THE SUBSTRATE SPECIFICITY OF L-ASPARAGINASE FROM *ALCALIGENES EUTROPHUS*

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1. Introduction

The tumor suppressive effects of L-asparaginase have been well documented [1]. All clinical studies reported to date have utilized the enzyme from *Escherichia coli.* However, continual therapy with the enzyme from this source has commonly resulted in problems of toxicity, including immunological sensitivity to the foreign protein [2], indicating the need for alternative sources of L-asparaginase. Towards that end we have been examining the serological and chemical properties of a variety of bacterial L-asparaginases. The present paper deals with some of the kinetic properties of L-asparaginase from *Alcaligenes eutrophus* (formely *Hydrogenomonas eutropha*) [3]. A comparison of the substrate specificities for L-asparaginase from *A. eutrophus* and *E. coli* showed striking differences.

2. Materials and methods

A. eutrophus (Strain No. NRRL B-2804) was the generous gift of Dr. Robert E. Peterson, United States Department of Agriculture, Agricultural Research Service, Northern Utilization and Research Division, Peoria, Illinois. The organism was grown on corn steep medium according to the procedure of Roberts et al. [4] and the L-asparaginase has been purified to homogeneity by procedures to be described in detail elsewhere [5]. The *E. coli* enzyme (30 units/mg) was obtained from Worthington Biochemical Corp., Freehold, New Jersey, USA and used without further purification. L-Asparagine was purchased from Mann Research Laboratories, New York; D-asparagine, L-glutamine, *N*-acetyl-L-asparagine, NADH, and ammonia-free bovine liver glutamic dehydrogenase in glycerol were from Sigma Chemical Co., St. Louis, Missouri; and L-alanyl-L-asparagine was obtained from International Chemical and Nuclear Corp., City of Industry, California.

Enzymatic activity was determined by the NADH dependent coupled assay procedure of Boyd [6] for both L-asparagine and the other substrates tested.

3. Results and discussion

The marked differences in the substrate specificities of *E. coli* and *A. euthropus* asparaginase can be seen in table 1. *E. coli* asparaginase has a sharp specificity for L-asparagine; D-asparagine and L-glutamine are poor substrates. The broader specificity of the *A. eutrophus* enzyme is shown by its activity with all three substrates. The glutaminase activity of the *A. eutrophus* asparaginase does not appear to be due to contamination since the preparation was judged to be homogeneous by isoelectric focusing, SDS-polyacrylamide electrophoresis and its sedimentation behavior.

Also of interest is the finding that while *N*-acetyl-L-asparagine is a poor substrate for both the *E. coli* and *A. eutrophus* enzymes, L-alanyl-L-asparagine is deamidated at rates comparable to free L-asparagine. To our knowledge this is the first report of purified L-asparaginases showing activity with dipeptides. No *N*-terminal asparagine peptides or peptides with internal asparagine residues were available for examination.

The presence of high glutaminase activity in the

Table 1
Relative activities of *A. eutrophus* and *E. coli* L-asparaginase toward various substrates[a]

Substrate	*A. eutrophus* L-asparaginase[b]		*E. coli* L-asparaginase[c]	
	ΔA_{340}/min	% Activity	ΔA_{340}/min	% Activity
L-Asparagine	0.831	100	0.821	100
D-Asparagine	0.437	53	0.086	10
L-Glutamine	0.748	90	0.031	4
N-Acetyl-L-asparagine	0.011	1	0.011	1
L-Alanyl-L-asparagine	0.858	103	0.771	94

[a] All substrates at 2 mM.
[b] K_m for L-asparagine: 0.9×10^{-3} M.
[c] K_m for L-asparagine: 1.7×10^{-4} M [6].

A. eutrophus asparaginase may be of particular significance in view of the recent report of the antineoplastic activity of purified bacterial glutaminases [7]. The only report of a purified L-asparaginase with glutaminase activity comparable to that found in the *A. eutrophus* enzyme is that of Ramadan et al. for a *Pseudomonas* asparaginase [8]. The unusual properties and ease of purification of the *Alcaligenes eutrophus* L-asparaginase suggest that this enzyme may prove to be an alternative to *E. coli* asparaginase for therapeutic purposes and evaluation of the antineoplastic and immunosuppressive activities are being undertaken.

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