic, linoleic and linolenic acid for the desaturating and/or elongating enzymes. Our own results ob-