

THE POLYPEPTIDE CHAIN MOLECULAR WEIGHT OF A MAMMALIAN HEXOKINASE

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1. Introduction

Hexokinase (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1) is responsible for initiating glucose metabolism in the mammalian cell and is therefore a potential site of regulation of glycolysis by allosteric effectors. In order to understand the mechanism of such regulation it is necessary to determine the number of subunits in the enzyme and to investigate the nature of their interactions. Considerable knowledge has been gained about such interactions in yeast hexokinase [1, 2] but little is known of the properties of mammalian hexokinases owing to their instability and difficulty of purification. The enzyme has been obtained previously in homogeneous form from bovine brain [3] but no detailed examination of its molecular properties has been reported.

A further complication encountered in mammalian tissues is the occurrence of four major isoenzyme forms. Type I hexokinase is the most widely distributed of these, type II is a form thought to be sensitive to insulin, type III is a substrate-inhibited form and type IV is commonly known as glucokinase [4]. For the purpose of the present investigation, type I hexokinase was purified from porcine heart (Easterby, unpublished work) and its polypeptide chain molecular weight determined. This revealed that native type I hexokinase from heart is a single polypeptide chain of 97,000 molecular weight which is subject under certain conditions to extensive polymerisation. This is in marked contrast to yeast hexokinases which exist as dimers of 108,000 molecular weight [5, 6] and show no tendency towards aggregation.

2. Materials and methods

Hexokinase was purified from porcine heart (Easterby, unpublished work) and had a specific activity of at least 60 units/mg (a unit being defined as the amount of enzyme catalysing the phosphorylation of 1 μ mole of glucose per min at 30°).

N,N'-Methylenebis-acrylamide, acrylamide, ammonium persulphate, sodium dodecyl sulphate (SDS), bromophenol blue, β -mercaptoethanol, dithiothreitol, ethylenediaminetetraacetic acid (EDTA) and lysozyme were obtained from British Drug Houses Ltd. (Poole, England). *N,N,N',N'*-Tetramethyl ethylenediamine was a product of Kodak Ltd. (London). Alcohol dehydrogenase (yeast), α -chymotrypsinogen A, serum albumin (human) and ovalbumin were obtained from Sigma Chemical Co. (London). Pyruvate kinase was a product of C.F. Boehringer and Sons (Mannheim, Germany). Sephadex G-200 was a product of Pharmacia (Uppsala, Sweden). All other reagents were British Drug Houses AnalaR grade.

Ultracentrifugation was conducted in a Spinco Model E analytical ultracentrifuge equipped with monochromator and photoelectric scanner on the absorption optics. All sedimentation velocity experiments were conducted at a rotor speed of 60,000 rpm and at a temperature of 10°. Low speed equilibrium ultracentrifugation was carried out according to the method of Van Holde and Baldwin [7]; high speed runs were performed as described by Yphantis [8]. Data obtained from the schlieren optical system were treated by the method of Lamm [9], data from the photoelectric scanner were analysed using the equation

$$M_w = \frac{2RT \times \text{d} \ln c / \text{d} r^2}{\omega^2 (1 - \bar{v} \rho)}$$

For the purpose of preliminary investigations a partial specific volume of 0.74 was assumed for the enzyme.

SDS-polyacrylamide electrophoresis was performed as described by Weber and Osborn [10]. It was found necessary to keep samples in SDS containing buffers at room temperature for 24 hr to ensure complete denaturation and binding of SDS.

Hexokinase was maleylated by stirring a solution containing 3 mg of enzyme in 0.2 M K_2HPO_4 , 1 mM EDTA onto 2 mg of maleic anhydride. The solution was stirred at room temperature for a further 20 min and then dialysed against the same buffer containing 5 mM mercaptoethanol.

3. Results

Type I hexokinase isolated from porcine heart was shown to be homogeneous by starch gel electrophoresis,

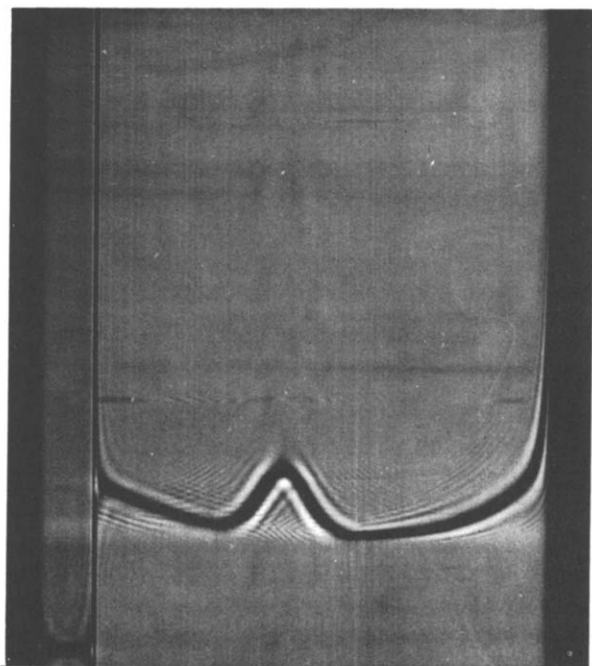


Fig. 1. Sedimentation velocity of heart hexokinase. Hexokinase sediments as a single, homogeneous boundary of $S_{20,w}$ 5.3 S. The photograph was taken 90 min after reaching a rotor speed of 60,000 rpm. Buffer was phosphate, pH 8, ionic strength 0.1, 0.1 M glucose, 5 mM EDTA. Temperature was 10° and wire angle 60°. Protein concentration was 2.5 mg/ml.

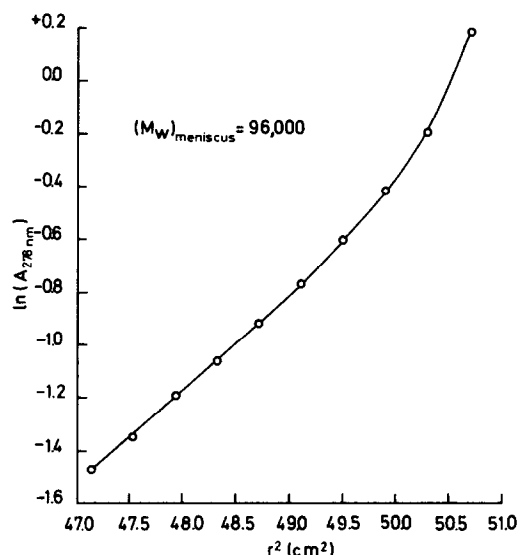


Fig. 2. Low speed sedimentation equilibrium of heart hexokinase. The diagram shows the presence of polymeric hexokinase. The meniscal molecular weight is close to that expected for the hexokinase monomer. The protein concentration was 0.5 mg/ml, rotor speed 8,000 rpm and temperature 10°. The buffer was phosphate, pH 8, containing glucose and mercaptoethanol.

gel filtration and ultracentrifugation (fig. 1). On electrophoresis hexokinase migrated as a single protein band at all pH values in the range 5–9 and was isoelectric in acetate at pH 6. At high protein concentrations on polyacrylamide gels there was some evidence of polymer formation.

At pH 8 and 0.1 ionic strength in phosphate containing 0.1 M glucose and 5 mM mercaptoethanol, hexokinase sedimented as a single boundary of $S_{20,w}$ 5.3 S (fig. 1). The sedimentation properties were independent of the presence of mercaptoethanol and glucose and also of pH in the range 7–9. On investigation of the molecular weight by low speed equilibrium centrifugation [7] the sample appeared heterogeneous as indicated by an increase in molecular weight through the solution (fig. 2). The molecular weight at the solution meniscus was dependent on rotor speed and protein concentration. Thus at 8,000 rpm and 3 mg/ml the meniscal molecular weight was 105,000 while at 0.5 mg/ml it fell to 96,000 (fig. 2). When the high speed method of Yphantis [8] was used to examine the molecular weight at the solution meniscus the

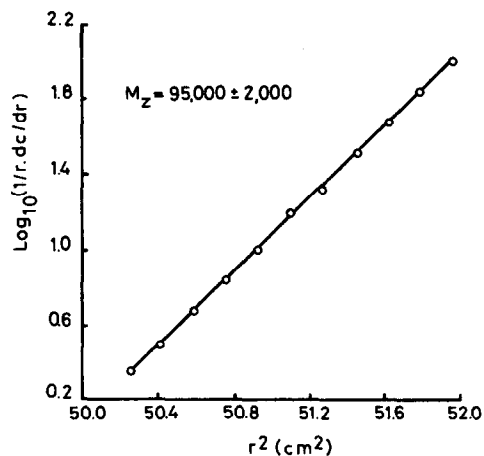


Fig. 3. High speed sedimentation equilibrium. The diagram demonstrates the homogeneity of material at the solution meniscus. Protein concentration was 2 mg/ml, rotor speed 20,000 rpm and temperature 10° . The buffer was phosphate, pH 8, containing mercaptoethanol and glucose.

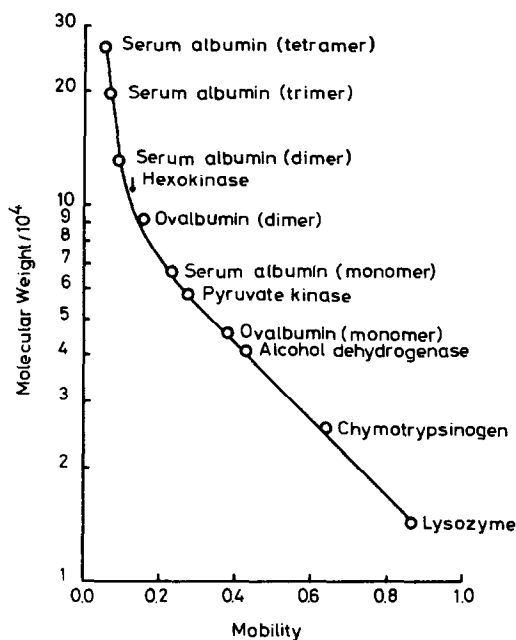


Fig. 4. SDS-polyacrylamide gel electrophoresis of heart hexokinase. The diagram demonstrates the presence of a single polypeptide chain of 97,000 molecular weight. Mobilities are expressed relative to bromophenol blue marker dye.

material appeared homogeneous with molecular weight $95,000 \pm 2,000$ (fig. 3). Similarly, molecular weight determination by measurement of sedimentation and diffusion coefficients gave a molecular weight of 97,000. This suggested that during sedimentation equilibrium experiments a slow polymerisation of a monomer of about 97,000 molecular weight was occurring. The monomeric form of the enzyme was stable when frozen but polymerisation occurred at 10° during the attainment of sedimentation equilibrium. Similarly during purification of the enzyme at 4° the slow formation of polymers was observed. These polymers eluted from columns of Sephadex G-200 in the void volume. Neither mercaptoethanol nor dithiothreitol prevented polymerisation and it is therefore unlikely that disulphide linkages were the primary cause of the effect.

The polypeptide chain molecular weight of the enzyme was determined by electrophoresis in SDS-polyacrylamide gels [10]. Initially two protein bands were observed corresponding to molecular weights of 92,000 and 100,000. If the enzyme was heat-treated (100° for 5 min) prior to dialysis against SDS-containing buffer, a single band of $97,000 \pm 5,000$ molecular weight was observed (fig. 4). No species corresponding to a subunit of the native enzyme was detectable. Hexokinase ran as a very sharply defined band on SDS-polyacrylamide and this was also thought to be indicative of a high molecular weight polypeptide chain.

When the subunit size of the enzyme was investigated by maleylation [11] and centrifugation in 0.2 M K_2HPO_4 a single sedimenting boundary of $S_{20,w} 3.8$ S was observed. No decrease in molecular weight was observed and the maleyl-enzyme continued to polymerise in a manner similar to native hexokinase.

Unlike yeast hexokinase [1] the heart enzyme was not dissociated by increasing ionic strength. In the presence of molar KCl $S_{20,w}$ was 5.0 S.

4. Discussion

The above data suggest that native heart hexokinase has a molecular weight of 97,000 and is subject to slow polymerisation reactions. The ineffectiveness of thiol-containing reagents in preventing polymerisation indicates the absence of disulphide linkages as a

primary cause of polymerisation (although they may contribute towards the stability of polymers). The ready formation of polymers at higher temperatures suggests the possible involvement of hydrophobic bonds.

From the behaviour of hexokinase in the presence of SDS it is concluded that the minimum polypeptide chain molecular weight is 97,000 and that the native enzyme is consequently a single polypeptide chain. Similarly, the behaviour of the maleyl-enzyme is consistent with this idea. The reduction in sedimentation coefficient on a maleylation is probably insufficient to be the result of dissociation to a half molecule or smaller species and is most likely the result of unfolding of the polypeptide chain. The appearance of two hexokinase species in SDS in the absence of heat treatment suggests a minor contamination of the preparation by a protease capable of degrading the denatured enzyme. This was also found to be the case with yeast hexokinase and may be a more widely occurring phenomenon [5]. There was also some evidence of a slow reduction in the molecular weight of the maleyl-enzyme which might also have been occasioned by proteolysis.

The absence of any marked effect of salt on the dissociation of heart hexokinase suggests that unlike the yeast enzyme [1] this species does not contain subunits stabilised by ionic interactions.

The fact that the polypeptide chain molecular weight of heart hexokinase is almost twice that of the enzyme [5, 6] points to the possible evolution of heart hexokinase from an ancestral dimer by a process of gene duplication. If this is the case the present enzyme might be expected to have two active centres on a single polypeptide chain. Moreover, the sites of

intersubunit interaction in the ancestral form would be duplicated in the present enzyme and might lead to polymer formation by extensive heterologous interactions.

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