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PRELIMINARY X-RAY DIFFRACTION DATA FOR CHICKEN MUSCLE GLYCEROL 3-PHOSPHATE DEHYDROGENASE

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SUMMARY: Single crystals of chicken muscle glycerol 3-phosphate dehydrogenase have been obtained which are suitable for a high resolution structure analysis. The crystals are triclinic with smallest cell dimensions of a=58.9 Å b=54.5 Å c=58.5 Å $\alpha=91^{o}$ $\beta=95^{o}$ $\delta=89^{o}$ but show a substantial degree of pseudo symmetry which indicates the presence of at least an approximate and possibly exact dyad axis relating the two subunits in the asymmetric unit.

INTRODUCTION

Glycerol 3-Phosphate Dehydrogenase (EC 1.1.1.8) exists as two isoenzymes in most higher organisms, one predominant in the liver and the other in muscle (1). Both forms have a molecular weight of 75,000 (2) although they are widely different in kinetic parameters (3). The form present in muscle operates in concert with muscle type lactate dehydrogenase to regenerate NAD during anaerobic glycolysis as its primary physiological role. The enzyme exists as a dimer composed of two subunits that biochemical evidence indicates are identical (4,5). The protein is crystallized from ammonium sulfate as the final stage of its preparation, but until this time has not been obtained in a form suitable for X-ray diffraction analysis.

MATERIALS AND METHODS

We have purified glycerol 3-phosphate dehydrogenase from chicken muscle using conventional isolation procedures (3) and crystallized it for diffraction analysis by the following method.

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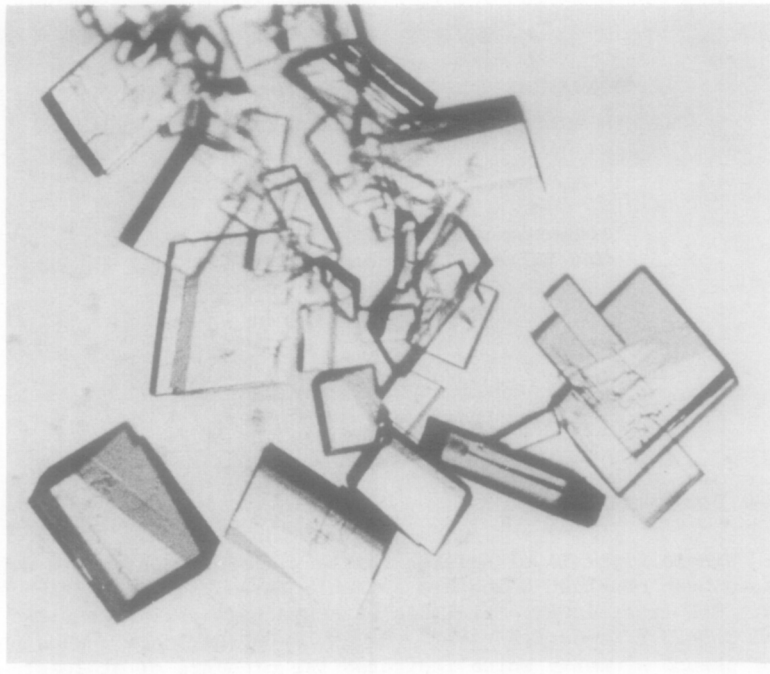

FIGURE 1. Crystals of glycerol 3-phosphate dehydrogenase from chicken muscle seen under a low power light microscope. Crystals were grown by vapor diffusion from 10% PEG 6000.

The microcrystalline protein in 80% saturated ammonium sulfate was dissolved by addition of water and simultaneously dialyzed and concentrated by reduced pressure in conical collodion bags against a 20 mM. Tris-HCl solution at pH 7.6 containing 1mM EDTA and 5 mM β-mercaptoethanol. The final protein concentration was 14 mg/ml. To each chamber of a nine well depression plate was added 10 µl of 10% polyethylene glycol (PEG) 6000. These plates were equilibrated against 25 ml reservoirs of 10% PEG 6000 in plastic boxes at 25° C. After three days to a week, crystals like those shown in figure 1 were formed. The crystals appear to be monoclinic prisms and in some cases reach dimensions greater than 0.5 mm on all edges. Twinning occurred in a large number of instances.

Crystals were mounted in quartz capillaries by conventional procedures along with a small quantity of mother liquor to maintain hydration and sealed with dental wax. X-ray diffraction patterns were recorded on Buerger precession cameras with a crystal to film distance of 75mm and precession angles of 14°. The X-ray source was an Elliott GX-6 rotating anode operated at 40 kV and 40mA with a focal spot size of 200 μ^2 and Cuk_{α} radiation. Exposure times were 12 to 18 hours duration.

RESULTS AND DISCUSSION

A systematic search of reciprocal space around several different crystallographic directions did not reveal the presence of any symmetry other

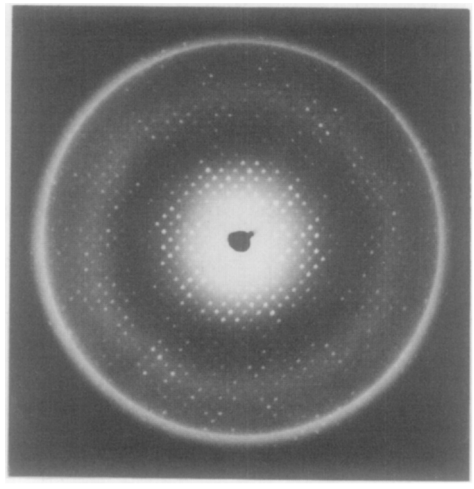

FIGURE 2. Precession X-ray diffraction photograph (μ=14°) of the zero level of the h0l zone of the reciprocal lattice from triclinic crystals of glycerol 3-phosphate dehydrogenase. Crystal to film distance was 75 mm with CuK_α radiation and an exposure time of 16 hours.

than Friedel symmetry within the reciprocal lattice in spite of the fact that two reciprocal cell edges were found to be virtually identical in length. The only crystal system consistent with all of the observed diffraction patterns is triclinic with a unit cell of a=58.9 Å b=54.5 Å c=58.5 Å α=91° β=95° δ=89°. The unit cell is very nearly compatible with monoclinic symmetry suggesting the possibility of a pseudo or exact dyad axis in the cell. Indeed an examination of the major zones of reciprocal space do indicate that a twofold axis is present, and that it is directed along the 110 direction in real space.

The volume of the unit cell is 1.95 x 10^5 A^3 and if one assumes that there is one glycerol 3-phosphate dehydrogenase dimer of 75,000 daltons in the cell, then the volume to mass ratio calculates to be 2.57 A^3/dalton. This is near the center of the range found for most globular proteins, and almost all other dehydrogenases (6). The pseudo dyad indicated in the diffraction pattern must relate the two subunits within the triclinic

cells' asymmetric unit. Since the dyad is not crystallographic it can not be stated that the two subunits are identical, but it does suggest that they are either identical or nearly identical and that they are related by an exact or near exact twofold axis of symmetry. The failure of the unit cell to incorporate the molecular symmetry into its crystallographic symmetry could be due to a deviation from identity or symmetry in the dimeric molecule, but more likely originates from packing considerations.

The crystals can be reproducibly grown, are mechanically stable, diffract to at least 2.5 Å in precession photographs and show no more than average sensitivity to X-radiation. Thus they appear to be good candidates for a high resolution structure analysis.

ACKNOWLEDGEMENT

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