elastases was suggested by their specific activities of 1630 elastase caseinolytic units/mg of NYS 64-332 elastase (S.D. ± 215 for 10 determinations) and 1550 elastase caseinolytic units/mg of NYS 66-319 elastase (mean of 3 determinations). The respective distribution coefficients of 0.59 and 0.61 for NYS 64-332 and NYS 66-319 elastases on gel filtration in Sephadex G-100 suggest that the observed differences on ion-exchange chromatography and polyacrylamide electrophoresis are not attributable to association reactions. Thus, both elastases possess approximately the same molecular weights and proteolytic properties and are immunochemically indistinguishable. They differ in electrostatic charges as indicated by disc electrophoresis and ion-exchange chromatography. These differences in net charge do not produce immunologic distinctions.

This research was supported by Grant HE-09902 from the National Heart Institute, National Institutes of Health, and by the U.S. Army Medical Research and Development Command (DADA17-69-G-9284).

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Received June 30th, 1969

Biochim. Biophys. Acta, 191 (1969) 179–181

BBA 63406

Esters of phenols as substrates for pancreatic lipase

Esters of phenol[1], *p*-nitrophenol, *β*-naphthol[2], 4-methyl umbelliferone[3,4], and fluorescein[5] have been used as substrates for "lipase" in colorimetric or fluorimetric analytical methods. It has also been reported that lipases, and in particular pancreatic lipase, show maximal activity against the medium chain length esters of these phenols[2–4]. If we agree to give the name lipase (EC 3.1.1.3) only to those enzymes which attack emulsified long-chain triglycerides, typically triolein or olive oil[6], we may doubt whether phenol esters will be appropriate substitutes for the natural substrate of such enzymes. I found that *p*-nitrophenyl oleate and *β*-naphthyl oleate are only slowly hydrolyzed by crude porcine pancreatic lipase and that phenyl oleate is not at all hydrolyzed[7]. BARROWMAN AND BORGSTRÖM[8] have recently conducted semi-quantitative assays on a number of phenol esters as substrates for the lipase and for an esterase of rat pancreatic juice. They found that triolein, *β*-naphthyl laurate and oleate, and *p*-nitrophenyl laurate were rapidly hydrolyzed by the lipase, whereas 4-

methyl umbelliferone decanoate was a moderately active substrate, and fluorescein esters were not attacked.

The availability of a pure preparation of the best-known lipase, porcine pancreatic lipase, has prompted me to determine the maximum rates of hydrolysis of several of the recommended phenol esters in comparison to triolein, under conditions[9] which are nearly optimal for triolein as well as for a number of other substrates, *e.g.* tributyrin and substituted benzyl esters[7]. Cholesterol oleate, which has never been exposed to pancreatic lipase of undisputable purity, was also tested.

Fluorescein dibutyrate, *p*-nitrophenyl octanoate and dodecanoate, and 4-methyl umbelliferone octanoate and dodecanoate were purchased from Nutritional Biochemicals Co. (Cleveland). The other esters were synthesized *via* the acid chlorides. All substrates were purified by chromatography on neutral aluminum oxide Woelm (Alupharm Chemicals, New Orleans)[10].

Porcine pancreatic lipase was prepared by ion-exchange and Sephadex chromatography[12,13] and had a specific activity of 5000 against triolein emulsified with gum arabic[11]. The purity of the preparation has been demonstrated by standard procedures and convincingly by its separation into two isoenzymes of equal specific activity[12,13].

The assay system was that described by BENZONANA AND DESNUELLE[9]: final concentrations: 0.1 M NaCl, 0.5 mM $CaCl_2$, 4.5 mM sodium deoxycholate; total volume, 15 ml; temp., 37°; pH 9.0. The substrates were added after sonic emulsification (20 kcycles, 50 W, 30 sec) with the 2-fold volume of 32 mM deoxycholate[9]. To the 4-methyl umbelliferone octanoate 25% of the dodecanoate were added to give a liquid substrate. The fatty acids released by the action of the lipase were titrated (Radiometer, Copenhagen) under nitrogen and with stirring at maximal speed, and with continuous recording. The linear rates during the first few minutes were measured. Maximum velocities of lipolysis were established by varying the amounts of substrate emulsion (between 0.2 and 0.8 ml) (Table I).

The following conclusions can be drawn: (1) The esters of phenols are poor substrates for porcine pancreatic lipase. The rate of hydrolysis of even the most active esters is less than 2% of that of triolein. (2) Esters of fluorescein are not attacked by

TABLE I

MAXIMUM VELOCITIES OF LIPOLYSIS OF DIFFERENT SUBSTRATES

Maximum velocities, V, of lipolysis are given relative to V of triolein = 100.

Substrate	V
Triolein	100
p-Nitrophenyl octanoate	0.6
p-Nitrophenyl dodecanoate	1.7
p-Nitrophenyl oleate	1.6
β-Naphthyl nonanoate	1.3
β-Naphthyl oleate	1.4
Phenyl oleate	0.4
4-Methyl umbelliferone octanoate	0.3
4-Methyl umbelliferone oleate	0.3
Fluorescein dibutyrate	<0.02
Fluorescein dioleate	<0.02
Cholesteryl oleate	<0.03

pancreatic lipase. This is not unexpected since these esters have a zwitter-ionic structure in contrast to the neutral structure of triglycerides. (3) Cholesteryl oleate is resistant to pancreatic lipase. It behaves, predictably, like an ester of a non-activated secondary alcohol[7]. (4) Rates of hydrolysis are not higher for medium chain-length esters than for oleic esters. Where higher rates have been reported[2–4], enzymes other than pancreatic lipase may have been measured. Medium chain-length esters appear, therefore, to be quite unsuitable for the detection or assay of pancreatic lipase. (5) The possibility remains that completely insoluble esters, like β-naphthyl oleate, though being rather poor substrates, will satisfy the requirement of specificity, *i.e.* will not be hydrolyzed by any enzymes except lipases. They could thus be used for quantitative determinations of lipases; however, the identity of the enzyme should always be verified by the hydrolysis of triolein or olive oil.

I thank Dr. P. Desnuelle for his hospitality at the Institut de Chimie Biologique at Marseille, and Dr. R. Verger for his advice in the preparation of the pancreatic lipase.

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Received June 2nd, 1969

Biochim. Biophys. Acta, 191 (1969) 181–183

BBA 63412

4-Methylumbelliferyl phosphate as a substrate for lysosomal acid phosphatase

In their classical work on lysosomes APPELMANS *et al.*[1] used β-glycerophosphate as substrate for acid phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2). This gave good localisation of the enzyme in the lysosome fraction with a high degree of latent activity. Other workers[2–4] have used *p*-nitrophenyl phosphate for assaying the same marker enzyme, although it was shown by NEIL AND HORNER[5] and confirmed by BROTELLE AND WATTIAUX[6] that microsomal phosphatase is capable