INTERACTION OF D-α-METHYLASPARTIC ACID WITH ASPARTATE AMINOTRANSFERASE

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1. Introduction

DL-α-methylaspartic acid inhibits aspartate aminotransferase by competing with amino acid substrates [1]. It is known to combine with the enzyme to give a characteristic spectrum which has been used to measure the thermodynamic [2,3] and kinetic [4] parameters of the interaction. In each study using α-methyl-aspartate, the D-form was assumed to be unreactive because only the L-isomers of the natural substrates are known to bind. This assumption was not tested because only the racemic mixture was available at the time.

2. Results and discussion

The α-subform of supernatant aspartate aminotransferase was prepared from pig heart as previously described [5]. Enzyme concentrations were determined spectrophotometrically using either a molar extinction coefficient per active site of 8.05×10^3 M^{-1} cm^{-1} at 362 nm or molar extinction coefficient per active site of 7.16×10^4 M^{-1} cm^{-1} at 280 nm [6].

D-α-methylaspartic acid was the gift of Dr David Miller of Oberlin College. DL-α-methylaspartic acid was obtained from Calbiochem. Aliquots of D-α-methlyaspartate were added to aspartate aminotransferase to a final concentration of 39 mM. Then DL-α-methylaspartic acid was added to a final concentration of 19 mM. The spectrum was recorded after each addition. All experiments were done in 0.1 M $Na_4P_2O_7$, pH 8.0, at 25°C.

The results are shown in fig.1. No interaction between the enzyme and the D-isomer is visible to a concentration of 39 mM, a concentration known to be near saturation for the racemic mixture. (K = 420 M^{-1} under these conditions [3]).

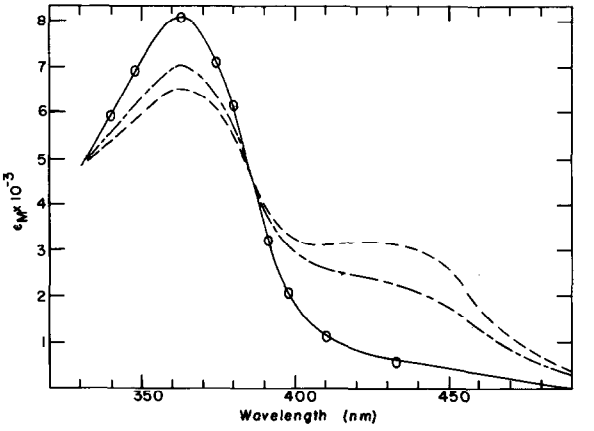

Fig.1. Absorption spectrum of aspartate aminotransferase in the presence of various amounts of D-α-methylaspartic acid at pH 8.00, 25°C. (———) No additions, (—o—o—o—) 39.3 mM D-α-methylaspartate (– – –) 7.1 mM DL-α-methylaspartate (– · –) 19.0 mM DL-α-methylaspartate

D-amino acids are believed not to bind to the enzyme. A single report of binding by D-aspartate exists [7]. The reported dissociation constant is rather large (102 ± 14 mM) and is based on inhibition studies. D-aspartate was found to serve as a competitive inhibitor with respect to aspartate. However, in the experiment the ionic strength was held constant by variation of phosphate concentration. Phosphate is known to serve as an activator of this enzyme [8,9]. Although this activation was not analyzed in the same fashion in each report, both agree that phosphate

modifies the Michaelis constant for aspartate. It is therefore possible to simulate competitive inhibition by D-aspartate by decreasing the phosphate level while simultaneously increasing amino acid levels, thereby increasing the observed Michaelis constant for aspartate. While this may not be a complete explanation of the effect observed, it is probably an important element of it.

The observed failure of D-α-methyl aspartate to generate spectral changes in aspartate aminotransferase is consistent with the general failure to observe inhibition or spectral changes with D-amino acids. As discussed the report of D-aspartate inhibition may be accounted for by considering the effect of phosphate on the reaction.

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