AMINO ACID COMPOSITION OF CYTOPLASMIC YEAST PYRUVATE DECARBOXYLASE

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1. Introduction

Cytoplasmic yeast pyruvate decarboxylase (EC. 4.1.1.1) has been isolated as a pure (but still amorphous) protein [1]. For a variety of measurements on this enzyme, the knowledge of its amino acid composition was required. The results of the total amino acid analysis, carried out on 4 different enzyme preparations from 2 yeast strains, are given and discussed in this paper.

2. Materials and methods

Pyruvate decarboxylase was isolated essentially as described earlier [1] from *Saccharomyces carlsbergensis*:

(a) fresh brewer's yeast, strain Weihenstephan T34, obtained from Ganter-Brauerei, D-78 Freiburg, with a slight modification of the gel filtration procedure;

(b) dried brewer's yeast, regular strain, obtained from Anheuser-Busch Inc., St. Louis, Mo., USA.

The best fractions of 3 different enzyme preparations from source (a) and one from source (b) were exhaustively dialysed against water at 3° for 2–3 days and freeze-dried. Two thirds of the original specific activity of 62, 59, and 74 units/mg [1] for (a) (the highest specific activity reached as yet was 85 units/mg) and 60 units/mg for (b) were lost during this procedure, the apoenzyme lost all activity by this treatment. 7–10 mg of the resulting hygroscopic white powder were hydrolysed in a sealed vial with 2 ml of 6 N HCl under 10–20 mm Hg of purified N_2 at 110° for (a) 24, 48, 72 and 96 hr; and 20 hr for (b). After evaporation of the solvent, the residue was dissolved in citrate buffer, pH 2.2, and freed of insoluble material by (a) filtration; (b) centrifugation. Aliquots of the resulting solution were applied to a two-column Beckman Unichrom amino acid analyser under standard conditions. For valine, leucine and isoleucine, liberated more slowly than the other amino acids, the constant yields obtained after 48, 72 and 96 hr of hydrolysis were taken. The values of unstable amino acids (serine, threonine and tyrosine and to a lesser extent, methionine and arginine) were obtained by graphic extrapolation to zero time on semilogarithmic graph paper. For exact determination of cysteine/cystine, several samples of (a) were subjected to performic acid oxidation 22 hr prior to hydrolysis. The values found for ammonia tended to be too high, mainly due to decomposition of several amino acids; and hence were not included in the results section. Tryptophan was determined separately by (a) spectrophotometry in 6 M guanidinium chloride (purchased from Sigma Chemical Co., St. Louis, Mo.) at 280 and 288 nm [3] (producing an additional tyrosine value which confirmed the one found by the other method); (b) alkaline hydrolysis [4] followed by standard amino acid analysis.

3. Results and discussion

The mole per cent of amino acids, found in yeast pyruvate decarboxylase, are compiled in table 1. Based

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Table 1
Mole % of amino acid residues found in yeast pyruvate decarboxylase.

Amino acid	(a)*	(b)	Amino acid	(a)*	(b)
Lysine	6.18	6.85	Alanine	9.74	9.71
Histidine	2.12	2.28	Cysteine/cystine	0.85	0.78
Arginine	2.49	2.80	Valine	7.85	6.43**
Aspartate	10.36	10.47	Methionine	2.02	2.10
Threonine	7.22	7.42	Isoleucine	6.39	5.85**
Serine	4.81	5.06	Leucine	9.63	9.63
Glutamate	9.48	10.08	Tyrosine	2.85	2.94
Proline	4.75	4.64	Phenylalanine	4.09	4.02
Glycine	7.75	7.58	Tryptophan	1.42	1.36

* Average of 12 analyses, taken from 3 enzyme preparations (a).
** Too low because of incomplete hydrolysis (only 20 hr).

Table 2
Number of amino acid residues per molecule of pyruvate decarboxylase*.

Amino acid	(a)	(b)	Most probable value	Amino acid	(a)	(b)	Most probable value
Lysine	96	104	100	Alanine	152	148	150
Hystidine	32	34	34	Cysteine/ cystine	14	12	14
Arginine	38	42	40	Valine	122	98**	122
Aspartate	162	158	160	Methionine	32	32	32
Threonine	112	112	112	Isoleucine	100	88**	100
Serine	76	76	76	Leucine	148	146	148
Glutamate	148	152	150	Tyrosine	46	44	46
Proline	74	70	72	Phenylalanine	64	62	62
Glycine	120	116	118	Tryptophan	22	20	22
Total	1558	1514	1558	Mol. Weight	170,000	166,000	170,000

* Calculated from table 1 assuming 170,000 as the molecular weight.
** Too low because of incomplete hydrolysis (only 20 hr).

on these values, the total content of the common amino acids was calculated for a molecular weight of 170,000 and two subunits [5], which were assumed to be identical (table 2). The numbers in the last column of this table should be a very close approximation to the true amino acid composition of this enzyme. Almost no differences exceeding the normal limits of experimental error were found between the several enzyme preparations, even from different strains of yeast.

The amino acid composition found can be considered rather normal for a soluble globular protein. The most striking feature is the low value of cysteine/cystine. The relatively high content of hydrophobic amino acids (circa 54%) may be taken as an indication of a low ratio of surface area to internal volume, resulting in a compact structure, probably close to spherical. Measurements of the radius of gyration [6] confirmed this interpretation. The relatively high values for aromatic amino acids explain why the absorbance near 280 nm was found somewhat higher [7] than in average proteins (serum albumin). The observed percentages of proline, serine, and threonine (together 16.7%) suggest a quite low degree of helicity [8,9]. Measurements of α-helix content of the intact

holoenzyme by different physical methods [7] gave values between 20 and 30%, and a little less for the undenatured apoenzyme. From the values in table 2, a partial specific volume for the enzyme of (a) 0.742 and (b) 0.737 was calculated [10,11]. This is in the normal range for globular proteins.

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