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Enzymatic Hydrolysis of N-Acylated 1-Aminophosphonic Acids

V. K. Svedas^a; E. V. Kozlova^a; D. A. Mironenko^a; V. P. Kukhar^a; T. N. Kasheva^a; V. A. Solodenko^a; A. N. Belozersky^a

^a Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow University, Moscow, USSR

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ENZYMATIC HYDROLYSIS OF N-ACYLATED 1-AMINOPHOSPHONIC ACIDS

V.K.ŠVEDAS, E.V.KOZLOVA, D.A.MIRONENKO, V.P.KUKHAR,
T.N.KASHEVA, and V.A.SOLODENKO
A.N.Belozersky Laboratory of Molecular Biology and
Bioorganic Chemistry, Moscow University, Moscow
119899, USSR

Penicillin acylase from *E.coli* (EC 3.5.1.11) was found to hydrolyse N-phenylacetylated 1-aminoalkylphosphonic acids and their esters. Enzyme preferentially converts the R-form of the substrates: the ratios of the bimolecular rate constants of penicillin acylase-catalysed hydrolysis of R- and S- forms of 1-(N-phenylacetamino)-ethylphosphonic acid and its dimethyl- and diisopropyl- esters are 58000, 2600, 1800; these derivatives were shown to have the greatest values of the catalytic constants for enzymatic hydrolysis of all known substrates of penicillin acylase: 237, 148, and 134 s^{-1} ; corresponding values of Michaelis constants are 3.7×10^{-5} , 6.8×10^{-4} , and 6.2×10^{-4} M. The kinetics of the enzymatic hydrolysis of 1-(N-phenylacetamino)-ethylphosphonic acid was investigated up to high degrees of conversion. The inhibition of penicillin acylase by high concentrations of the R-form of the substrate (with substrate inhibition constant 0.07 M) and competitive inhibition by the reaction product phenylacetic acid ($K_i = 3.5 \times 10^{-5}$ M) was observed. Penicillin acylase was shown to possess quite broad substrate specificity among N-acylated 1-aminoalkylphosphonic acids and was found to be capable of hydrolysing 1-(N-phenylacetamino)-substituted 2-phenylethyl-, 1-phenylmethyl- and 3-methylbutylphosphonic acids with high efficiency and enantioselectivity.