SUBUNIT AND AMINO ACID COMPOSITION OF L-ARGININE DEIMINASE OF *PSEUDOMONAS PUTIDA*

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1. Introduction

The preparation and some enzymatic properties of crystalline L-arginine deiminase (L-arginine imino-hydrolase, EC 3.5.3.6) of *Pseudomonas putida* have been described [1,2]. The properties of this enzyme are different from those of *Streptococcus* enzyme [3] in inhibition by carbonyl reagents, and from those of *Mycoplasma hominis* [4] and *Mycoplasma arthritidis* [5] in molecular size.

This paper describes the subunit structure, and amino acid composition of L-arginine deiminase of *Ps. putida*. It also describes the identification of the amino- and carboxyl-terminal amino acids of this enzyme, and quantitation of these residues.

2. Materials and methods

Crystalline L-arginine deiminase of *Ps. putida* was prepared as in [2]. All chemicals unless otherwise specified were Katayama (Osaka) certified reagent grade. Phenylthiohydantoin derivatives of all the amino acids were obtained from Seikagaku Kogyo (Osaka). SDS–polyacrylamide gel electrophoresis was carried out by the method in [6]. The enzyme (100 μg) was treated with 1% SDS with or without 1% β-mercaptoethanol in 0.1 M sodium phosphate buffer (pH 7.2) at 37°C for 2 h and placed on the top of gel. Electrophoresis was performed at room temperature at 8 mA/tube for 7 h. Amino- and carboxyl-terminal amino acid were determined, respectively, by the phenylisothiocyanate procedure in its three-stage form and by hydrazinolysis as in [7]. Amino acid composition was determined as below. Samples were mixed with an equal volume of constant boiling hydrochloric acid, and hydrolyzed at 110°C for 24–96 h in evacuated sealed Pyrex tubes. The hydrolyzate was evaporated to dryness twice in a rotary evaporator at 50°C. The residue was dissolved in 0.2 M citrate buffer (pH 2.2) and analyzed in the Hitachi amino acid analyzer, model KLA-3. Cysteine was determined as carboxymethylcystesine after carboxymethylation of the enzyme with iodoacetic acid by the method in [8]. Tryptophan was determined spectrophotometrically [9] and by the *p*-dimethylaminobenzaldehyde method [10].

3. Results and discussion

3.1. *Subunit studies*

SDS–polyacrylamide gel electrophoresis revealed that the enzyme dissociated into a single protein band corresponding to mol. wt 54 000 both in the presence [2] and the absence of β-mercaptoethanol. Since the active enzyme was estimated to be mol. wt 120 000 from sedimentation equilibrium and gel filtration studies [1], L-arginine deiminase of *Ps. putida* is considered to be a dimeric enzyme.

3.2. *Terminal amino acid*

Phenylthiocarbamylation of the enzyme led to recovery of 89% tyrosine/mol enzyme on hydrolysis. A qualitative determination of the amino-terminal amino acid by the Sanger method yielded a single spot, corresponding in R_F values to dinitrophenyl-tyrosine. It is evident from the findings that L-arginine

deiminase has a single amino-terminal amino acid, tyrosine. The recovery of added PTH-tyrosine was 79% and the observed tyrosine recovery from the deiminase was corrected on this basis.

Duplicate determinations of carboxyl-terminal amino acid were made on the protein alone and on the protein plus added amounts of tyrosine. The recovery of added tyrosine in the presence of enzyme was 46% and the observed tyrosine recovery from the deiminase was corrected on the basis. The average figure, 0.80 mol tyrosine/polypeptide (mol. wt 54 000), appears to be low, but it is likely that the 10 h reaction period was insufficient for the protein as it seems to be for others [11]. Of the other amino acid, glycine, serine and valine were present in only trace amounts. The analyses for amino- and carboxyl-terminal amino acid of L-arginine deiminase suggest that this enzyme is composed of apparently identical subunit.

3.3. *Amino acid composition*

Table 1 summarizes the results of amino acid analyses obtained for 4 periods of acid hydrolysis. The minimum molecular weight on the basis of the tryptophan content is ~54 000, which agrees closely with the molecular weight obtained by gel electrophoresis.

The results suggest that L-arginine deiminase (mol. wt 120 000) contains 2 apparently identical subunits (mol. wt 54 000) and that there are no interchain disulfide bonds. This observation is similar with that on the subunit structure of the enzyme of *Mycoplasma arthritidis* [12], but molecular weight of subunit and native enzyme of *Pseudomonas* are somewhat larger than those of *Mycoplasma*. Major difference is the amino-terminal amino acid, that is, tyrosine for the *Pseudomonas* and alanine for the *Mycoplasma*. The fact that the methionine content is the same [12] suggests the possibility that the amino acid sequence of both enzymes may be common in part.

In anaerobes like *Mycoplasma*, L-arginine deiminase is a member of arginine dihydrolase system and plays a role in energy acquisition from L-arginine [4], but in aerobes like *Pseudomonas* the role of L-arginine deiminase is obscure since L-citrulline is not further metabolized [13]. It is possible that L-arginine deiminase is evolved from a common ancestor to different enzymes conserving a subunit structure and possibly amino acid sequence.

Table 1
Amino acid composition of L-arginine deiminase of *Ps. putida*

Amino acid	Extrapolated value (tryptophan = 6.0)	No. residues (integral) mol/monomer
Tryptophan	6.0,[a] 6.1[b]	6
Lysine	27.9	28
Histidine	13.7	14
Arginine	27.3	27
Aspartic acid	49.8	50
Threonine[c]	29.9	30
Serine[c]	20.9	21
Glutamic acid	48.0	48
Proline	32.4	32
Glycine	48.0	48
Alanine	32.1	32
Half-cystine	5.7[d]	6
Valine	39.0	39
Methionine	11.2	11
Isoleucine	25.9	26
Leucine	43.3	43
Tyrosine	13.1	13
Phenylalanine	18.3	18
Total		492

[a] From spectrophotometric values
[b] Values obtained by the *p*-dimethylaminobenzaldehyde method
[c] Extrapolated to zero time of hydrolysis
[d] Carboxymethylcysteine recovered from hydrolysates of reduced, carboxymethylated derivative

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