01.2-04 OPTIMISATION OF THE ANOMALOUS SCATTERING OF THE PLATINUM L_{III} ABSORPTION EDGE IN THE $K_2Pt(CN)_4$ HEAVY ATOM DERIVATIVE OF SINGLE CRYSTALS OF 6-PHOSPHO - GLUCONATE DEHYDROGENASE FROM SHEEP LIVER AND BACILLUS STEAROTHERMPHILUS

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The tuneable diffractometer, XII at the European Molecular Biology Laboratory (EMBL) outstation, Hamburg and the storage ring DORIS (working at 3.18 GeV, 100mA) have been used to collect complete oscillation camera 3-D film data on the high energy side of the platinum L_{III} absorption edge from the $K_2Pt(CN)_4$ derivative of crystalline sheep liver 6-phospho-gluconate dehydrogenase (6-PGDH). Analysis of the refined unit cell dimensions gives an accurate estimate of the wavelength used of 1.003 ± 0.007Å. A typical merging R factor (on intensity) between weak and strong films on a film pack is 7% for data extending to ≈ 3Å resolution, actually more accurate than similar data collected on a conventional X-ray source. The EXAFS station also at EMBL was used to measure the absorption curve in transmission of the L_{III} edge of the inorganic sample $K_2Pt(CN)_4$ alone to estimate the values of f" above and below the edge as well as at the white line (position of strong absorption) which are 9, 4 and 19e^- respectively. The XII workstation provides an intense beam with reasonable exposure times (with photographic film) due to the point focussing set up but a $\delta\lambda/\lambda \approx 5 \times 10^{-3}$ capable of sampling the L_{III} edge such that f" can take a maximum value of 9e^-. The average Friedel difference for the data collected for a protein of this size is expected to be 6% at λ = 1.003Å which compares with 2.5%. 12.9% and 4.6% if data had been taken below the edge, at the white line and at the CuKα (1.5418Å) wavelength. Phase determination of structure factors with this data is in progress and can be compared with phases determined by conventional multiple isomorphous replacement (Adams, Helliwell and Bugg (1977) J. Mol. Biol. 112, 183). This work will serve to establish the efficacy of the method. Data have also been collected on an unknown crystalline structure, Bacillus Stearothermophilus 6-PGDH) both from native, unmodified crystals (at 1.66Å) and a platinum derivative at 1Å. The space group of these crystals ($P3_121$) is not as favourable as the sheep liver case since Friedel equivalents do not occur on the same film. This data will be compared with the sheep liver data described above. A description of the apparatus used to collect the data will also be given.

01.3-01 MOLECULAR REPLACEMENT STUDIES ON DES-PENTA-PEPTIDE INSULIN.

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Insulin from which the 5 C-terminal B chain residues have been removed (known as des-penta-peptide insulin or DPPI) retains significant biological activity and a high affinity for insulin antibodies. The modification, however, abolishes the molecule's ability to aggregate as in 2Zn insulin and it remains monomeric at high concentrations and neutral pH. The DPPI molecule's structure is of great interest since all biochemical evidence indicates that insulin acts as a monomer in the body.

The crystals are usually small, and small variations in the crystallisation conditions often change the space group. The forms for which data are available are described in Table 1.

The data for the Pig I form was kindly sent by the group of the Institute of Biophysics, Academica Sinica, Peking, China. The Patterson maps for the Beef 2 form show that the two molecules in the asymmetric unit have an approximate repeat at ½c. The addition of zinc acetate to this form gives Beef 3.

We have looked for non-crystallographic symmetry in the Beef 1 form; and for matches between all possible pairs of observed intensities.

Calculated structure factors obtained from placing a suitably tailored insulin molecule into a large P1 cell have been matched with each form. Although the very close packing of the asymmetric units has hindered the analyses it has been possible to extract a consistent set of minima.

To position the molecule in the different cells, R factor searches have been carried out. The C2 forms give sensible results which are consistent with each other. The closest packing seems to be around the 2_1 axis.

It is hoped that this molecular orientation will give phases accurate enough to place the omitted residues, and to allow us to start refinement.

TABLE 1 - DPPI CRYSTAL DATA

Species	Pig 1	Sheep	Beef 1	Beef 2	Beef 3	Model Cell
Space-group	C2	C2	P212121	C2	P21	P1
a	58.7	53.9	27.81	52.74	53.0	36.0
b	27.9	25.9	43.52	26.11	25.7	42.0
c	24.0	25.6	57.77	51.64	26.0	60.0
Beta	100.6	93.8	90.0	93.41	93.8	90.0
Asymmetric vol. ($Å^3$)	9659	8606	17477	17750	17678	
Molecules/ asym. unit	1	1	2	2	2	1
Resolution	4.0Å	4.0Å	2.15Å	2.5Å		