ARGININE AS A SUBSTRATE BINDING SITE IN ASPARTATE AMINOTRANSFERASE

H. F. Gilbert and Marion H. O'Leary

Department of Chemistry, University of Wisconsin
Madison, Wisconsin 53706

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Modification of one or two arginine residues in pig-heart cytoplasmic aspartate aminotransferase with 1,2-cyclohexanedione nearly abolishes its catalytic activity and abolishes its ability to bind dicarboxylic acids. The modification is competitively inhibited by glutaric acid. Modification of the enzyme causes no change in its ability to transaminate alanine, but causes a tenfold increase in the Michaelis constant and a 10^4 fold decrease in the rate of transamination of aspartate. These results indicate that the binding site for the β-carboxyl group of aspartic acid is an arginine residue.

INTRODUCTION

Aspartate aminotransferase (EC 2.6.1.1) catalyzes the pyridoxal 5'-phosphate dependent transamination of amino- and keto-dicarboxylic acids. The mechanism of action of the enzyme has been studied extensively (1), but the mode of binding of the substrate to the enzyme is not well understood. One carboxyl group of the substrate is apparently bound to the protonated imine nitrogen of the enzyme-coenzyme Schiff base (2), but the binding site for the other carboxyl group has not been identified. We present evidence that an arginine residue of the enzyme is responsible for binding the β or γ carboxyl group of the substrate.

Of the several reagents which have been used for the modification of arginine residues in proteins, 1,2-cyclohexanedione is one of the most useful (3). In the presence of borate the modification is rapid, specific, and reversible. Quantitation can be achieved by amino acid analysis.

MATERIALS AND METHODS

Reagent grade materials were used throughout. UV spectra were recorded on a Cary 15 spectrophotometer equipped with a 0.1 absorbance scale. Enzyme assays were performed on a Gilford model 222 spectrophotometer by the method of Jenkins, et al. (4).

The α subform of cytoplasmic aspartate aminotransferase from pig heart was prepared by the method of Martinez-Carrion, et al. (5). The specific activity of the purified enzyme was approximately 240 μmoles oxaloacetate $min^{-1}mg^{-1}$ and the absorbance ratio A_{430}/A_{340} was 2.5-3.0 at pH 5.4 (0.1 M acetate). Results are calculated based on a subunit molecular weight of 46,500 and $E^{1\%}_{280} = 14.0$ (5).

Aspartate aminotransferase was modified with 1,2-cyclohexanedione according to the procedure of Patthy and Smith (6). The enzyme (0.15 mg/ml) was treated with 0.05 M 1,2-cyclohexanedione in 0.2 M sodium borate buffer, pH 8.5 at 21°. For amino acid analysis the modified enzyme was subjected to dialysis followed by acid hydrolysis in the presence of mercaptoethanol (3). Amino acid analysis was carried out on a Beckman 120C amino acid analyzer.

RESULTS

Treatment of the pyridoxal form of aspartate aminotransferase with 0.05 M 1,2-cyclohexanedione in borate buffer, pH 8.5 for 100 min virtually abolishes the catalytic activity of the enzyme. Amino acid analysis of the modified protein reveals that 2.4 arginine residues are modified per 46,500 daltons. No other amino acids are affected. Approximately 70% of the activity of the enzyme can be restored by treatment with 0.2 M hydroxylamine at pH 7.0 under nitrogen at 37° in 16 hr (3).

The loss of catalytic activity of the enzyme on treatment with 1,2-cyclohexanedione is first-order in 1,2-cyclohexanedione and first-order in enzyme. The rate constant for activity loss at 21° in 0.2 M sodium borate buffer, pH 8.5, is 0.80 $M^{-1}min^{-1}$. The rate of activity loss is lower in the presence of glutaric acid, a competitive inhibitor of transamination (7). Measurements of the modification rate in the presence of various concentrations of glutaric acid reveal that the inhibition is competitive, with an inhibition constant of 25 mM. The constant for

inhibition of transamination by glutarate is 18 mM (8).

Following gel filtration, the modified enzyme shows a uv absorption maximum at 362 nm and an absorbance ratio $A_{362}:A_{280}$ of 0.10, compared to a ratio of 0.13 for the native enzyme. Thus, the pyridoxal cofactor appears to be intact in the modified enzyme. Aspartate aminotransferase shows a large spectral change on binding succinate and other dicarboxylic acids (2). No such spectral change is observed with the modified enzyme at pH 8.0.

Although the modified enzyme does not catalyze transamination between amino- and ketoacids at a measurable rate, treatment of this material with aspartate, cysteine sulfinate, or alanine for 10 min causes the absorbance at 362 nm to be replaced slowly by a peak at 330 nm. Thus, the modified enzyme is still capable of undergoing slow transamination, although transaminations with aspartate and with cysteine sulfinate are more than 10^4 slower than in the native enzyme. The transamination of aspartate follows saturation kinetics, with a Michaelis constant for aspartate of 25 mM; the Michaelis constant for aspartate with the native enzyme is 1.5 mM (1). Transamination with alanine is about 60% faster in the modified enzyme than in the native enzyme.

DISCUSSION

Modification of one or two arginine residues in aspartate aminotransferase reduces the activity of the enzyme toward normal substrates by a factor of more than 10^4 but does not entirely abolish the ability of the enzyme to undergo transamination with appropriate substrates. The modification is competitively inhibited by glutarate, an inhibitor of transamination, and the inhibition constant is similar to that observed for transamination in the presence of glutarate. On modification, the enzyme loses its ability to bind succinate. Thus, it appears that the essential

arginine is involved in substrate binding.

The most striking feature of the modified enzyme is its activity toward various substrates. Although all of the enzyme is kinetically competent, the rate at which the enzyme transaminates with aspartate is reduced by more than 10^4 compared to the native enzyme, and the Michaelis constant is increased by more than a factor of ten. Alanine, on the other hand, is transaminated at approximately the same rate by native and modified enzyme. Thus, the essential arginine provides the binding site for the β or γ carboxyl group of the substrate. Modification of this arginine converts the enzyme into a nonspecific transaminase of very low activity which transaminates alanine, aspartate, and cysteine sulfinate at approximately the same rate. The very low activity of the enzyme probably arises from the failure of these substrates to achieve an optimum conformation for reaction.

The lack of reactivity of the α carboxyl binding site toward 1,2-cyclohexanedione, toward lysine reagents (9), and toward histidine reagents (10), together with the effects of substrate and inhibitor binding on the spectrum of the pyridoxal chromophore (2), suggest that the α-carboxyl binding site is, as has been suggested previously (2), the imine nitrogen of the enzyme-pyridoxal 5'-phosphate Schiff base.

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Editorial Note (9/8/75): After this article was received, an article by Riordan and Scandurra (BBRC 66, 417-424) appeared which reached similar conclusions. The present findings that transaminase activity for alanine is unchanged by modification while that for aspartate is greatly decreased, reinforces the view that modification involves specifically the binding site for the β-carboxyl group of aspartate.

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