SOME HOMOGENEITY STUDIES ON COMMERCIAL PREPARATIONS OF ALLEGEDLY CRYSTALLINE PHOSPHOMANNOSE ISOMERASE*

J. R. Feramisco[†,1], B. E. Tilley[‡], W. R. Conn[‡], R. W. Gracy[‡,2], and E. A. Noltmann[†,3]

Department of Biochemistry, University of California, Riverside, California 92502

and

Department of Chemistry, North Texas State University, Denton, Texas 76203

Received September 18,1973

Summary--Commercial preparations of what is labeled as "Crystalline Phosphomannose Isomerase" were subjected to chromatographic, electrophoretic and ultracentrifugal analysis to determine the degree of their homogeneity. The results suggest that these preparations do not contain more than 5 to 10% of their total protein content as phosphomannose isomerase and that they must owe their crystallinity to other enzymatic or nonenzymatic protein(s). In both column chromatography and polyacrylamide gel electrophoresis 10 to 16 different protein components were discernible, only one of which had phosphomannose isomerase activity. Sedimentation velocity ultracentrifugation allowed the distinction of only two uniformly shaped schlieren peaks, reflecting the unsatisfactory nature of this technique as a tool for probing the purity of grossly inhomogeneous protein solutions.

Some time ago, the senior authors of this communication reported on the isolation and characterization of phosphomannose isomerase (D-mannose-6-phosphate ketol-isomerase, EC 5.3.1.8) from Fleischmann's Dry Yeast (1-3). The final product of the isolation procedure possessed a specific activity of over 800 I.U., assayed (1) at 30° (4), per mg of protein, corresponding to a 1600-fold increase over the original autolysate, and was judged homogeneous by several criteria (1). However, attempts to crystallize the enzyme were not successful. Since then, at least three commercial suppliers have engaged in the sale of what is described as "Crystalline Phosphomannose Isomerase"

*This work was supported in part by USPHS Grants AM 07203 and AM 14638 and a grant from The Robert A. Welch Foundation (B-502).

†Department of Biochemistry, University of California, Riverside, California.

‡Department of Chemistry, North Texas State University, Denton, Texas.

[1]Summer Student in the National Science Foundation Undergraduate Research Participation Program, GY 10550.

[2]US Public Health Career Development Awardee (K04 AM 70198).

[3]To whom inquiries may be directed.

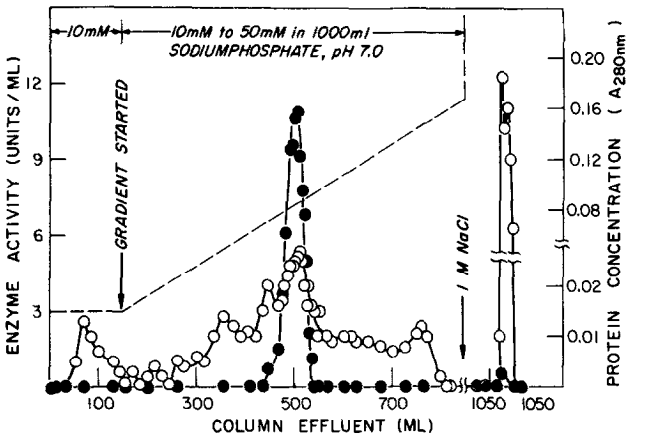

Fig. 1: Chromatography of "Crystalline Phosphomannose Isomerase" from yeast (Boehringer, Lot 7512105) on DEAE-cellulose. Enzyme (10 mg, 63 I.U. per mg) was applied after extensive dialysis against 10 mM sodium - 0.5 mM cobaltous acetate, pH 7.0. Elution by sodium phosphate gradient, 10 mM (500 ml) to 50 mM (500 ml), pH 7.0; flow rate, 40 ml per hr. Other conditions as described in the text. Closed circles represent enzyme activity, open circles A_{280nm}.

(Boehringer, Sigma, PL Biochemicals) of specific activities ranging from 40 to 80 I.U. per mg of protein, i.e. approximately 5 to 10% of what had been found for our preparation from Fleischmann's yeast. The reason for this discrepancy could be either that the commercial enzyme is also homogeneous, but per se has a lower specific activity than the preparation described earlier (1), or that the commercially available crystalline suspension consists primarily of some other protein(s) merely contaminated with phosphomannose isomerase activity. Which of the two is true may be of little significance as long as such preparations are used solely as crude enzyme reagent. If the second alternative prevails, however, commercial phosphomannose isomerase does not appear to be suitable for protein studies or investigations of its catalytic specificity. Since studies on the mechanism and the specificity of this enzyme are currently pursued in several laboratories (e.g., Reference 5) it was deemed desirable to determine the degree of homogeneity of commercially available "crystalline" phosphomannose isomerase.

MATERIALS AND METHODS

Preparations of "Crystalline Phosphomannose Isomerase" from yeast were

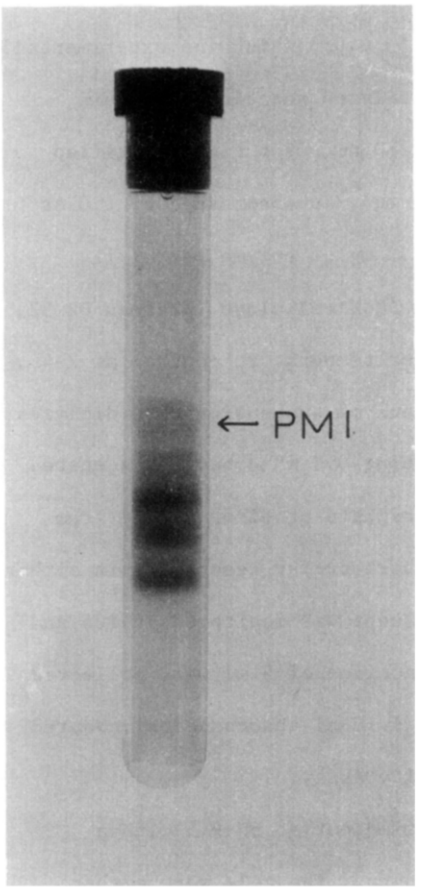

Fig. 2: Polyacrylamide gel electrophoresis of "Crystalline Phosphomannose Isomerase" from yeast (Sigma, Lot 102C-3171). Enzyme (100 μg, 75 I.U. per mg) was subjected to electrophoresis for 5 hours at 2 milliamps per gel. Gels were run in pairs, one of which was stained for protein, the other sliced into 1-mm sections and assayed for phosphomannose isomerase (PMI) activity. Anode was at the bottom of the gel.

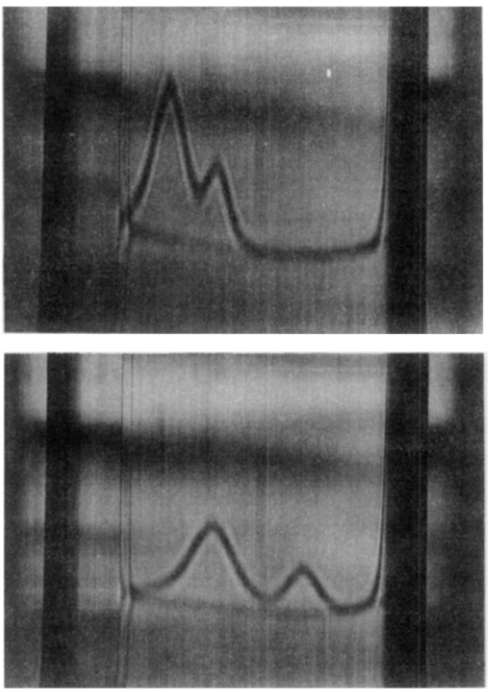

Fig. 3: Schlieren pattern of an analytical ultracentrifuge sedimentation velocity run. "Crystalline Phosphomannose Isomerase" (Boehringer, Lot 7511204, 4.1 mg per ml, 40 I.U. per mg) equilibrated with 10 mM triethanolamine - 0.1 M NaCl buffer, pH 7.5, was centrifuged at 60,000 rpm in an An-D rotor. The photographs shown were taken at 47 (top) and 85 minutes (bottom), respectively, after reaching full rotor speed.

purchased from both Boehringer (Lots 7511204 and 7512105) and Sigma (Lots 102C-3170 and 102C-3171). Enzyme activity was assayed at 30° by the spectrophotometric assay described previously which utilizes phosphoglucose isomerase and glucose 6-phosphate dehydrogenase as coupling enzymes (1). Enzyme units (I.U.) in this communication refer always to micromoles of substrate turnover

per minute either measured at 30° or, where originally given for 25°, converted to 30° by multiplication with the factor 1.20 (± 0.02) which was experimentally determined for the phosphomannose isomerase catalyzed reaction. Protein concentrations are expressed as absorbance at 280 nm. For highly purified yeast phosphomannose isomerase (~ 800 I.U. per mg), an absorbance of 1.0 at 280 nm for a 10-mm light path corresponds to 1.2 mg of enzyme per ml (1).

Chromatographic analyses were performed on DEAE cellulose (Whatman DE 32, 1 meq per g) columns (2.5 x 50 cm), jacketed for temperature control at 2-4°. Prior to chromatography, 10-mg samples of the enzyme suspension were dialyzed against 10 mM sodium phosphate, pH 7.0, containing 0.5 mM cobaltous acetate. Columns were developed with a linear sodium phosphate gradient either from 10 to 100 mM, or from 10 to 50 mM, of pH 7.0, both buffer reservoirs in either case containing 500 ml. Absorbance of the effluent was monitored at 206 and 280 nm with the aid of an LKB Uvicord III. Fractions of 5 ml were collected in a refrigerated fraction collector, their individual absorbancies measured at 280 nm, and aliquots taken for enzyme activity assay.

Polyacrylamide gel electrophoresis was carried out at pH 9.5 with a current of 2 milliamps per gel applied for 5 hours. The gels were either stained for protein with 0.1% Buffalo Black in methanol:acetic acid:water (45:10:45) or sliced into 1-mm sections which were assayed for phosphomannose isomerase after extraction with 50 mM triethanolamine, pH 8.5. Sedimentation velocity ultracentrifugation experiments were performed at 12.2° with a Beckman-Spinco Model E analytical ultracentrifuge with use of a double-sector cell to allow display of the baseline pattern.

RESULTS AND DISCUSSION

Figure 1 shows the elution pattern obtained on chromatography of Boehringer phosphomannose isomerase on a DEAE cellulose column developed with a gradient from 10 to 50 mM sodium phosphate, pH 7.0. At least 10 protein species could be distinguished only one of which contained phosphomannose isomerase. In

the peak tube of this fraction the specific activity was found to exceed 400 I.U per mg of protein. With a sodium phosphate gradient from 10 to 100 mM, the resolution was less pronounced but phosphomannose isomerase activity was also found in only one protein peak. Similar results were obtained with phosphomannose isomerase samples from Sigma.

It should be mentioned that all of the commercial preparations of phosphomannose isomerase were dialyzed against buffer containing 0.5 mM cobaltous acetate to obtain maximally active enzyme prior to chromatography. For reasons unknown, all of the commercial samples contain 1 mM EDTA although yeast phosphomannose isomerase had been shown to be a zinc metallo-enzyme from which the essential zinc can be removed by treatment with EDTA (2). Divalent cobalt had been found previously to be most efficient in restoring enzyme activity (2).

Polyacrylamide gel electrophoresis (Figure 2) yielded 9 to 10 protein bands from Sigma phosphomannose isomerase (specific activity 70 I.U. per mg) and up to 16 bands from a sample of the Boehringer enzyme (specific activity 40 I.U. per mg). Again, in each instance, only one of the bands possessed phosphomannose isomerase activity.

In contrast to the multiplicity of protein components resolved by either column chromatography or gel electrophoresis, analytical sedimentation velocity ultracentrifugation gave only two rather symmetrically appearing schlieren peaks (Figure 3). The finding that the other techniques allowed distinction of a considerably larger number of protein species is an impressive example of the unsatisfactory resolving power of velocity sedimentation ultracentrifugation and its unsuitability as a tool for proving homogeneity of protein solutions.

It is of interest to add the comment that during the purification procedure of yeast phosphomannose isomerase (1) at two points crystalline material is obtained in fractions that are discarded. In "Fraction VI" (1) crystallization is deliberately induced to remove contaminating protein prior to chromatography on Sephadex. In addition, an unknown fraction eluting immediately after

phosphomannose isomerase on DEAE cellulose column chromatography was found to crystallize spontaneously when brought to 0.53 saturation with ammonium sulfate (6). It is therefore not unlikely that the crystalline material appearing in commercial preparations of phosphomannose isomerase represents either one or both of these proteins. It is quite definite, however, that based upon previously published data (1) phosphomannose isomerase constitutes at best 10% of the total protein that is sold commercially as "Crystalline Phosphomannose Isomerase".

REFERENCES

1. Gracy, R. W., and Noltmann, E. A. (1968) J. Biol. Chem. 243, 3161-3168.
2. Gracy, R. W., and Noltmann, E. A. (1968) J. Biol. Chem. 243, 4109-4116.
3. Gracy, R. W., and Noltmann, E. A. (1968) J. Biol. Chem. 243, 5410-5419.
4. Enzyme Nomenclature, Recommendations (1964) of the International Union of Biochemistry, Elsevier Publishing Company, Amsterdam, 1965.
5. Rose, I. A., O'Connell, E. L., and Schray, K. J. (1973) J. Biol. Chem. 248, 2232-2234.
6. Gracy, R. W. (1968), Ph.D. Dissertation, University of California, Riverside