PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Structure Determination of Rabbit Phosphoglucomutase

R. K. Wierenga, D. G. Lewis, M. G. Rossmann and W. J. Ray

Phil. Trans. R. Soc. Lond. B 1981 **293**, 205-208
doi: 10.1098/rstb.1981.0073

Phil. Trans. R. Soc. Lond. B **293**, 205–208 (1981)
Printed in Great Britain

The structure determination of rabbit phosphoglucomutase

By R. K. Wierenga, D. G. Lewis, M. G. Rossmann and W. J. Ray, Jr
Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907, *U.S.A.*

Tetragonal crystals of rabbit phosphoglucomutase have been grown from solutions containing ammonium sulphate, polyethylene glycol solution and enzyme. There are two molecules, each of relative molecular mass 64 000 per asymmetric unit. A rotation function suggests that these are related by a twofold axis. X-ray diffraction data for five heavy-atom derivatives and native crystals have been collected by using oscillation photography. A tentative and partial solution of the $KAu(CN)_2$ sites has been obtained. The enzyme in the native crystals is phosphorylated, but the phosphate can be removed without harm to the crystals. Similarly the essential Mg^{2+} ion can be removed or replaced by Zn^{2+}. The enzyme is active in the native crystals.

Introduction

Rabbit phosphoglucomutase (PGM) catalyses the interconversion of glucose-1-phosphate (Glc-1-P) and glucose-6-phosphate (Glc-6-P). The active species is the phosphoenzyme in which a phosphate is bound to a serine (figure 1). Bound Mg^{2+} is essential for activity. The relative molecular mass of one molecule is 64 000, and it contains only one polypeptide chain. The amino acid sequence is being determined by one of us (W.J.R.) and is about 60 % complete.

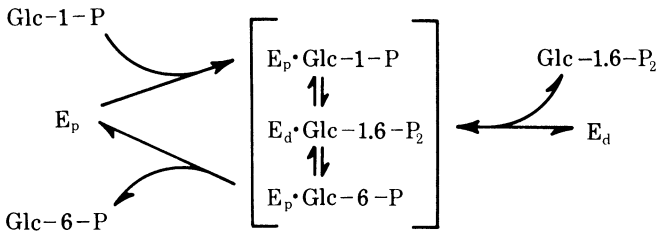

Figure 1. Reaction scheme for the interconversion between Glc-1-P and Glc-6-P. Glc-1,6-P_2 is tightly bound to the dephosphoenzyme.

Crystal growth and characteristics

Large crystals (figure 2) are grown in a two-step procedure. Seeds are obtained by vapour diffusion from a solution containing 10 mg/ml protein, 46 % ammonium sulphate, 3.3 % polyethylene glycol (PEG-400), 16 mM Mg^{2+}, 1 mM EDTA and 50 mM morpholino ethylene sulphuric acid. They are then transferred to a solution containing 4.5 % PEG-400, and harvested when the ammonium sulphate concentration is around 52.5 %.

Native crystals show visible changes in their diffraction patterns after 15 h exposure on an Elliott rotating anode tube operating at 35 kV and 35 mA. Nevertheless, reflexions can be observed to about 2.3 Å resolution.

The space group of the crystals is $P4_12_12$ or $P4_32_12$ with cell dimensions $a = b = 174$ Å and

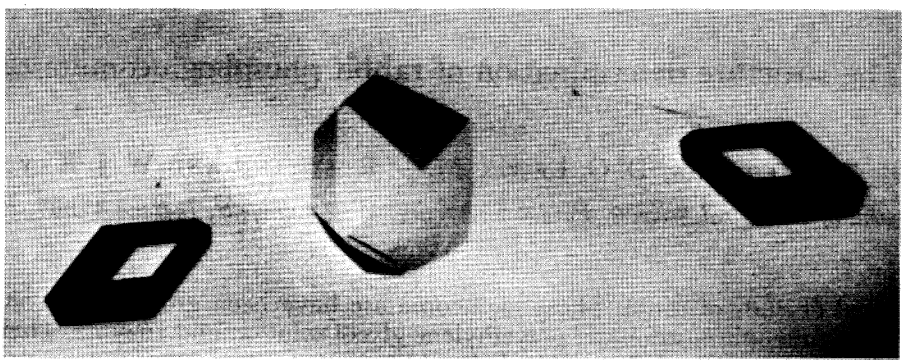

FIGURE 2. Crystals of PGM grown by vapour diffusion from ammonium sulphate and PEG. Their maximum diameter is approximately 1 mm.

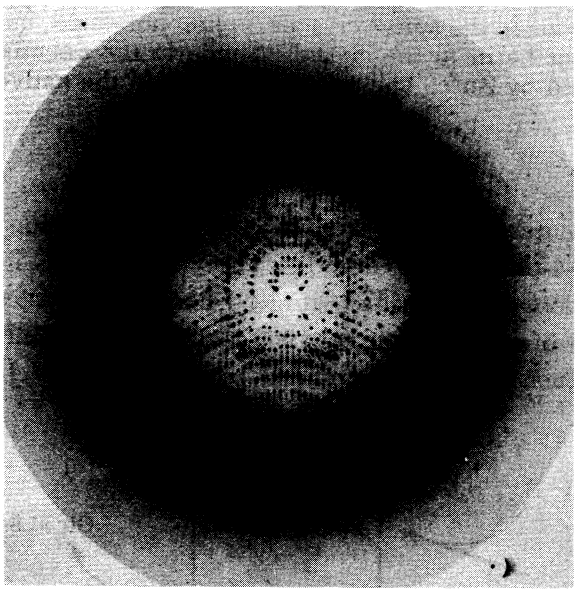

FIGURE 3. An oscillation photograph from a native PGM crystal. The scan angle was 1.1° and the exposure time was 8 h with Cu K_α radiation from a rotating anode generator operated at 35 kV and 35 mA.

TABLE 1. DATA COLLECTION RESULTS

compound†	soaking time/h	concentration mM	number of crystals	resolution	R_{merge}‡	a.d.§	R_{diff}¶
native	—	—	28	2.7	8.8	0.035	—
osmate	87	2.0	7	3.5	8.6	0.168	15.2
PHMB	36	0.2	6	3.9	9.4	0.263	19.8
EMP	36	0.2	8	3.9	9.5	0.308	25.3
gold	72	0.5	8	3.6	9.1	0.082	15.3
uranyl	72	7.0	9	3.5	8.5	0.240	14.3

† Osmate, $K_2OsO_4 \cdot 2H_2O$; PHMB, *p*-hydroxylmercury (II) benzoate; EMP, ethylmercury (II) phosphate; gold, $KAu(CN)_2$; uranyl, $K_3UO_2F_5$.

‡ $R_{merge} = [100\ \Sigma\Sigma|(I_{hi} - \bar{I}_h)|]/\Sigma_h\Sigma_i\bar{I}_h$, where I_{hi} is the ith observation of reflexion h within a given data set and $\bar{I}_h$ is the mean of all observations.

§ A.d. coefficient $= \Sigma\delta_i\delta_j/\sqrt{(\Sigma\delta_i^2\Sigma\delta_j^2)}$, where δ_i and δ_j are anomalous dispersion differences measured on films i and j, respectively.

¶ $R_{diff} = [100\ \Sigma|(F_{nat} - F_{cpd})|]/\Sigma|F_{nat}|$.

c = 101 Å. There are two molecules per asymmetric unit. The cell volume per mole (V_m) is 3 Å per mass unit, with 60% of the cell being occupied by solvent.

Diffraction data collection

A native data set to 2.7 Å resolution, and five heavy-atom derivative data sets to better than 4 Å resolution, have been collected (table 1) by oscillation photography (figure 3). Anomalous dispersion data have been analysed. It shows the smallest coefficient (see footnote 3 to table 1) for native data and the largest coefficient for EMP. The latter compound also shows the largest differences with respect to native crystals.

Data collection to 3 Å resolution for the gold derivative is now in progress because a tentative heavy-atom determination has been achieved.

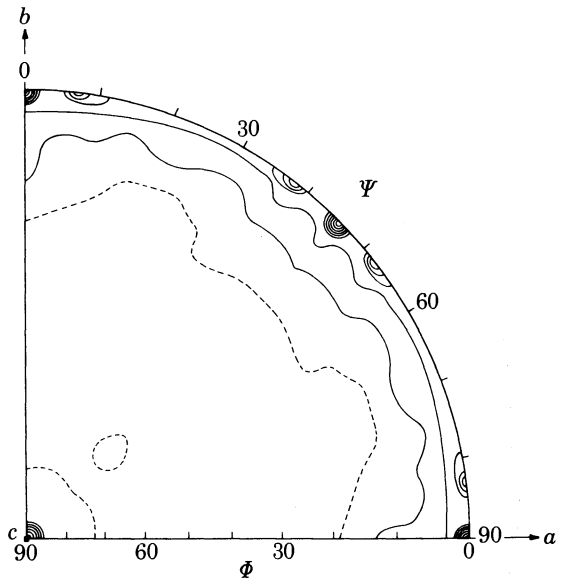

Figure 4. A self-rotation function in the K = 180° plane. Data were between 6 and 10 Å resolution. The radius of integration was 60 Å.

Structure determination

A self-rotation function search for a twofold axis, which would relate the two independent molecules in the asymmetric unit, produced a unique peak 10° away from a crystallographic twofold axis (figure 4). A vector search map of a 5 Å resolution difference Patterson for the gold derivative showed one dominant peak. This site was used to find a second site of lesser occupancy. However, these two sites have not yet yielded a solution to the heavy atom positions of the other derivatives.

The active centre in the crystals

The enzyme can be assayed by measuring the production of Glu-6-P in a coupled reaction with Glu-6-P dehydrogenase and NADP. This procedure was used to show that the activity of

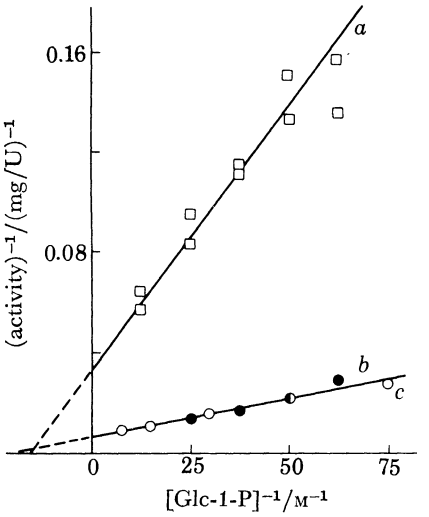

FIGURE 5. Lineweaver–Burk plots obtained with (*a*) microcrystals (smallest dimension 3 μm) and (*b*, *c*) enzyme in 60 % ammonium sulphate: (*b*) supernatant enzyme; (*c*) soluble enzyme.

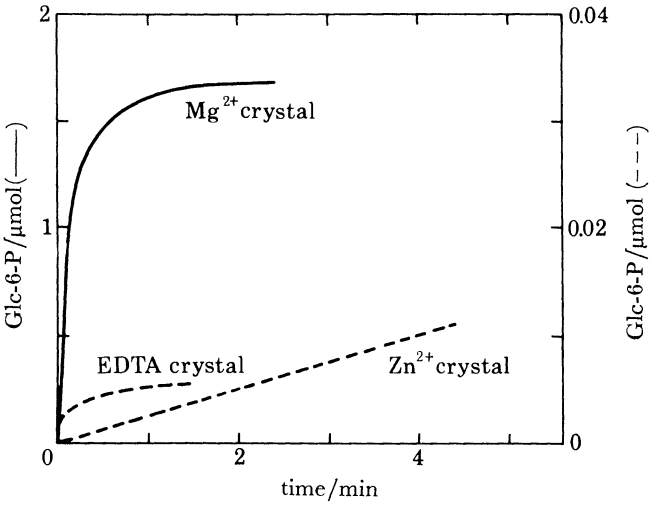

FIGURE 6. The active site bivalent metal ion can be removed or replaced in the crystals.

the enzyme in the crystals is similar to that of the enzyme in solution (figure 5). Similarly it was possible to show that the active-site phosphate can be removed from the crystals. Finally it has been demonstrated (figure 6) that the active-site Mg^{2+} ion can be either removed with EDTA or replaced by Zn^{2+}.

This work has been supported by grants from the N.I.H. and N.S.F. to W.J.R. and M.G.R.

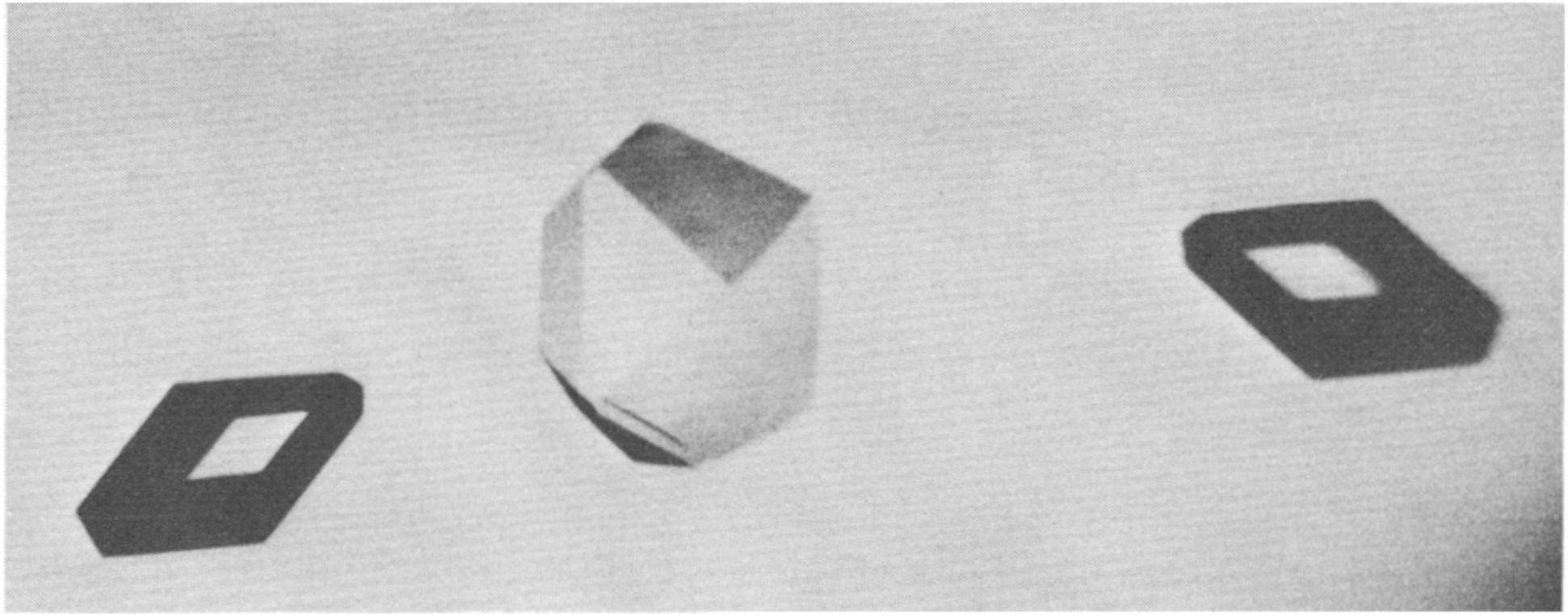

FIGURE 2. Crystals of PGM grown by vapour diffusion from ammonium sulphate and PEG. Their maximum diameter is approximately 1 mm.

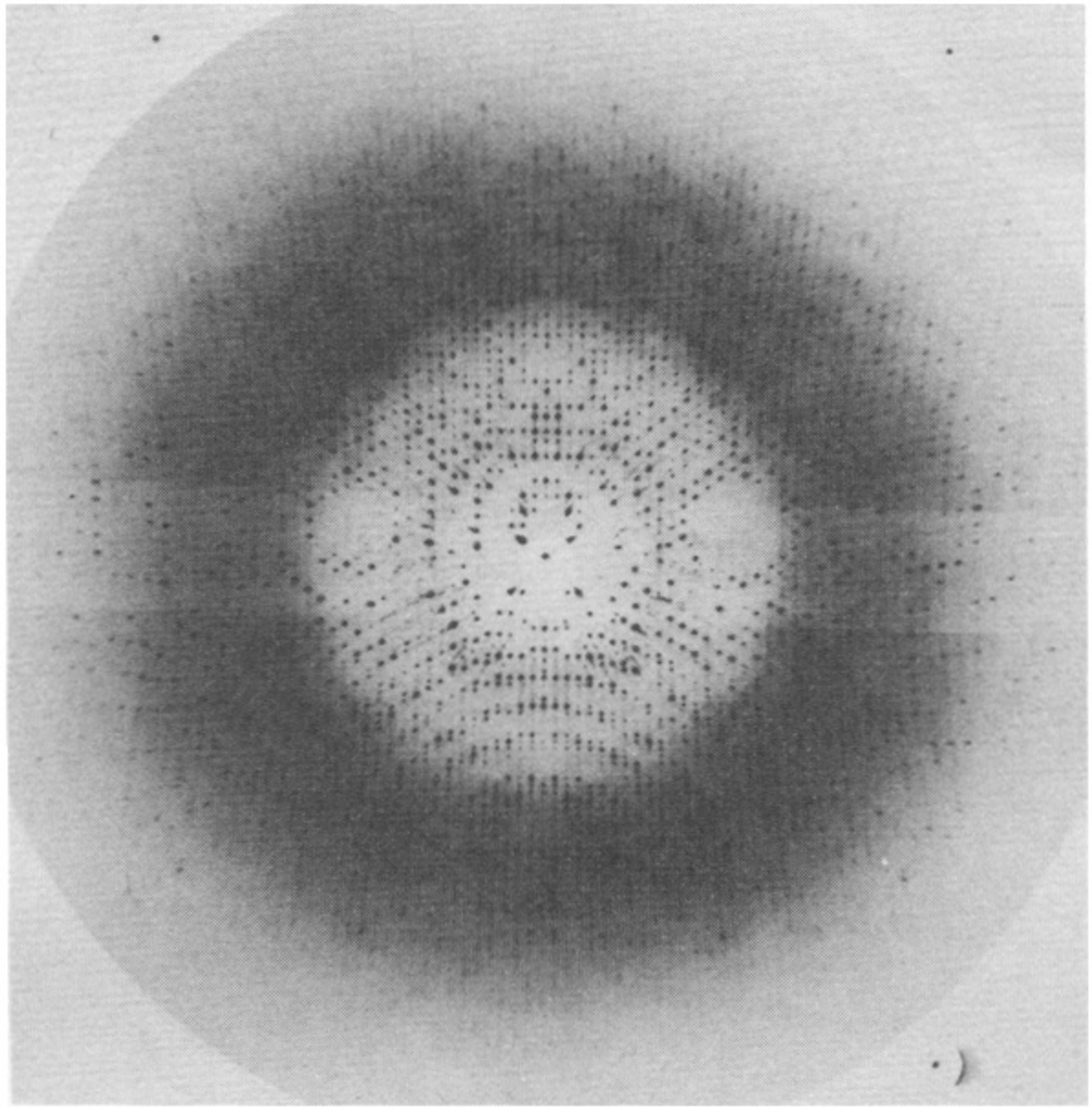

FIGURE 3. An oscillation photograph from a native PGM crystal. The scan angle was 1.1° and the exposure time was 8 h with Cu K_α radiation from a rotating anode generator operated at 35 kV and 35 mA.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES