NUCLEOTIDE EFFECTORS OF ASPARTASE[1]

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Nucleotide-sensitive preparations of aspartase have been obtained for the first time. With highly purified enzyme fractions, AMP or IMP displays kinetic effects characteristic of an allosteric activator. The kinetic curve shifts from cooperative to Michaelis-Menten, V_{max} is unchanged, and K_m decreases.

Introduction

Bacterial aspartase (L-aspartate ammonia-lyase, EC 4.3.1.1), recently shown to exist as a tetramer (Williams and Lartigue, 1967), has long been the center of controversy regarding the role of nucleosides and nucleotides in the reaction:

$$\text{L-aspartate} \rightleftarrows \text{fumarate} + NH_4^+$$

Whereas the aspartase activity of aged whole cells could be markedly and reproducibly increased by the addition of adenosine or AMP to cell suspensions, the same activation could not be obtained with cell-free extracts. The numerous reports concerning these observations were summarized by Ellfolk in 1956. In time, the activation was attributed to secondary effects.

Results and Discussion

In 1959 one of us succeeded for the first time in preparing from Bacterium cadaveris, strain Gale, cell-free aspartase fractions, the activity of which was greatly enhanced by the addition of certain nucleosides and nucleotides (Scott, 1959). In this unpublished report, aspartase was obtained from bacterial

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sonic lysates and purified according to a scheme including: pH fractionation, ammonium sulfate and protamine sulfate fractionation, alumina Cγ gel treatment, calcium phosphate gel treatment, and DEAE-cellulose treatment. The final specific activity was 8.3 μmoles of fumarate per min per mg of protein at pH 7.2 and 25°. A portion of this fraction was dialyzed against 10 mM EDTA, pH 7.2 for four days at 4°, at the end of which time its activity was measured in the presence of various nucleotides and nucleosides (Table I).

Table I

Effect of Nucleoside and Nucleotide Additions on Aspartase Activity[a]

Addition	Final Conc. of Added Substance	Protein Conc.	Per Cent Activity[b]
	mM	μg/ml	
Exp. 1			
None	------	6.0	100
IMP	0.167	6.0	180
AMP	0.167	6.0	154
Adenosine	0.167	6.0	150
Exp. 2			
None	------	133.0	100
GDP	0.333	133.0	136
GTP	0.333	133.0	40
UTP	0.333	133.0	77

[a]These data are the most significant of those obtained in a large screening experiment (Scott, 1959).

[b]Assay system: 80 mM L-aspartate, pH 7.2. Activity measured as rate of fumarate production at 240 mμ in the Beckman spectrophotometer, Model DU.

The addition of IMP showed marked stimulation, AMP and adenosine were slightly less effective, and GDP showed some stimulation. GTP and UTP were inhibitory.

At the time this work was done the hypothesis that IMP served as an intermediate ammonia acceptor and AMP as an intermediate ammonia source was explored.

Incubating AMP or IMP and aspartase with or without substrate did not result in detectible interconversion of cofactors. Adenylosuccinate, a likely intermediate in the proposed mechanism, was synthesized by the method of Carter and Cohen (1956). Although this preparation was utilized by a yeast extract containing adenylosuccinase, neither crude nor purified aspartase degraded the adenylosuccinate. Allosteric effectors were then unknown, so there seemed to be no clear explanation of the cofactor function.

Recent studies on the kinetics of the aspartase reaction have revealed cooperative effects of substrate which are especially pronounced at pH values above 8.0 (Williams and Lartigue, 1967). Concentration-velocity plots are not truly hyperbolic within the pH 6.0 to 7.5 range, but drift upward at the high concentration end. Above pH 7.5 a distinct sigmoidicity appears which is increasingly pronounced as pH rises. These results, along with the polymeric structure of aspartase, suggested that the system might truly be subject to nucleotide regulation, as are so many other enzymes possessing quaternary structure.

Initial attempts at Louisiana State University to repeat the nucleotide activation experiments described above were unsuccessful, but within the last year nucleotide-sensitive fractions have been obtained. Sonic lysates of B. cadaveris, strain Gale, were purified according to the method of Williams and Lartigue (1967). In an additional step, fractions from the Ecteola cellulose column were loaded onto a DEAE-Sephadex A-50 column and eluted with a 0 - 0.5 M KCl gradient in 0.05 M KPO_4 buffer, pH 7.0. Final specific activity at pH 7.0 and 25° was 20 μmoles of fumarate per min per mg, representing a 100-fold purification from starting material. Disc electrophoresis on polyacrylamide gel showed about 80% major component with five faint minor bands.

The addition of AMP or IMP at 0.333 mM final concentration at pH 8.7 removed nearly all traces of sigmoidicity from the velocity-concentration curve. As may be seen in Fig. 1, the corresponding Lineweaver-Burk plot for the system containing IMP was nearly linear, whereas in the absence of effector, the curve was para-

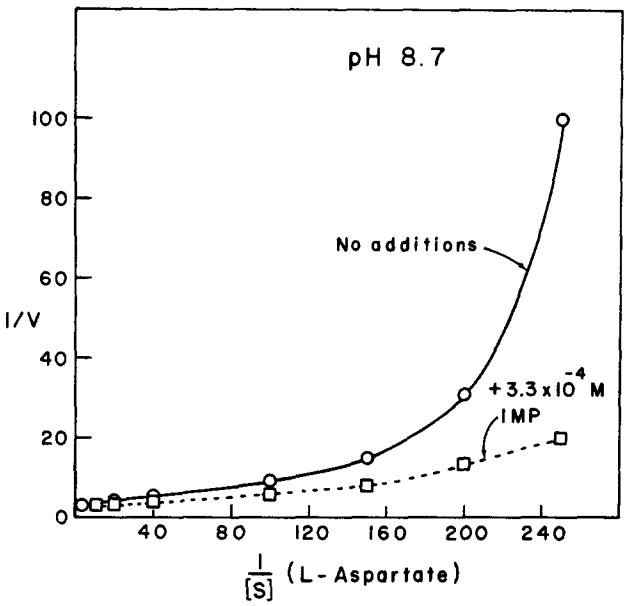

Fig. 1. Lineweaver-Burk plot for aspartase system, with and without IMP. All assays were carried out in 0.05 M Tris-HCl buffer, pH 8.7, containing 2 mM $MgSO_4$ and 0.1 mM EDTA. Protein content was 2.3 µg per ml. Velocity was measured in units of ΔA_{240} per min. Substrate concentration is in moles per liter. Temperature, 25°.

bolic. Replacement of IMP with AMP gave identical results. In the light of present knowledge, this is the typical pattern of heterotropic action of a nucleotide effector in which V_{max} is unchanged, K_m decreases, and the kinetic curve shifts from cooperative to Michaelis-Menten (Frieden, 1967; Kirtley and Koshland, 1967). At pH 7.0 the effect of AMP and IMP was much less dramatic. A Hill plot for data obtained at pH 8.7 in the absence of nucleotide had a slope of 2.0, strengthening the case for a multisite system.

Present pursuit of this problem is directed toward an improved method of preparation of nucleotide-sensitive aspartase. Many preparations show no response to the addition of either AMP or IMP, possibly because of unremoved traces of nucleic acid. Experiments on GTP inhibition are in progress.

References

Carter, C. E., and Cohen, L. H., J. Biol. Chem., 222, 17 (1956).
Ellfolk, N., Ann. Acad. Sci. Fennicae, Ser. A., II, 79, 1 (1956).
Frieden, C., J. Biol. Chem., 242, 4045 (1967).
Kirtley, M. E., and Koshland, D. E., Jr., J. Biol. Chem., 242, 4192 (1967).
Williams, V. R. and Lartigue, D. J., J. Biol. Chem., 242, 2973 (1967).
Scott, R. M., Ph. D. Dissertation, University of Illinois, 1959; University of Michigan Microfilms No. 59, 4566.