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HYPOXANTHINE-GUANINE PHOSPHORIBOSYLTRANSFERASE FROM BEEF BRAIN: A TRIMER.

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SUMMARY: Hypoxanthine-guanine phosphoribosyl isolated from beef brain was reacted with cross-linking reagents in order to establish the number of subunits that constitute the native protein. The results obtained from experiments with dimethylsuberimidate and gluteraldehyde in the absence and in the presence of substrates all indicate that the native structure is a trimer.

INTRODUCTION

Hypoxanthine-guanine phosphoribosyltransferase from many mammalian sources has been isolated and characterized. Gel filtration studies of the enzyme from Chinese hamster brain (1), and mouse liver (2) indicate that the enzyme from these sources exists as a trimer of native molecular weight 78,000 - 80,000. Sedimentation equilibrium centrifugation studies of the human erythrocyte enzyme (3,4) also indicate a trimer of molecular weight 81,000 - 85,000. However, sedimentation studies of the human lymphoblast enzyme (5) indicated that the enzyme existed as a dimer in a low ionic strength buffer and as a tetramer in a high ionic strength buffer. Strauss, et al. (6) found that heat treatment of the enzyme from human erythrocytes or Chinese hamster liver in the presence of the substrate, P-Rib-PP, resulted in molecular weights of 110,000 and 105,000, respectively, as determined by electrophoretic mobility as a function of acrylamide concentration. This would indicate the enzyme exists as a tetramer. Without heat treatment in the presence of P-Rib-PP, the human erythrocyte enzyme appeared to be of a trimeric structure.

Gel filtration studies of the enzyme from beef brain were carried out by Paulus, et al. (7) and the evidence indicated a trimeric structure. The authors determined a molecular weight of 86,000 for the beef brain enzyme by Sephadex G-100 chromatography in a buffer containing 50mM Tris-HCl, pH 7.4, 10mM $MgCl_2$, 1mM monothioglycerol, 25mM KCl, and 0.1M P-Rib-PP and a molecular

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weight of 85,000 in the absence of P-Rib-PP. These studies of the beef brain enzyme suggest a trimeric structure.

Thus conflicting evidence exists as to the true subunit structures of mammalian hypoxanthine-guanine phosphoribosyltransferases. Crosslinking studies of the purified beef brain enzyme were performed with the hope that the results would clarify the situation.

MATERIALS AND METHODS

GMP, P-Rib-PP (Na salt), and dimethylsuberimidate were obtained from Sigma. Acrylamide (Sigma) and bisacrylamide (Eastman) were recrystallized from acetone. Glutaraldehyde was purchased from Polysciences, Inc.

Enzyme Purification - The enzyme was purified according to the procedure of Paulus, et al. (7). The purification consisted of a heat treatment of the crude supernatant, a 40-80% ammonium sulfate fractionation, affinity chromatography on GMP-Sepharose, and ion exchange chromatography on CM-Sephadex.

Crosslinking of Hypoxanthine-guanine phosphoribosyltransferase - The purified enzyme was crosslinked with dimethylsuberimidate according to the procedure of Davies and Stark (8). To 100µl of the enzyme in 50mM triethanolamine, pH 8.0, was added 50µl of a solution consisting of 9mg/ml dimethylsuberimidate in 0.6M triethanolamine, pH 8.5. Crosslinking was allowed to occur at room temperature for 5 to 31 hours. Samples were analyzed by electrophoresis either immediately or after storage at -20°C.

Crosslinking of beef brain hypoxanthine-guanine phosphoribosyltransferase by glutaraldehyde was done according to the procedure of Holden and Kelley (9). To 100µl aliquots of the enzyme in 50mM triethanolamine, pH 8.0, was added 50µl of a solution of 1.6% glutaraldehyde in 0.6M triethanolamine, pH 8.5. The reaction was stopped after 30 seconds or 30 minutes by freezing in liquid nitrogen.

Sodium Dodecyl Sulfate Gel Electrophoresis - Cylindrical gels were run in the discontinuous buffer system of Laemli (10). The 10% acrylamide separating gels were cast in 0.5cm i.d. glass tubes to a height of 9.0cm. A 0.25ml aliquot of 5% acrylamide stacking gel was cast on top of each separating gel. Samples were dissociated and reduced by heating for 2 minutes in a boiling water bath in a buffer consisting of 0.045M Tris-HCl, pH 6.8, 10% glycerol, 1% sodium dodecyl sulfate, 1% β-mercaptoethanol and 0.01% bromophenol blue. With 12 gels in the electrophoresis chamber, electrophoresis was performed at 20mA constant current for 4 hours. Gels were stained according to the procedure of Weber and Osborn (11) and scanned using an ISCO model 1310 gel scanner in conjunction with an ISCO model UA-5 absorbance monitor.

RESULTS AND DISCUSSION

The results of crosslinking hypoxanthine-guanine phosphoribosyltransferase with dimethylsuberimidate are shown in Figure 1. The uncrosslinked control shows

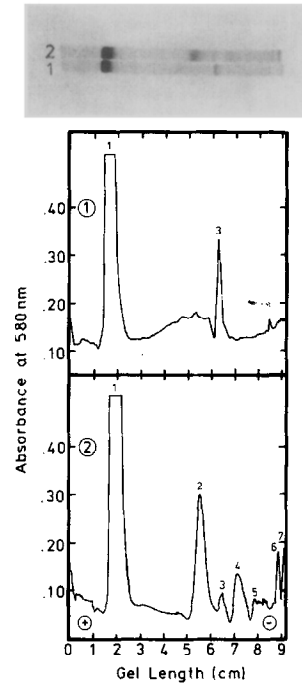


Figure 1. SDS Polyacrylamide Gel Electrophoresis of Hypoxanthine-Guanine Phosphoribosyltransferase Crosslinked with Dimethylsuberimidate

The beef brain enzyme was crosslinked with dimethylsuberimidate and subjected to SDS polyacrylamide gel electrophoresis as described under Methods. Gel 1, uncrosslinked control; Gel 2, beef brain enzyme crosslinked with dimethylsuberimidate for 10 hours. The circled numbers in the scans indicate the gel number. The protein bands are numbered in order of increasing molecular weight.

the enzyme monomer (molecular weight 27,000) and a minor contaminant which shows up very faintly in the crosslinked gels.

Scans of the gel containing crosslinked and uncrosslinked beef brain hypoxanthine-guanine phosphoribosyltransferase are also shown in Figure 1. Seven possible bands are present in the crosslinked enzyme gel. Bands 1 and 3 correspond to the enzyme monomer and the minor contaminant that is present. Bands 2 and 4 are presumably dimer and trimer while band 5 might be a tetramer. In order to determine if the major protein species observed are actually integral multiples of the monomer, the logarithm of the electrophoretic mobility of each species was plotted against its expected molecular weight. The monomer molecular weight was 27,000, calculated from a standard curve constructed from protein

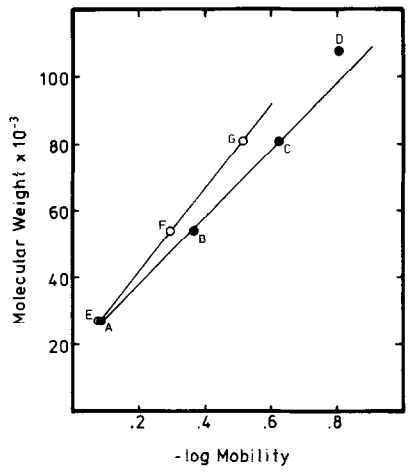


Figure 2. Plots of the Logarithm of Mobility versus Expected Molecular Weight for Crosslinked Hypoxanthine-Guanine Phosphoribosyltransferase.

The logarithms of the mobility of the protein species observed after crosslinking with dimethylsuberimidate (●) and glutaraldehyde (○) were plotted against the expected molecular weights for the monomer (27,000), dimer (54,000), trimer (81,000), and tetramer (108,000).

standards that were subjected to electrophoresis simultaneously with the cross-linked samples. Expected molecular weights of a dimer, trimer and tetramer would be 54,000, 81,000 and 108,000 respectively. As Figure 2 shows, bands 1, 2, and 4 (points A, B, C) do indeed fall on a straight line and correspond to monomer, dimer, and trimer. Band 5 does not, however, fall on this straight line (point D) and thus is not likely to be a tetramer. Bands 6 and 7 do not correspond to a tetramer (data not shown). The presence of sharp, high molecular weight bands near the tops of gels was noted also by Holden and Kelley (9) with dimethyl-suberimidate crosslinked human enzyme. They suggested the bands represent higher molecular weight forms of the enzyme. They may, however, be molecular weight multiples of the minor contaminants which are present in both the human enzyme and the beef brain enzyme.

Crosslinking with glutaraldehyde was performed to further investigate the subunit structure of beef brain enzyme. The results are shown in Figure 3. In this case, only three bands were present suggesting that the beef brain enzyme

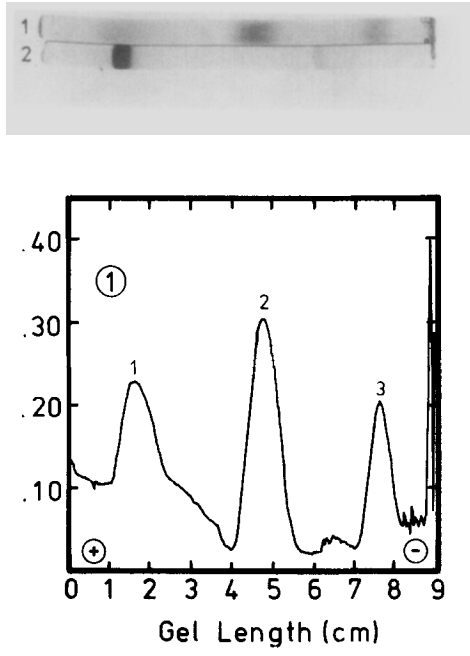
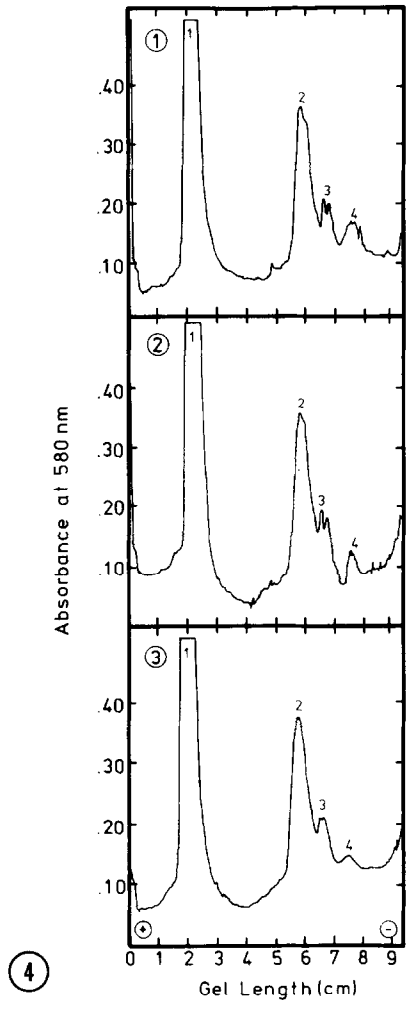


Figure 3. SDS Polyacrylamide Gel Electrophoresis and Gel Scan of Hypoxanthine-Guanine Phosphoribosyltransferase Crosslinked with Glutaraldehyde.

The beef brain enzyme was crosslinked with glutaraldehyde and subjected to SDS polyacrylamide gel electrophoresis as described under Methods. Gel 1, enzyme crosslinked with glutaraldehyde; Gel 2, uncrosslinked control. The scan of Gel 1 shows the protein bands numbered in order of increasing subunit molecular weight.

exists as a trimer. This is confirmed by the plot shown in Figure 2. Bands 1, 2, and 3 (points E, F, and G respectively) do indeed correspond to a monomer, dimer, and trimer since they all fall on a straight line.

The apparent molecular weights of the protein species obtained when beef brain hypoxanthine-guanine phosphoribosyltransferase was crosslinked with dimethylsuberimidate were higher than when the enzyme was crosslinked with glutaraldehyde; the dimer and trimer of dimethylsuberimidate crosslinked enzyme had smaller relative mobilities than the glutaraldehyde crosslinked enzyme (note the difference in slopes in Figure 2). This difference was also noted with the human enzyme by Holden and Kelley (9) who suggested it may be due to the different degrees of crosslinking within the molecular. If glutaraldehyde introduces more crosslinks



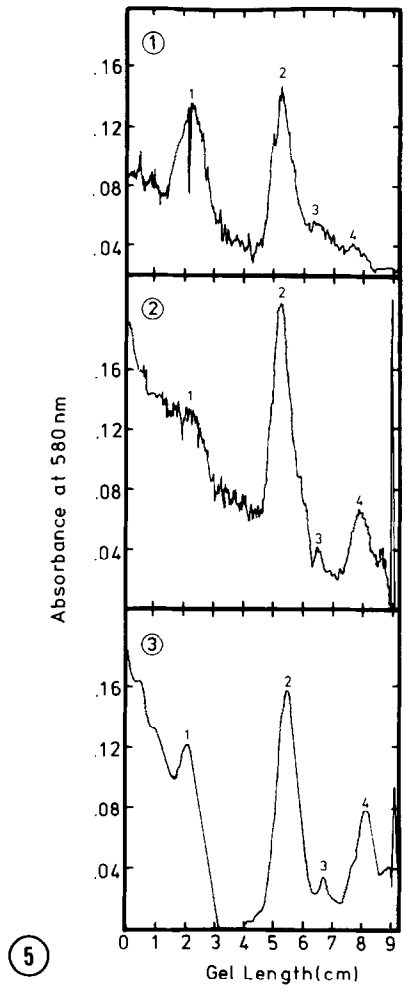


Figure 4. Scans of SDS Polyacrylamide Gels Containing Hypoxanthine-Guanine Phosphoribosyltransferase Crosslinked with Dimethylsuberimidate in the Presence of P-Rib-PP or GMP.

The beef brain enzyme was crosslinked with dimethylsuberimidate in the presence of the indicated substrates, then subjected to SDS polyacrylamide gel electrophoresis as described under Methods. The protein bands are numbered in order of increasing subunit molecular weight. Gel 1, no additional substrates; Gel 2, 1mM P-Rib-PP; Gel 3, 5mM GMP.

Figure 5. Scans of SDS Polyacrylamide Gels Containing Hypoxanthine-Guanine Phosphoribosyltransferase Crosslinked with Glutaraldehyde in the Presence or Absence of P-Rib-PP.

The beef brain enzyme was crosslinked with glutaraldehyde in the presence or absence of 0.2mM P-Rib-PP, then subjected to SDS polyacrylamide gel electrophoresis as described under Methods. The protein bands are numbered in order of increasing subunit molecular weight. Gel 1, 30 second corsslinking time in the absence of P-Rib-PP; Gel 2, 30 minute crosslinking time in the absence of P-Rib-PP; Gel 3, 30 minute crosslinking time in the presence of 0.2mM P-Rib-PP.

per molecule, the molecule would be more compact and therefore should have a greater mobility on sodium dodecyl sufate gels.

The crosslinking studies thus far suggest that beef brain hypoxanthine-guanine phosphoribosyltransferase exists as a trimer. However, all these studies were performed on purified enzyme which had been stored in the presence of 0.1mM P-Rib-PP. Since Strauss, et al. (6) found that the presence of P-Rib-PP influenced the state of aggregation of the human erythrocyte enzyme, the crosslinking pattern of beef brain hypoxanthine-guanine phosphoribosyltransferase which had been stored without P-Rib-PP was investigated.

Figure 4 shows the scans of gels of beef brain hypoxanthine-guanine phosphoribosyltransferase crosslinked with dimethylsuberimidate in the presence of 1mM P-Rib-PP (gel 2), 5mM GMP (gel 3) and in the absence of any substrate (gel 1) There is no significant difference in the observed patterns of banding. Band 3 again corresponds to the contaminant seen in uncrosslinked beef brain enzyme samples. Monomer, dimer, and trimer bands of the enzyme are present.

Scans of the gel containing the beef brain enzyme crosslinked with glutaraldehyde in the presence and in the absence of 0.2mM P-Rib-PP are shown in Figure 5. Comparison of gel 2 with gel 3 again indicates no significant differences in the banding patterns. It is of interest to note, however, that a 30 second crosslinking reaction time (gel 1) shows the formation of very little trimer (band 4) compared to the 30 minute reaction time (gel 2).

The results of crosslinking of beef brain hypoxanthine-guanine phosphoribosyltransferase with dimethylsuberimidate and glutaraldehyde indicate that the presence of the substrates, P-Rib-PP or GMP, does not affect the state of aggregation of the enzyme. These results are in agreement with the results obtained by Holden and Kelley (9) for the human erythrocyte enzyme crosslinked by dimethylsuberimidate or glutaraldehyde. Additionally, all crosslinking studies carried out on the beef brain enzyme support a trimeric structure for the enzyme. No evidence was found to indicate the presence of a tetrameric form of the enzyme.

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