

# Crystallisation and preliminary analysis of glucose isomerase from *Streptomyces albus*

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The glucose isomerase of *Streptomyces albus* has been crystallised from a dilute solution of magnesium chloride buffered at a pH of 6.8-7.0. The crystals are in the space group I222 with cell dimensions  $a=93.9$  Å,  $b=99.5$  Å and  $c=102.9$  Å. There is one monomer of the tetrameric molecule per asymmetric unit of the crystal and the packing density is  $2.93$  Å<sup>3</sup>·Da<sup>-1</sup>. The tetramer sits on the 222 symmetry point of the crystal. Native data have been recorded to a resolution of 1.9 Å and the crystals diffract to about 1.5 Å. The  $\alpha$ -carbon coordinates of the *Arthrobacter* glucose isomerase and the backbone coordinates of the *S. olivochromogenes* enzyme determined by other groups have been oriented in the present cell. The structure is currently being refined. The binding of several metal ions to the two metal sites has been analysed.

Enzyme crystallization; Glucose isomerase; Amino acid sequence; X-ray analysis; (*Streptomyces albus*)

## 1. INTRODUCTION

The use of D-(+)-xylose as a food source by bacteria requires three initial steps: transport of xylose into the cell, its isomerisation from the aldo form to the keto sugar xylulose, followed by phosphorylation to give D-xylulose 5-phosphate. The isomerisation is catalysed by D-xylose ketol-isomerase (EC 5.3.1.5) in the cytoplasm of the cell [1]. The enzyme is of interest in the commercial production of ethanol.

While D-xylose is the preferred substrate for the enzyme, it is also able to isomerise D-ribose to D-ribulose and D-glucose to D-fructose [2]. The glucose to fructose conversion is of great commercial importance: fructose-enriched corn syrup is extensively used in the soft-drink and related industries. Hence, as in the present paper, the enzyme is often referred to as glucose isomerase.

Glucose isomerase can be obtained from a great

number of microorganisms [3-5]. The enzyme studied in our laboratories was isolated from *Streptomyces albus*. It has a molecular mass of 172 kDa and consists of four identical subunits of 43 kDa [6]. The enzyme has an isoelectric point of 4.3. Addition of Mg<sup>2+</sup> is necessary for the activity of the protein, whereas Co<sup>2+</sup> stabilises the tetrameric structure.

The sequences of the gene coding for glucose isomerase from the four organisms, *Bacillus subtilis* [7], *Escherichia coli* [8], *Ampulariella* sp. strain 3876 [9] and *S. violaceoniger* [10] have been published and are aligned in fig.1. The alignment was carried out by a combination of the use of the program ALIGN [11] and manual methods. Few deletions and insertions are required to give the alignment shown in the figure; the exact positions of some of these cannot be established without 3-dimensional information on the structures. The *Streptomyces* and *Ampulariella* sequences are much more similar to each other than to that of either the *B. subtilis* or *E. coli* proteins. In addition, the latter both have an extra 49 (*E. coli*) or 56 (*B. subtilis*) residues at the N-terminus. A number of residues are conserved in all four sequences.

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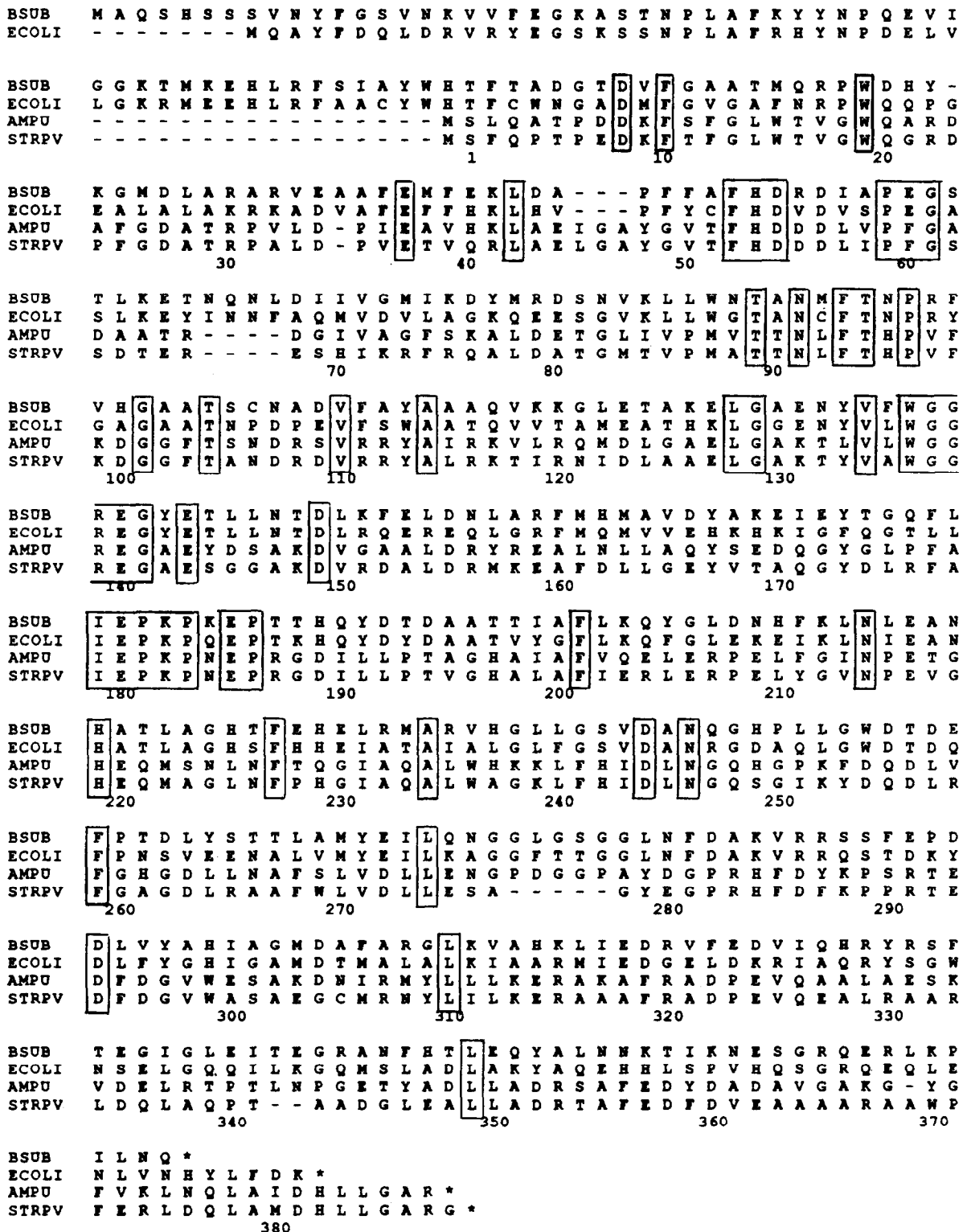


Fig.1. The aligned sequences of glucose isomerase from *B. subtilis* (BSUB), *E. coli* (ECOLI), *Ampullariella* (AMPU) and *Streptomyces violaceoniger* (STRPV). The references are given in the text. The conventional single-letter code is used for the amino acids. Residues conserved in at least 3 of the 4 sequences are indicated by boxes.

These can be expected to play important roles in the structure or function of the enzyme.

The sequence of the *S. albus* enzyme is being determined (Witzel and colleagues, personal communication). Ten of the thirteen N-terminal amino acids are identical to those of the *S. violaceoniger* protein and all are identical to the published N-terminal sequence of the *S. violaceus-ruber* enzyme [12]. The sequences of other peptides currently being determined have at least this degree of homology. These observations suggest a high sequence homology and close similarity in tertiary structure within the *Streptomyces* species.

The *Streptomyces* and *E. coli* enzymes are active in the presence of  $Mg^{2+}$ , but show increased activity on addition of  $Co^{2+}$ . The *Arthrobacter* protein does not depend on  $Co^{2+}$  for optimal activity [13]. Recent EXAFS studies have shown that the  $Co^{2+}$  co-ordination is octahedral rather than tetrahedral [14].

X-ray structures have been reported for four glucose isomerases. Carrell and co-workers [15,16] have determined the structure of the *S. rubiginosus* enzyme at 3 Å resolution. Petsko et al. [17,18], in studies of *S. olivochromogenes*, have also reported a 3 Å structure. Blow and colleagues [19] have analysed the structure of the *Arthrobacter* B3278 isomerase at 2.5 Å resolution, and Rey et al. [20] have recently discussed the structure of glucose isomerase from *Actinoplanes missouriensis*. The *Arthrobacter* and *S. rubiginosus* structures have been compared by the two groups, and show considerable homology [16]. The three-dimensional structure of the *S. olivochromogenes* enzyme is very similar to those of *S. rubiginosus* and *Actinoplanes*. In all four studies the protein exists as a tetramer with 222 symmetry. For *Arthrobacter* and *S. olivochromogenes* there are two monomers per asymmetric unit and for *S. rubiginosus* only one, whereas in *Actinoplanes* the crystal asymmetric unit contains the whole tetramer. The polypeptide in the monomer folds into an 8-stranded, anti-parallel  $\beta$ -barrel, with the strands connected by  $\alpha$ -helices, as found in triose-phosphate isomerase and several other proteins, with an arm-like loop extending away from the barrel. This loop is involved in inter-monomer contacts in the tetramer. Crystallographic data have recently been reported for the *S. violaceoniger* enzyme [21].

We have obtained large single crystals of the isomerase from *S. albus*. These diffract to at least 1.6 Å. The crystallisation and a preliminary three-dimensional analysis are reported here.

## 2. MATERIALS AND METHODS

Glucose isomerase was obtained from Kali as a microcrystalline slurry. A solution of the enzyme was applied to an AcA 34 gel filtration column (2 × 120 cm), which had been previously equilibrated with double-distilled water. After gel filtration the enzyme shows only one band on an SDS-polyacrylamide gel stained with Coomassie brilliant blue.

Crystals were grown by a batch technique. To 400  $\mu$ l enzyme solution in water, with a protein concentration of 90–150 mg/ml, were added 50  $\mu$ l of 1 M  $MgCl_2$  and 50  $\mu$ l of 1 M Tris or Pipes buffer, pH 6.8–7.0. No  $Co^{2+}$  was added.

Crystals were mounted in thin-walled glass capillaries of 1.5 mm diameter. Initial crystallographic characterization of the crystals was carried out using an Enraf-Nonius precession camera mounted on an Elliot GX18 rotating anode operating at 2 kW.

From a single crystal three-dimensional data were collected using synchrotron radiation at the EMBL X31 beam-line in HASYLAB at the DORIS storage ring (DESY, Hamburg). Data were recorded on CEA-Reflex 25 X-ray film, using flat-plate cassettes with three film packs. The crystal-to-film distance was 56 mm and the wavelength of the radiation 1.283 Å. The oscillation range per exposure was 1.5°. A second crystal was used to record data to a limiting resolution of 3.0 Å with a crystal-to-film distance of 110 mm and wavelength 1.488 Å, using short exposures to record the high-intensity, low-resolution terms. Details of the data collection will be published later.

Samarium binding was studied by soaking pregrown crystals previously soaked in 0.1 M EDTA in the normal crystallisation buffer, with the 50  $\mu$ l of 1 M  $MgCl_2$  being replaced by 0.02 M  $SmCl_3$ , to give a high molar excess of samarium over protein. Europium binding was studied by co-crystallising metal-free isomerase from the usual buffer with the 50  $\mu$ l of 1 M  $MgCl_2$  being replaced by the relevant amount of  $EuCl_3$  to give a 1:1 or 2:1 ratio of  $Eu^{3+}$  to monomer of protein for Eu1 and Eu2, respectively (see table 2 for nomenclature). Pb2 was similarly prepared by co-crystallising with a molar ratio of 2  $Pb(AcO)_2$  per monomer. Data were collected on either film or Fuji imaging plate and processed to 5 Å resolution for initial analysis.

The films were digitised on an Optronics Photoscan P-1000 microdensitometer. The images were processed using the MOSCO film processing system. Subsequent calculations were carried out using the CCP4 suite of programs. Both the MOSCO and CCP4 programs were supplied by the SERC Daresbury Laboratory. All computations were performed either on the EMBL Hamburg VAX 11/750 or MicroVAX II (Digital Equipment Corp.) computers. The program FRODO [22] running on an Evans and Sutherland PS330 interactive graphics station was used for inspection and interpretation of the electron density maps.

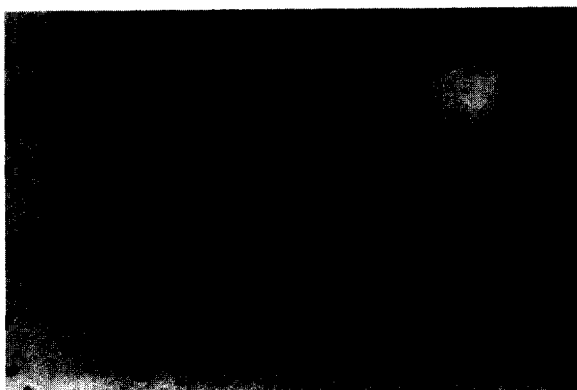


Fig.2. Crystals of glucose isomerase. Bar, 0.5 mm.

### 3. RESULTS AND DISCUSSION

With the conditions described above, crystals appeared in the batch medium after a few hours, growing to maximum dimensions of 0.8–1.0 mm<sup>3</sup> within 1–2 days. They are usually isometric and have rhombic dodecahedral morphology (fig.2). With higher protein concentrations, crystals up to 3 mm could be obtained.

Precession photographs (fig.3) allow us to identify the space group as I222 or I2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>, with the cell parameters listed in table 1. The expected 222 symmetry of the tetramer indicates that I222 is the true space group. The crystals are clearly isomorphous with those of form C of the glucose isomerase

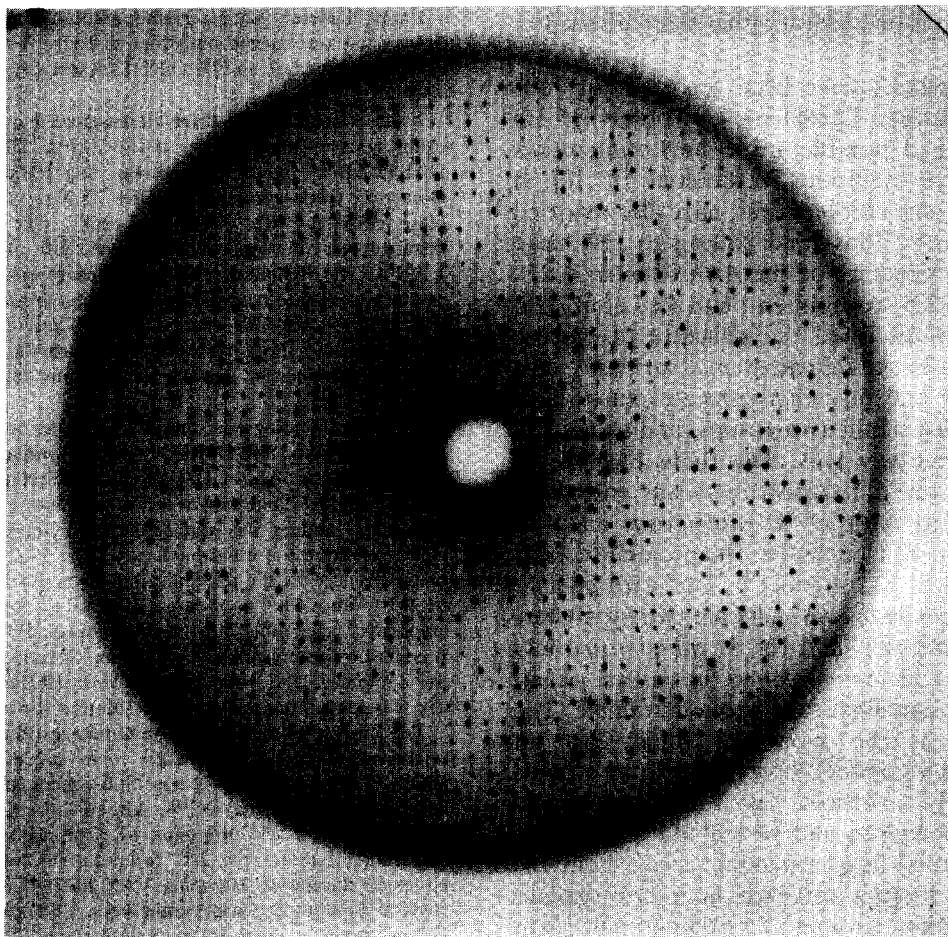


Fig.3. A 15° precession photograph of the *h0l* layer of an *S. albus* glucose isomerase crystal.

Table 1  
Data for the crystals of glucose isomerase reported to date

Species	Space group	<i>a</i>	<i>b</i>	<i>c</i>	<i>V<sub>m</sub></i>	Monomers per asymmetric unit
<i>Streptomyces albus</i>	I222	93.9	99.5	102.9	2.93	1
<i>S. rubiginosus</i> [13]						
Form A	I222	93.9	99.4	102.7	2.9	1
Form B	I222	94.5	98.9	87.0	2.5	1
Form C	I222	93.9	99.6	102.9	2.9	1
<i>S. olivochromogenes</i> [15]	P22 <sub>1</sub> 2 <sub>1</sub>	98.7	93.9	87.7	2.6	2
<i>S. violaceoniger</i> [21]	P4 <sub>1</sub> 2 <sub>1</sub> 2	140.0	134.0	134.0	2.0	4
<i>Arthrobacter</i> B3278 [17]						
Form 1	P3 <sub>1</sub> 21	105.8	105.8	153.6	2.7	2
Form 2	C222	139.9	141.1	83.7	2.2	2
Form 3	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	90.6	108.2	83.9	2.2	2
<i>Actinoplanes missouriensis</i> [20]	P3 <sub>2</sub> 21	143.0	143.0	231.5	3.9	4

from *S. rubiginosus* being studied by Carrell and co-workers [15] (table 1). For the *S. albus* enzyme there is again one monomer of the protein per asymmetric unit, with the tetramer lying on a point of 222 crystallographic symmetry. As for *S. rubiginosus* the molecule has perfect 222 symmetry in the crystals being studied. Data for the other crystals reported to date are also included in table 1 for comparison.

The crystals give excellent diffraction. Data have been recorded to a nominal resolution of 1.9 Å from a single crystal using synchrotron radiation. 90° of data were recorded from a crystal rotating roughly about the [110] direction. The crystal is sufficiently misset from a major axis for there to be effectively no 'blind-region' due to the crystallographic symmetry. For the first 60° of rotation the data extended to the limit of the flat-plate cassettes at 1.9 Å. During the last 30° of rotation the resolution gradually fell to around 2.2 Å. Another crystal was used to record 90° of data to 3.0 Å with much shorter exposures: this allowed the recording of those low-resolution, high-intensity reflections which were saturated on the high-resolution films. The value of the merging *R*<sub>I</sub> for all data is 7.7%.

We have not used isomorphous replacement to solve the phase problem. Carrell et al. [15]

reported some difficulty in obtaining heavy-atom derivatives and our early attempts confirmed this observation. However, Blow and colleagues kindly provided us with the *C<sub>α</sub>* coordinates of the two monomers of the *Arthrobacter* enzyme. We placed these in the I222 cell, about the 222 symmetry point. There is no translation problem as the position of the 222 point is defined in both the unit cell and the *Arthrobacter* coordinates.

The orientation problem is also minimised. There are only six possible orientations of the 222

Table 2  
Data on metal-binding sites

	Site	Occupancy	<i>x</i>	<i>y</i>	<i>z</i>
Sm	1	4.7	0.139	0.145	0.077
	2	4.6	0.082	0.139	0.064
Eu2	1	4.0	0.134	0.146	0.079
	2	2.1	0.086	0.136	0.067
Eu1	1	2.7	0.136	0.143	0.078
Pb2	2	4.7	0.085	0.137	0.068

The metal positions determined by difference Patterson syntheses, difference Fourier syntheses and least-squares refinement at 5 Å resolution. The Sm derivative was prepared by soaking crystals in SmCl<sub>3</sub> (see text) and the others by co-crystallization with 1 or 2 equivalents of the appropriate metal compound

tetramer about the 222 crystallographic symmetry point. The  $C_\alpha$  coordinates were first extended to a more complete model incorporating the complete polypeptide backbone and  $C_\beta$  side chain atoms. This construction of a poly-Ala model was carried out manually on the PS330 graphics system. This model was also used in a rotation function calculation to establish the correct orientation of the

tetramer about the 222 symmetry point. Details of the data processing and orientation calculations will be published elsewhere.

The model was subjected to 6 cycles of refinement using the restrained least-squares minimisation of Konnert and Hendrickson [23], with fast Fourier algorithms for calculation of structure factors and gradients [24]. The  $R$  factor dropped to

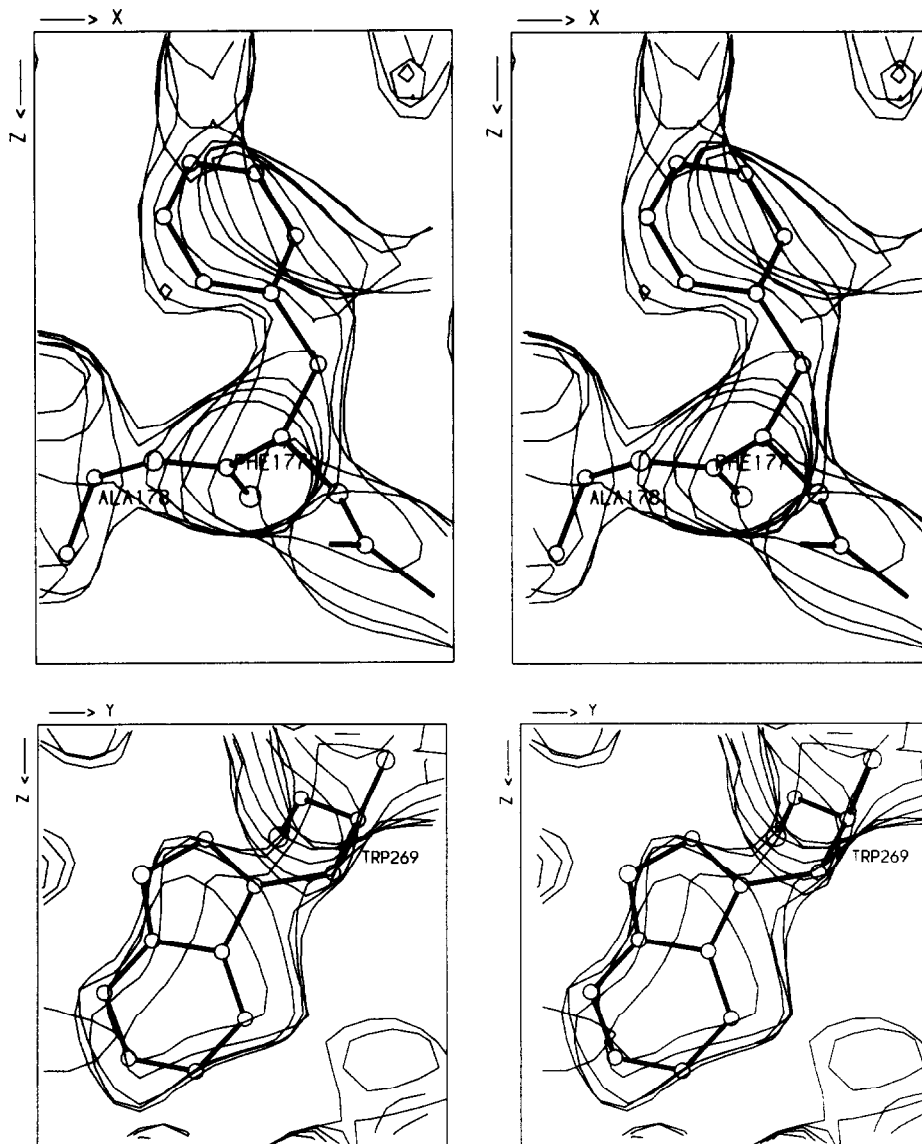


Fig. 4. Stereo views of superimposed sections of the electron density on 'omit' maps at 2.8 Å resolution around two aromatic residues, Phe-177 and Trp-269. The  $2|F_{\text{obs}}| - |F_{\text{calc}}|$  maps have been calculated with phases from the model omitting 15 residues, 175–190 and 260–275, respectively.

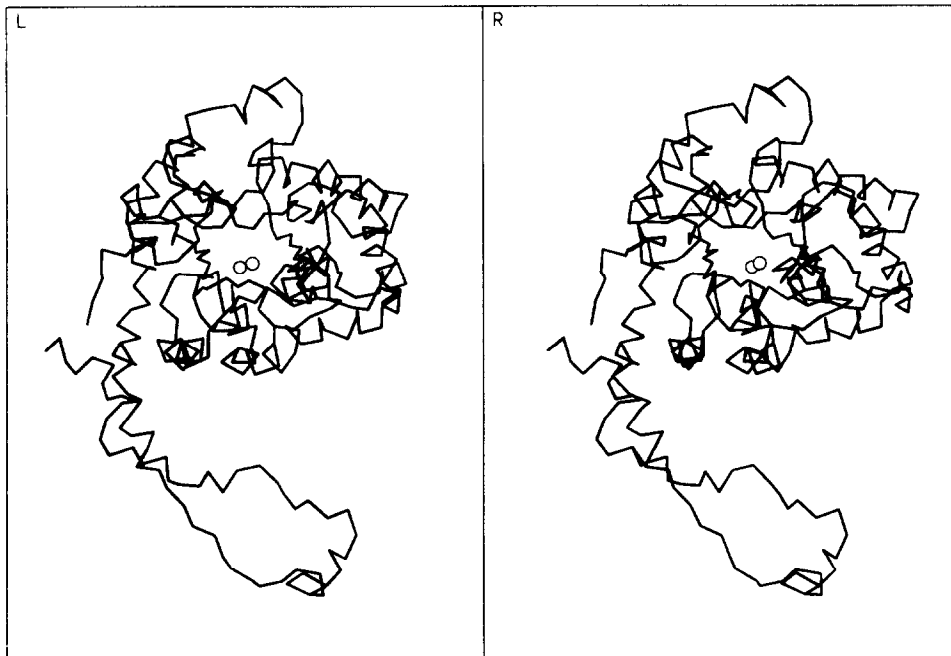


Fig.5. Stereo view of the backbone of a monomer of *S. albus* glucose isomerase with the position of the two metal sites shown.

36% at 2.9 Å, showing that we had indeed found the correct position of the model in the cell, particularly as only the  $C_{\beta}$  atoms of the side chains have been included. Significant density for many side chains could be observed in the electron density map.

Subsequently, Petsko and Farber provided us with the backbone coordinates of the *S. olivochromogenes* glucose isomerase. These were oriented in the cell as for *Arthrobacter* above. The model again refined after six cycles to an *R* factor of about 35%. Many side chains can clearly be seen in the  $2|F_{\text{obs}}| - |F_{\text{calc}}|$  synthesis and even better in 'omit' maps for which 15–20 residues have been excluded from the structure factor calculation. The density around two large aromatic side chains in such an omit map is shown in fig.4 for residues Phe-177 and Trp-269. The maps will certainly allow rebuilding and refinement of the model to proceed in a straightforward manner. In this rebuilding we are using the published sequence for the *S. violaceoniger* enzyme [10] modified with respect to *S. albus* as this is determined.

We have recently achieved successful binding of  $\text{Sm}^{3+}$  to crystals previously soaked for several

hours in a solution containing 0.1 M EDTA. X-ray data have been collected to 2.1 Å resolution from a single crystal soaked in 0.1 M  $\text{SmCl}_3$  using a wavelength close to the samarium absorption edge. The samarium binds at 2 sites per subunit of the enzyme which appear to be the same as those for europium reported in [17,20]. Preliminary data to 5 Å resolution have been collected for crystals obtained from metal-free enzyme co-crystallised with a 2:1 molar ratio of metal to monomer of enzyme for  $\text{Pb}^{2+}$  and  $\text{Eu}^{3+}$ , and for a 1:1 ratio of  $\text{Eu}^{3+}$ . The results of the binding studies are summarised in table 2.  $\text{Sm}^{3+}$  and  $\text{Eu}^{3+}$  bind at identical sites, with site 1 in table 2 having the higher occupancy. This is the only significant site in  $\text{Eu}^{3+}$  crystals with 1 equivalent of metal per monomer.  $\text{Pb}^{2+}$  binds only to site 2, in spite of there being 2 equivalents of metal per monomer. Details of the metal binding must await analysis at higher resolution. However, the conserved histidine at position 219 in the sequence and the conserved glutamic acid at 180 (fig.1) appear to be involved directly in binding to site 1. The positions of the two metal sites in the enzyme are shown in fig.5.

Future work will include rebuilding and refining

the present model to include the side chains and correct main chain loops, deletions and insertions. Extending the data to high resolution is also planned. We shall carry out studies of the substrate- and metal-binding sites of glucose isomerase, particularly in relation to the EXAFS studies on the  $\text{Co}^{2+}$  site already performed in our two laboratories.

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