A TRIMERIC STRUCTURE FOR MAMMALIAN PURINE NUCLEOSIDE PHOSPHORYLASE

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Received 4 April 1973

1. Introduction

Purine nucleoside phosphorylase (EC 2.4.1.2) catalyses the phosphorolytic cleavage of inosine to hypoxanthine and ribose 1-phosphate. This enzyme has been the subject of various studies [1–6] and has been purified from rat, pig [1] and calf [2] liver and from human erythrocytes [3, 4]. The molecular weight of the enzyme has been estimated as 84,000 by gel filtration chromatography using crude tissue extracts [5] and purified preparations from calf spleen and human erythrocytes [7]. Biochemical genetic studies of the human enzyme suggest that nucleoside phosphorylase is a trimeric molecule [6]. The present study undertook to investigate this suggestion by establishing the molecular weight of the native enzyme using ultracentrifugation and by estimating the molecular weight of the subunit after electrophoresis in sodium dodecyl sulphate [8].

2. Methods

A pure crystalline preparation of purine nucleoside phosphorylase (calf spleen) was purchased from Boehringer Ltd. The enzyme was dialysed exhaustively against 50 mM sodium phosphate, pH 7.5.

A Beckman Model E analytical ultracentrifuge equipped with interference optics was used for the sedimentation experiments. The molecular weight of the native enzyme was measured according to the meniscus depletion method described by Yphantis [9]. Nucleoside phosphorylase, 0.6 mg·ml^{-1}, in 50 mM phosphate pH 7.5 was centrifuged to equilibrium at 22,000 rpm at 20°.

The enzyme was carboxymethylated using 10 mM iodoacetic acid in 8.0 M urea, 0.1 M Tris-HCl pH 8.0 according to the method of Anderson et al. [10]. The amino acid composition of the carboxymethylated enzyme was determined by analysis using a Beckman 120 C automatic analyser. The protein was hydrolysed with 6 N HCl in a sealed evacuated tube at 105° for 24 hr, prior to analysis.

Cellulose acetate electrophoresis was carried out on Gelman Sepiapore II cellulose acetate strips at 250 V for 90 min using 0.06 M barbital buffer pH 8.6. Protein bands were detected with Ponceau S stain. 50 μg samples of the native protein in 0.01 M sodium phosphate pH 7.5 containing 0.1% sodium dodecyl sulphate and 1.0% mercaptoethanol were heated at 100° for 1 min. These samples were then electrophoresed in 7.5% polyacrylamide gels in the presence of 0.1% sodium dodecul sulphate at 4 mA per gel for 4 hr [8]. Protein bands were detected with Coomassie Blue.

3. Results

The homogeneity of the protein was examined by electrophoresis on cellulose acetate strip. A single pro-

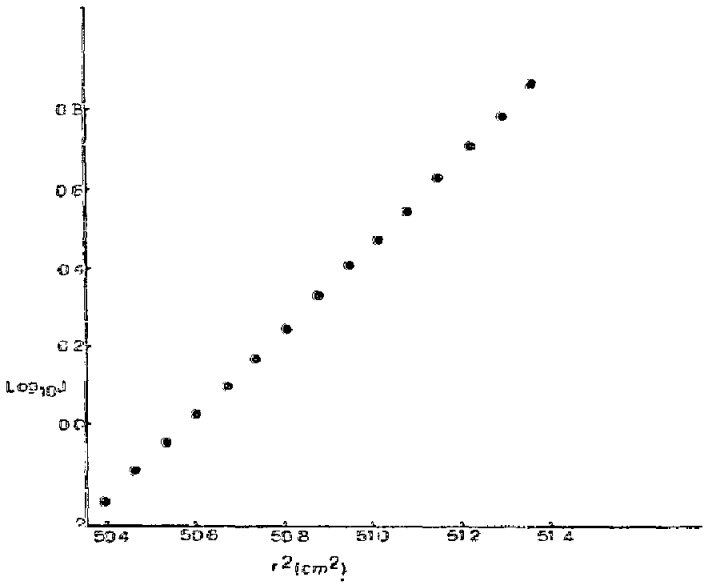

Fig. 1. A plot of $\log_{10} J$ versus r^2, where J is the fringe displacement at the corresponding radial position r.

Fig. 2. Polyacrylamide electrophoresis of calf spleen nucleoside phosphorylase in the presence of sodium dodecyl sulphate. A) Marker proteins, bovine serum albumen (68,000), rabbit glyceraldehyde 3-phosphate dehydrogenase (36,000), hen egg white lysozyme (14,000); subunit molecular weight shown in parentheses. B) Purine nucleoside phosphorylase. C) A mixture of purine nucleoside phosphorylase and marker proteins.

Table 1

Amino acid composition of calf spleen purine nucleoside phosphorylase based on a molecular weight of 84,600.

Amino acid	% total weight	(moles/mole)
Lysine	5.9	34.1
Histidine	3.5	19.1
Arginine	7.0	34.0
Carboxymethylcysteine	1.8	9.2
Aspartic acid	9.0	57.5
Threonine	4.9	34.6
Serine	6.4	51.8
Glutamic acid	14.73	84.7
Proline	4.7	34.8
Glycine	6.2	70.0
Alanine	4.9	46.1
Valine	6.3	45.8
Methionine	3.1	17.8
Isoleucine	3.1	19.7
Leucine	8.1	52.3
Tyrosine	4.2	19.7
Phenylalanine	6.2	31.5
Tryptophan	not determined	

tein band was observed which had moved a distance of 2.9 cm towards the anode after electrophoresis for 90 min.

The ultracentrifuge study provided data from which a plot of $\log_{10} J$ versus r^2 was constructed, where J is the fringe displacement at the corresponding radial position r, (fig. 1). The plot was linear (regression coeff. 0.999952) which can be taken as additional evidence of homogeneity. The partial specific volume ($\bar{v}$) of the protein was calculated to be 0.725 $ml \cdot g^{-1}$. from the percent total weight amino acid composition given in table 1. Using this value of $\bar{v}$ the molecular weight calculated from the slope of the $\log_{10} J$ versus r^2 plot was 84,600 ± 3,000 (estimated error). The amino acid composition of carboxymethylated purine nucleoside phosphorylase based on this molecular weight is given in table 1. Corrections which take into account the partial destruction of threonine and serine on hydrolysis and the incomplete hydrolysis of valine and isoleucine have been made.

Polyacrylamide gel electrophoresis of the native enzyme in the presence of sodium dodecyl sulphate resulted in a single protein band. The mobility of this band relative to the marker proteins (bovine serum albumen, rabbit glyceraldehyde 3-phosphate dehydrogenase and hen egg white lysozyme) corresponded to

a subunit molecular weight of 28,000. A photograph of typical polyacrylamide gels is shown in fig. 2. This estimate of the subunit molecular weight is in good agreement with the expected molecular weight of a subunit of a trimeric molecule of molecular weight 84,600.

4. Discussion and conclusions

The results of the present study on calf nucleoside phosphorylase (NP) are entirely consistent with those obtained previously from electrophoretic studies on genetic variants of human NP [6], from *in vitro* [6] and somatic cell [11] hybridisation experiments with mouse and human NP and from the substrate binding characteristics of purified human NP [7]. They lead to the firm conclusion that NP is a trimeric molecule with a total molecular weight in the region of 84,000 and a subunit size of about 28,000. Judging from a recent survey of the literature [12] structures of this kind are relatively uncommon since only seven possible examples of trimers were found among 109 different proteins exhibiting a multimeric structure and in most of these examples there appeared to be some doubt whether the protein was trimeric or tetrameric.

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