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Short Communications

On the Specificity of Arylesterases

KLAS-BERTIL AUGUSTINSSON and GUNNAR EKEDAHL

Institute of Organic Chemistry and Biochemistry, University of Stockholm, Sweden

By definition, arylesterases are enzymes which hydrolyse aromatic esters and are resistant to those organophosphorus compounds which strongly inhibit aliesterases and cholinesterases [1]. When this definition was introduced, little was known about the specificity of arylesterases, except for the fact that they hydrolyse phenyl esters at a higher rate than do aliesterases and cholinesterases, the acetate being more readily split than the propionate and butyrate. The present communication extends the specificity study to the description of the influence of the structure of the phenolic/alcoholic moiety of the substrate on the hydrolysis rate, catalysed by human plasma arylesterase.

A partly purified arylesterase preparation (nitrogen content, 13 %) from human plasma was used as enzyme source. It was free from cholinesterase activity. For comparison, a cholinesterase preparation (nitrogen content, 12.6 %) from the same source and free from arylesterase activity was also tested. The Warburg manometric technique was used for esterase determination at pH 7.4, during which the initial substrate concentration was 8 mM. A detergent (Tween 20) was used for solubilization of most esters, and in the concentration used (0.05 %) it had no significant influence on the activity measured. Esterase activity is expressed in μmoles of substrate hydrolysed per h per mg esterase preparation. Corrections were made for spontaneous hydrolysis of substrate, which did not exceed 0.6 μmoles per h in any case under the experimental conditions used.

Among a number of esters tested for enzymatic hydrolysis, the following were not hydrolysed by arylesterase:

benzyl acetate
benzyl benzoate
2-phenylethyl acetate
3-phenylpropyl acetate
ethyl phenylacetate
isoamyl phenylacetate
acetylsalicylate
ethyl benzoate
ethyl *p*-hydroxy-benzoate
ethyl salicylate
diethyl phthalate
diisoamyl phthalate
ethyl gallate
phenyl salicylate
ethyl acetate
isopropyl acetate
amyl acetate
2-ethoxyethyl acetate
vinyl crotonate

The influence of the structure of the phenolic/alcoholic radical of the substrate on the enzymatic hydrolysis is illustrated in Fig. 1, in which the hydrolysis rates of a series of acetic acid esters are compared for arylesterase and cholinesterase. Phenyl acetate (I) was hydrolysed at the highest rate among all esters tested. With the alicyclic analogue of phenyl acetate (cyclohexyl acetate, IV) no hydrolysis was detected. As regards the three cyclohexenyl acetates, it was found that the Δ^1-derivative (II) was hydrolysed but not the Δ^2- (III) and Δ^3-derivatives. This indicates that a double bond nearest to the ester linkage might be prerequisite for enzymatic hydrolysis. Further support for this idea was obtained when it was demonstrated that Δ^1-cyclopentenyl acetate(VI) was hydrolysed, but not cyclopentyl acetate (V). A comparatively high hydrolysis rate was also observed for 3-pyridyl acetate (XIII).

To test this structure-activity relationship further, the hydrolysis of certain

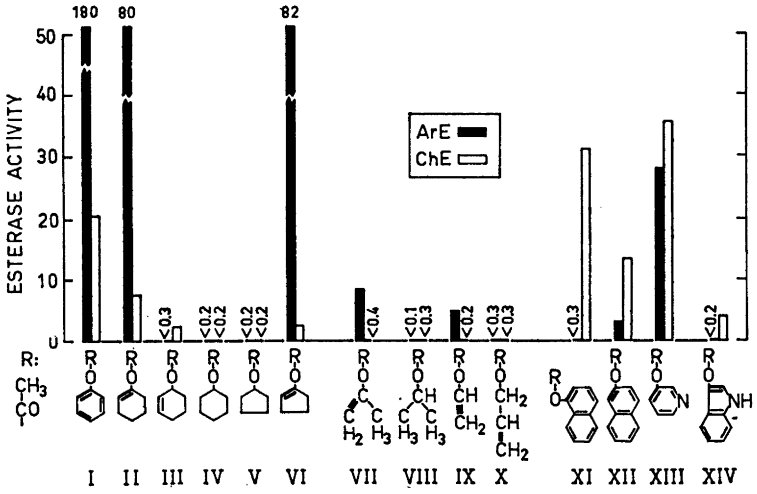

Fig. 1. The enzymatic hydrolysis of various acetates by purified preparations of arylesterase (ArE) and cholinesterase (ChE) from human plasma. Esterase activity is expressed in μmoles of ester hydrolysed per h per mg enzyme preparation.

simple aliphatic acetates were studied. Thus isopropenyl acetate (VII) was found to be split by arylesterase, but not isopropyl acetate (VIII). Moreover, vinyl acetate (IX) was attacked by this enzyme, but not allyl acetate (X).

When naphthyl acetates were tested as substrates, it was found that the 2-naphthyl ester (XII) was hydrolysed at a much higher rate by arylesterase than the 1-naphthyl ester (XI). The hydrolysis rates of these two esters were the reverse for cholinesterase. Steric hindrance is probably the explanation for the difference between the two esters *vis à vis* arylesterase. For the same reason, probably, indoxyl acetate (XIV) and acetyl salicylate were poor substrates for arylesterase.

Human plasma arylesterase was determined to be an acetylarylesterase, since acetates were hydrolysed more rapidly than propionates and butyrates, not only of the phenolic series, but also of the series of vinyl and indoxyl esters. Cholinesterase of human plasma differs in this respect from arylesterase, butyrates (choline, phenyl, indoxyl) being hydrolysed at a higher rate than the corresponding propionates and acetates. As regards arylesterase, substitution by electrophilic and nucleophilic groups in the acyl radical of the ester has a marked influence on the hydrolysis rate. This will be the subject of a following report in this field.

The results reported indicate that the active center of arylesterase contains a structure that reversibly reacts with a C=C group of the alcoholic moiety of an ester; the prerequisite for enzymatic hydrolysis is that this group is bond directly to the alcoholic oxygen atom. It should be mentioned that arylesterase is highly sensitive to mercaptide forming agents (*e.g.*, *p*-hydroxymercuribenzoate) and that the inhibition by these agents is reversed by cysteine [2]. We therefore suggest, as a tentative model for the mechanism of action of arylesterase, that the enzymatic hydrolysis involves the formation of a thioester intermediate from the enzyme SH and the acyl moiety of the substrate. The formation of this intermediate is dependent on the special character of the C-C bond nearest to the ester linkage, as was demonstrated above.

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1. Augustinsson, K.-B. *Nature* **181** (1958) 1786; *Acta Chem. Scand.* **13** (1959) 571.
2. Augustinsson, K.-B. *Ann. N. Y. Acad. Sci.* **94** (1961) 844.

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