CRYSTALLIZATION AND CRYSTALLOGRAPHIC DATA OF RABBIT MUSCLE PHOSPHORYLASE a AND b

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Received 23 December 1971

1. Introduction

Glycogen phosphorylase from rabbit muscle shows many of the characteristic properties of an interconvertible regulatory enzyme. The b form is dependent in its enzymatic activity upon the allosteric effector, AMP; all substrates show positive homotropic cooperativity [1–4], and the enzyme is converted by a specific kinase to the more active a form, much less influenced by AMP [4]. Both forms of the enzyme act on the substrate glycogen in the form of a dimer, each subunit having a molecular weight of 100,000 daltons. In the absence of the substrates, the dimers tend to aggregate to tetramers at higher protein concentrations; with the b form, this process is enhanced by AMP [5]. As crystals are usually obtained using saturation concentrations of AMP and Mg^{2+}, both forms should exist as tetrameric molecules within the crystals.

As parts of the primary structure are now known [6], and several binding sites of the molecule could be specifically labelled [7–10], the investigation of its tertiary and quaternary structure by X-ray crystallography seemed feasible. Some information on the molecular shape is available from small-angle X-ray scattering [11] and electron micrographs [12].

The X-ray data indicate a tetramer of the dimensions $55 \times 110 \times 123$ Å^3. From the electron micrographs a model is proposed which consists of 4 elongated subunits assembled according to point group symmetry 222 at the vertices of a tetrahedron. The approx. dimensions are $70 \times 120 \times 120$ Å^3.

This communication describes the growing of crystals of phosphorylase a and b suitable for X-ray crystallography and their crystallographic data.

2. Material and methods

Phosphorylase b was prepared from rabbit back and hind leg muscles according to Fischer and Krebs [13] and recrystallized 3–5 times. The last recrystallization step used a 10-fold concentration of AMP and a 5-fold concentration of Mg^{2+}. Phosphorylase a was prepared from the b form as described by Krebs and Fischer [14]. The phosphorylase b kinase used for these preparations was obtained from rabbit muscle following the procedure of Krebs and Fischer [15]. The a enzyme was recrystallized after dissolving the crystals in 0.03 M cysteine solution at pH 7.0, and addition of 0.1 M AMP solution to a final concentration of 0.01 M, and 1 M magnesium solution to 0.05 M. After slight turbidities had been centrifuged down at 20,000 rpm in a Sorvall SS 1 rotor for 30 min, the solution was cooled to 4°.

The specific enzymatic activities of phosphorylase a and b were determined as described previously [9], in the presence and absence of the effector AMP. Complete conversion to the a form was also established by analytical ultracentrifugation. S_{20w} values were determined in a Beckman model E ultracentrifuge equipped with Schlieren optics. The speed was 56,000 rpm. Bar angle was 75°. A valve type synthetic boundary cell was used. The solutions contained: 50 mM sodium glycerophosphate–HCl-buffer, pH 6.8, 1.5 mM AMP, and 7 mg/ml of the enzyme at 20°.

3. Results

3.1. *Crystallization conditions*

For the growing of larger crystals the b form crystals were dissolved by gentle warming. After centrifugation at 30,000 *g* for 1 hr, the solution was diluted to a protein concentration of 7–15 mg/ml by addition of a cysteine solution at pH 7.0, containing 0.01 M AMP, and 0.05 M magnesium acetate. One half of the solution was then diluted with saturated ammonium sulfate solution at pH 7.0, up to 0.25–0.32 saturation. After standing for one night at room temp the precipitated protein was centrifuged down at 30,000 *g* for 1 hr.

The protein solutions were now filled into the usual 4 cm capillary tubes of 2.5 mm internal diameter, closed with a piece of dialysis tubing at the bottom. The capillaries were placed into 10 ml of a 0.32 saturated ammonium sulfate solution, containing AMP and Mg^{2+} at the concentrations present within the protein solution, in counting vials. The vials were placed in a shallow water bath maintained at exactly 16 ± 1°. Prismatic crystals of up to 0.8 mm length formed during 20–90 days.

Phosphorylase a was set up for crystallization in the same manner. Preprecipitation with ammonium sulfate before the capillary crystallization stage used however only 0.15 final saturation. Prismatic crystals of suitable size (up to 1.2 mm in length) grew within 7–20 days.

3.2. *Crystals*

Phosphorylase b crystals occurred in 2 different modifications, one forming aggregates of long, thin needles, the other compact, prismatic crystals (fig. 1). In several crystallization capillaries needle-like crystals could be observed which were split, perpendicularly to their long axis, into many prismatic pieces, possibly indicating a transition of one modification into the other in the crystalline state. Phosphorylase a crystals are very similar to the prismatic b crystals.

3.3 *Crystallographic data*

Crystals of the prismatic modification of phosphorylase a and b were mounted in glass capillaries containing mother liquor. Precession photographs of the 3 zero and several higher order layers showed a monoclinic cell and symmetry. Systematic extinctions: $0k0$ present only for $k = 2n$. a: 119.4 Å. b: 188.8 Å. c: 88.1 Å. β: 108° 55′. V: 1.88×10^6 Å^3. Space group $P2_1$.

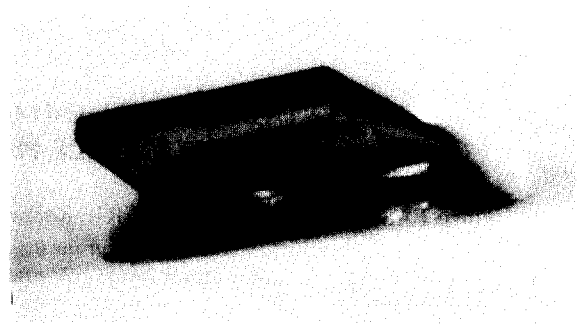

Fig. 1. Prismatic crystal of phosphorylase b with approx. dimensions 0.2 × 0.3 × 0.7 mm^3.

The errors are estimated as 0.5% in the linear dimensions and 10′ in the angle. No difference between phosphorylase a and b crystals could be observed in the cell dimensions. The intensity distribution of the X-ray reflexions is closely similar.

Assuming the most commonly obtained value in protein crystals of 2.15 Å^3/dalton for the packing density [18], the molecular weight of the asymmetric unit is calculated to 436,000 daltons. This is close to the value expected for a phosphorylase tetramer. The packing density calculated for a dimer molecular weight 200,000 would be approx. 4.7 Å^3/dalton, a highly improbable value.

Fig. 2 shows a 14° precession photograph of the $hk0$ layer of a phosphorylase b crystal. Reflexions are visible to the edge of the photograph corresponding to a resolution of 3.2 Å. Precession photographs without layer-line screen showed measurable reflexions to a resolution slightly better than 3.0 Å. The crystalline order appears to allow high resolution crystallographic investigations. The crystal modification described above is obviously closely similar to a modification of phosphorylase b described by Mathews [16]. His conclusions, however, concerning the state of aggregation of the phosphorylase molecules in the crystal differ from ours. Recently, Madsen et al. [17] also succeeded in growing large crystals of phosphorylase a very similar to ours.

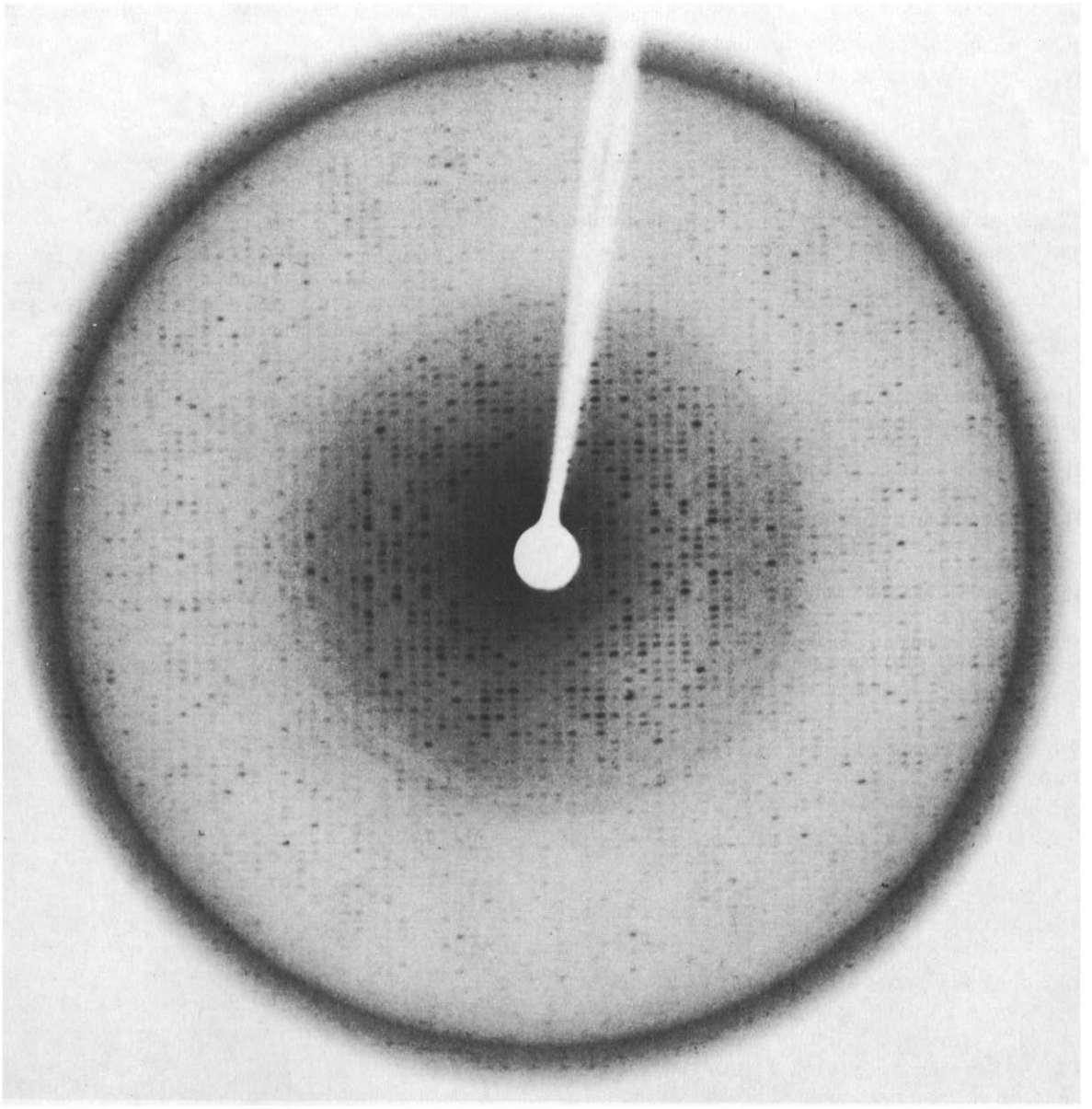

Fig. 2. *hk*0 Precession photograph of a phosphorylase b crystal, cuKα-radiation from a 1 kW X-ray tube, focus to crystal distance 170 mm, crystal to film distance 100 mm, 0.5 mm pinhole collimator, exposure time 25 hr.

4. Discussion

The crystals of phosphorylase a and b described above appear to be suitable for a crystallographic structure determination, although a crystal with an asymmetric unit containing a molecule of 400,000 daltons imposes severe problems with respect to data collection and phase determination. The close isomorphism of crystals of tetrameric phosphorylases a and b in the presence of AMP in sufficient concen-

tration provides evidence that the phosphorylation of a seryl residue in the phosphorylase b to a reaction is accompanied by minor structural alterations only.

Acknowledgements

This work was supported by grants from the Deutsche Forschungsgemeinschaft.

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