CRYSTALLIZATION AND CRYSTALLOGRAPHIC DATA OF *ESCHERICHIA COLI* MALTODEXTRIN PHOSPHORYLASE

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1. Introduction

In animals, the pyridoxal-5′-phosphate dependent enzyme phosphorylase (1,4-α-D-glucan:orthophosphate α-glucosyl transferase, EC 2.4.1.1) is controlled either by the binding of specific metabolites or by covalent modification of the enzyme, namely phosphorylation of serine residue by a specific kinase [1]. The animal enzymes consist of chemically identical subunits of approx. mol. wt 100 000, and the molecule exists in an association—dissociation equilibrium between dimer and tetramer. This equilibrium can be shifted by effectors or phosphorylation.

In bacteria, however, phosphorylase activity is controlled by induction, and the molecules, stable dimers consisting of 80 000—90 000 mol. wt subunits, exhibit straight Michaelis-Menten kinetics uninfluenced by metabolites, and are not subject to phosphorylation [2,3].

Crystallographic investigations of the 3-dimensional structure of rabbit muscle phosphorylase *a* [4] and *b* [5] have now both reached 3 Å resolution, and the amino acid sequence has been fully determined [6], so that complete structural information about both forms of the enzyme should be available soon. The structure analysis of an unregulated phosphorylase should then, apart from its own biochemical merits, greatly facilitate the analysis of the underlying molecular mechanisms of the regulatory processes mentioned above by serving as a 'neutral reference'.

Maltodextrin phosphorylase from *Escherichia coli* seems to be a suitable object for such a structure analysis since it can be obtained in large enough quantities with good purity and is biochemically well characterized. The dimer is mol. wt 164 000 [3]. Preliminary sequenching results indicate similarities to the rabbit muscle enzyme in the N-terminal region [7].

We have begun a crystallographic investigation of the structure of malto-dextrin phosphorylase from *E. coli*. Crystals have been grown and the space group and the cell dimensions have been determined. This communication described by growing of the crystals and the crystallographic data.

2. Experimental

To obtain reliably crystallizing protein the preparation described [3] had to be modified. The bacterial cells were disrupted, after incubation with lysozyme, by nitrogen decompression in a high pressure bomb. DNA digestion and heat step were replaced by precipitation with Polymin P. After ammonium sulfate fractionation a DEAE-cellulose column was introduced, followed by affinity chromatography on Sepharose bound glycogen. After two preparative crystallizations from ammonium sulfate the enzyme usually had a specific activity (cf. [3] for enzyme assay) of about 12 units/mg at 25°C (16 units/mg at 30°C). Details of the preparation procedure will be found [10].

Crystals were grown from ammonium sulfate at 8 mg/ml protein concentration in the range 1.8—2.1 M ammonium sulfate in 0.1 M sodium phosphate buffer containing 1 mM Cleland's reagent, 0.1 mM EDTA, and 5 μM pyridoxal phosphate, pH range 6.2—7.4.

The density of the crystals was measured in two ways:

1. By sedimentation through a step gradient made up of ammonium sulfate solutions of different densities. The crystals sank steadily through layers with less than the crystal density, but were sharply decelerated and came to a near stop when a layer of higher density than the crystals' was encountered. However, after enough salt from the surrounding solvent had diffused into the crystals they started to sink again, accelerating until they hit the next boundary.

Thus, by observing the behaviour of the crystals at different boundaries, the crystal density be narrowed down to a range of about ± 0.01 g/ml. The value obtained is an upper limit because of the salt uptake of the crystals in the upper layers. The advantage of the method is its suitability for relatively small crystals because it does not require transferring the crystals into a non-aqueous medium, its disadvantage is its relatively low accuracy.

2. The buoyant density of the crystals was determined in the usual way in a continuous gradient of pure chlorobenzene and a 1:1 mixture of chlorobenzene and bromobenzene. Crystals were transferred into the gradient within small droplets of mother liquor, then the surrounding liquid was removed through thin capillaries. The gradient was calibrated with small droplets of ammonium sulfate solutions of known density.

This method yields a lower limit value because of residual mother liquor surrounding the crystals.

3. Results

A seeding procedure allowed us to grow crystals of good size. Phosphorylase solutions were set up at pH 6.4 at the ammonium sulfate concentrations described above. After one day, many very small needles were growing in 2.1 M ammonium sulfate, and after a few days slightly larger needles appeared in the 2.0 M vials. The crystal needles were suspended in the liquid by swirling the vials gently, then allowed to settle again. About 1 μl supernatant liquid was transferred into the vials containing 1.8 M and 1.9 M ammonium sulfate, respectively.

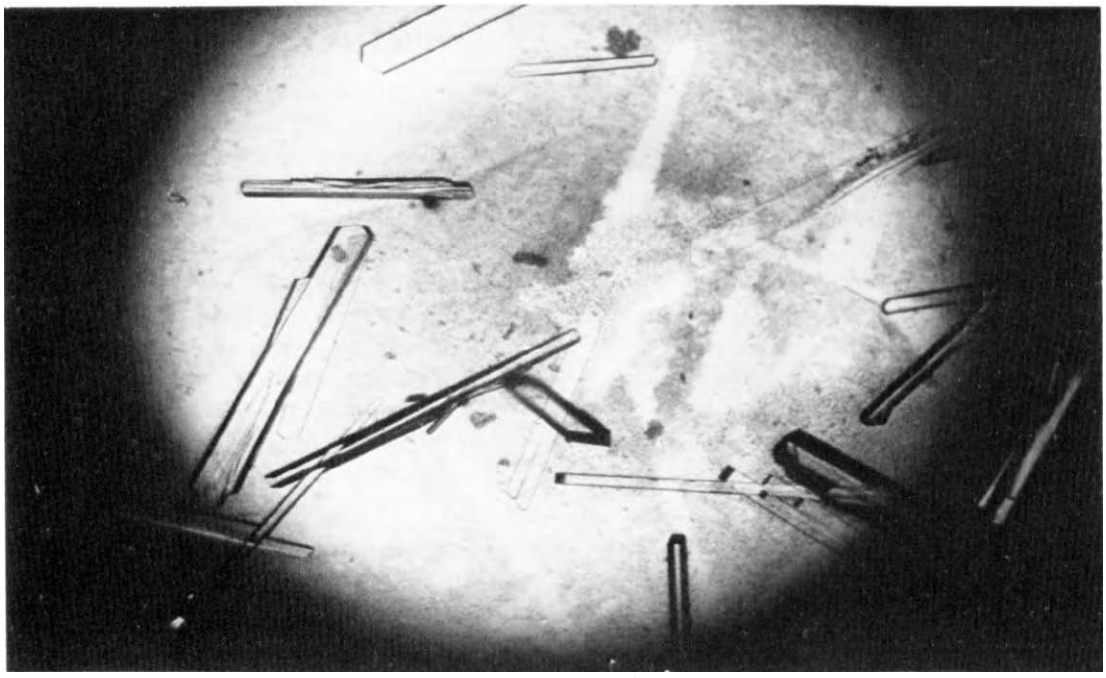

Fig.1. Crystals of *E. coli* maltodextrin phosphorylase grown from 1.8 M ammonium sulfate solution pH 6.4 (20-fold magnification).

After several months crystals up to 2 mm in length had grown from the 1.8 M solutions, and somewhat smaller ones from 1.9 M. The crystals are fragile flat needles (fig.1) which frequently grow in star-like conglomerates. Typically they are up to 2 mm long, up to 0.4 mm wide and up to 0.15 mm thick and exhibit optical activity in all directions.

The density of the smaller crystals grown from 1.9 M ammonium sulfate was determined using the step gradient method. A four layer gradient and a subsequent series of two layer gradients consisting of top layers of mother liquor density and bottom layers of densities from 1.18–1.22 g/ml allowed us to narrow down the value to 1.21 ± 0.01 g/ml. Some of the larger crystals grown from 1.8 M ammonium sulfate were subjected to the bromobenzene–chlorobenzene gradient. The buoyant density was found to be 1.196 ± 0.005 g/ml. Considering the limitations of each method and also the density difference between 1.8 M and 1.9 M ammonium sulfate solutions, the agreement is surprisingly good and the results can be combined to yield 'compromise values' of 1.20 and 1.205 ± 0.01 g/ml for crystals grown from 1.8 M and 1.9 M ammonium sulfate, respectively.

The partial specific volume of the protein was calculated [8] from the amino acid composition [3] to be $\bar{v}$ = 0.730 ml/g, corresponding to a density of the pure protein of 1.370 g/ml. The dimeric molecule has then a volume of 199 000 Å^3, corresponding to sphere of 72.4 Å diameter. From $\bar{v}$ and the crystal density it can be calculated that the crystals consist of 28% (by vol.) protein and 72% solvent which explains their fragility. X-Ray still photographs show that the crystals diffract out to and beyond 3 Å resolution. Unfortunately, they exhibit an anisotropic disorder with a mosaic spread of 0.5–2°, depending on the individual crystal. The diffracted intensities are so small that only precession photographs of up to 9° precession angle (5 Å resolution) can be reasonably well exposed within the crystal lifetime in the beam.

Precession photographs of the three principal zones show an orthorhombic unit cell and symmetry. This is borne out by inspection of the higher order rings on screenless precession photographs. Systematic absences are observed in photographs of the *hkO* reciprocal lattice plane (fig.2) for all reflections $h + k \neq 2n$ and in *hOl* photographs (fig.3) for the

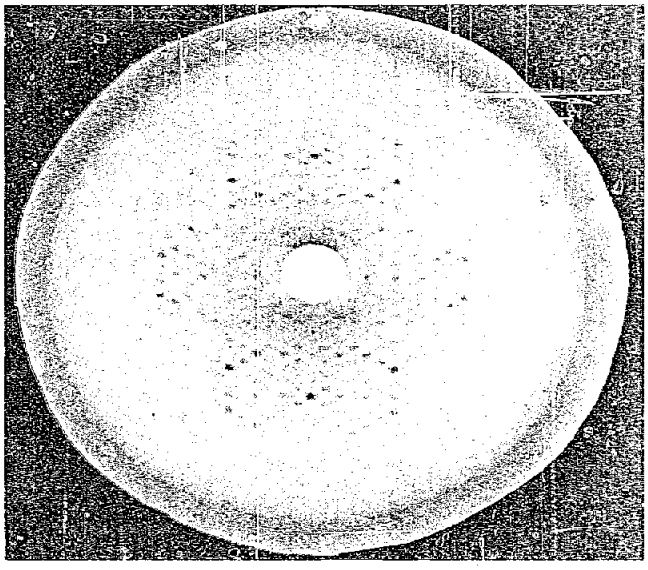

Fig.2. *hkO* Precession photograph of a phosphorylase crystal, precession angle 9°, crystal-to-film distance 100 mm, monochromatized Cu Kα radiation from a rotating anode tube operated at 40 kV and 100 mA, exposure time 36 h.

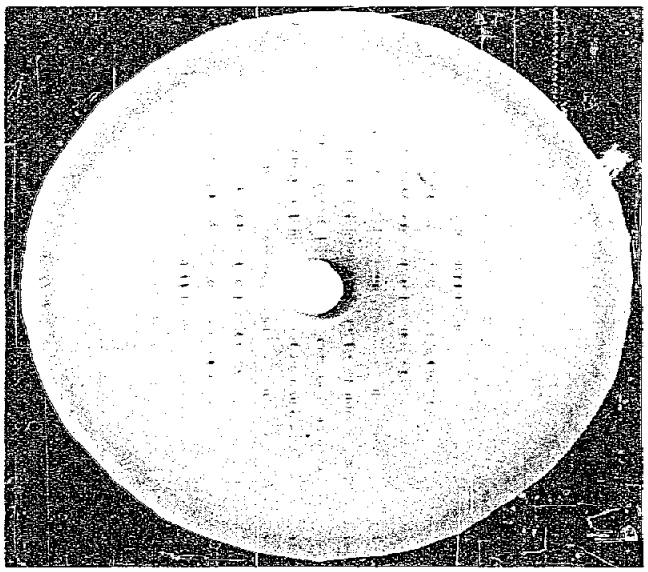

Fig.3. *hOl* Precession photograph, details as in fig.2.

OOl class of reflections ($l \neq 2n$). The space group is, therefore, C 2 2 2_1 which has eight general positions. The unit cell dimensions were found to be $a = 104.8$ Å, $b = 191.5$ Å, $c = 297$ Å, $V = 5.96 \times 10^6$ Å^3.

The cell lengths are accurate to within ± 0.5%. The volume of the asymmetric unit is then 745 000 Å^3 of which 28% = 208 000 Å^3 is protein. This value corresponds well with the above calculated volume of the dimeric molecule. Therefore, the asymmetric unit contains one molecule of phosphorylase (mol. wt 164 000), and the packing density is 4.54 Å^3/dalton which is quite high compared with other values [9].

4. Discussion

The crystals of *E. coli* maltodextrin phosphorylase appear to be suitable for a crystallographic structure determination, at least to low resolution. The anisotropic disorder prevents data collection using the screenless rotation method, but we hope that crystals of better order can be grown soon. At present, data are being collected using screened precession photography to 5 Å resolution. The technical problems encountered are not trivial due to the large unit cell with high solvent content and especially due to the extremely long *c*-axis.

Solving the structure, once good data are available, seems to be relatively straightforward since the two high resolution structures of rabbit muscle phosphorylase can be used in a molecular replacement procedure. It can be expected that the two molecules are structurally similar enough to make this method feasible.

The crystal data as of now do not contribute to the biochemical knowledge of *E. coli* phosphorylase, but they are in agreement with it. Crystal density and crystallographic data are consistent with molecular weight and protein density. The two chemically identical subunits are not crystallographically equivalent, so nothing can be said at the present time about their structural similarity or dissimilarity.

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