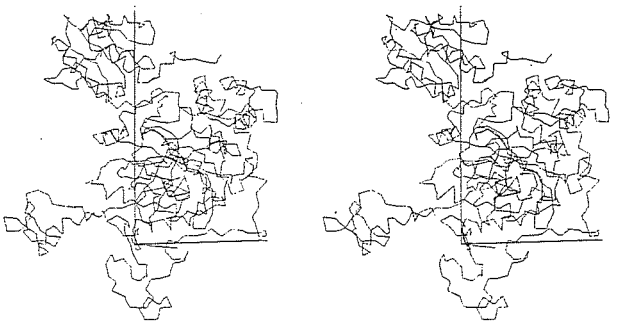
02.1-18 THE STRUCTURE AND HEME ENVIRONMENT OF BEEF LIVER CATALASE AT 2.5 Å RESOLUTION. By Mathur R. N. Murthy, Thomas J. Reid III, Andrew Sicignano, Nobuo Tanaka, W. Donald L. Musick and Michael G. Rossmann, Departments of Biochemistry and Biological Sciences, Purdue University, West Lafayette, Indiana 47907, USA.

Most of the amino acid side chains of beef liver catalase were clearly identifiable in the 2.5 Å resolution electron density map and are in good agreement with the sequence (W. A. Schroeder et al., Arch. Biochem. Biophys. 131, 653-655, 1969). The tertiary structure of one subunit consists of a large anti-parallel β-pleated sheet domain with helical insertions followed by a smaller domain containing four α-helices. The heme group is buried at least 20 Å below the molecular surface and is accessible by a channel lined with hydrophobic residues. The proximal ligand is tyrosine 357 while histidine 74 and Asn 147 are the important residues on the distal side of the heme. The inhibitor 3-amino-1,2,4-triazole, which has been shown to covalently bond to His 74, can be built into the heme cavity with its N(2) atom coordinated to the heme iron. The N-terminal domain and a smaller one of 70 residues packed in 4 successive helices. The third, C-terminal domain, comprising 160 residues, is similar in structure to flavodoxin.

The Bijvoet-difference Fourier map based on an additional measurement of anomalous scattering on a native catalase crystal showed that the heme group (one per subunit) is situated within the N-terminal domain. The distances between iron atoms in different subunits were found to be 31, 35 and 46 Å.

Crystals of P.vitale catalase inhibited by 3-amino-1:2:4-triazole (AT) were obtained and 6 Å resolution data were collected. The difference Fourier map showed that the binding site of AT is situated about 5 Å of the heme group and its center is shifted to a periphery of the porphyrin.

More detailed description of the active site could be done when the sequence is known.



02.1-19 STRUCTURE OF CATALASE FROM PENICILLIUM VITALE AT 3.0 Å RESOLUTION. By B.K.Vainshtein, V.R.Melik-Adamyan, V.V.Barynin, A.A. Vagin, A.I.Grebenko. Institute of Crystallography of USSR Acad.Sci., Moscow, USSR.

Catalase (H_2O_2:H_2O_2-oxidoreductase, EC 1.11.1.6) is an enzyme responsible for decomposition of hydrogen peroxide to molecular oxygen and water. Crystals of catalase from fungus Penicillium vitale (MW 280000) were grown in a preparative ultracentrifuge and belonged to space group $P3_121$ with 1/2 molecule per asymmetric unit. The unit cell parameters are a= 144.4 Å, c=133.8 Å. Intensities were collected on a four-circle diffractometer. Phase determination was based on data for two derivatives ($K_3UO_2F_5$, $K_2Pt(NO_2)_4$) up to 3.0 Å and for two others (K_2PtCl_4, $Pb(CH_3COO)_2$) up to 4.5 Å. The 6 Å electron density map and heavy-atom sites have led to a recognition of 4 equivalent protein subunits related in pairs by a 2-fold non-crystallographic axis (Vainshtein et al., Dokl. Akad.Nauk S.S.S.R.(1979) 246,220). After two cycles of phase refinement based on the method proposed by G.Bricogne (Acta Cryst.(1976) A32, 832) an improved electron density map made it possible to trace the polypeptide chain (Vainshtein et al.,Dokl.Akad.Nauk S.S.S.R. (1980) 250,242) and find preliminary co-ordinates of 654 α-carbon atoms.

The polypeptide chain in the P.vitale catalase subunit forms three domains. About 350 residues starting from the N-terminus constitute the largest domain. It contains a β-sheet of 8 strands with helical insertions between them, forming a distorted barrel. An irregular region of about 60 residues forms a connection between

02.1-20 THE STRUCTURE OF CYTOCHROME C3 FROM DESULFOVIBRIO VULGARIS, MIYAZAKI AT 2.5 Å RESOLUTION. By Y. Higuchi,* S. Bando,* M. Kusunoki,* Y. Matsuura,* N. Yasuoka,* M. Kakudo,* T. Yamanaka,** T. Yagi,*** and H. Iinokuchi**** *Institute for Protein Research, Osaka University, Suita, 565, **Faculty of Science, University, Toyonaka, Osaka, 560 ***Department of Chemistry, Faculty of Education, Shizuoka University, 422, ****Institute for Molecular Science, Okazaki, 444, JAPAN

Cytochrome c3, an electron carrier to hydrogenase [EC1.12.2.1] in sulfate-reducing bacteria has an unusually low redox potential, which seems to be attributed to the fact that it has four heme groups. Some kinds of cytochrome c3 have been isolated and characterized from various strains of sulfate reducing bacteria to date. One of them, cytochrome c3 from Desulfovibrio desulfuricans, Norway has been investigated by X-ray diffraction method, and the preliminary result was reported(Haser et al.(1979) Nature 282, 806).

Here, a brief description of the result of our X-ray structure determination of cytochrome c3 isolated from Desulfovibrio vulgaris, Miyazaki at 2.5 Å resolution is presented. The amino acid sequence study has been completed(Shinkai et al.(1980) J. Biochem. 87, 1747) and the 72.3 % of the sequence differs from that of Strain Norway.

The protein was isolated and purified according to the procedure of Yagi & Maruyama. Crystals of good quality have been obtained by the vapor diffusion method(Bando et al. (1979) J. Biochem. 86, 269). Heavy atom derivatives were prepared by the soaking method. Several kinds of heavy atom reagents were examined, among which Na2PtCl6, K2Pt(SCN)6, K3UO2F5 and K2IrCl6 gave the best results. Three-dimensional X-ray diffraction data were collected up to 2.5 A resolution at 11°C. The protein phases were determined by multiple isomorphous replacement method. The average figure of merit of 0.77 for 4605 reflections was obtained.

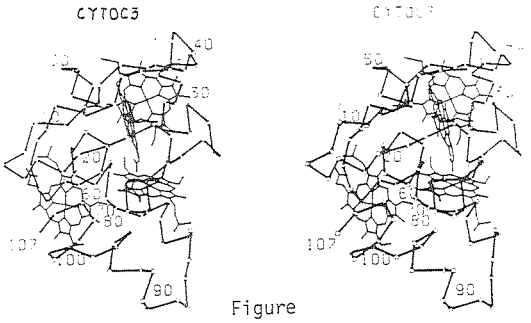
The electron density map was of excellent quality and it was easy to follow the peptide chain in the Richards' box, and Kendrew-type model (1Å=2cm) was constructed referring to the primary structure already reported. Four hemes and amino acid residues including side groups could be located on the map, except for some atoms in side groups of Ala 1, Pro 2 and Lys 3 which may be ascribed to the positional disorder in N-terminal residues. The stereo drawings of connected α-carbon coordinates with heme groups are

shown in the Figure. The overall dimensions of the molecule are approximately 33 X 39 X 34 Å. The heme-heme distances and angles are listed in the Table.

The structure of cytochrome c3 from D. desulfuricans, Norway, has been reported by Haser et al. Since they did not give any stereo drawing, precise comparison of our structure to theirs was not possible. It seems that the peptide back-bone of these two proteins differs significantly. This would be the reflection of only 27.7 % homology between them. Nevertheless, the overall shapes, especially the relative orientations of four hemes, resemble each other. It could be reasonable to assume that only the conformation of hemes is important in the structures of cytochrome c3 family, and the variations of amino acid residues, or even the deletion and insertion of peptides are allowable as long as the relative heme orientations are kept unchanged. This assumption could be confirmed if more structural information on other cytochrome c3 family proteins became available.

Table	Heme	Distance (Å) 1	2	3	4
Heme-Heme Distances	1	-	16.3	18.1	12.8
(upper right) and	2	71	-	12.4	16.2
Heme-Heme Angles	3	22	89	-	11.3
(lower left)	4	80	64	82	-

Angle (°)



Figure

02.1-21 THE STRUCTURE OF A BACTERIAL CYTOCHROME C_4 AND ITS RELATION TO OTHER CYTOCHROMES. By L. Sawyer and C.L. Jones, Napier College, Colinton Road, Edinburgh, A.M. Damas and R.O. Gould, Chemistry Department, Edinburgh University, and M.M. Harding, I.P.I. Chemistry Department, University of Liverpool, U.K.

The bacterial cytochrome c_4 from *Pseudomonas aeruginosa* has 181 amino acids, two haem groups, and a sequence which suggests that it is a 'covalent dimer' of two typical, 'short' cytochrome c segments. A crystal structure determination should show the relation of the protein chain folding of these parts to each other and to that of other cytochromes.

Hexagonal crystals of cytochrome c_4 were obtained from 2M ammonium sulphate with a = 62.38, c = 174.4 Å, space group $P6_522$ (see below), Z = 12. An electron density map at 5 Å resolution has been calculated using intensity data (CAD_4 diffractometer), including anomalous differences, from the native crystal, a $UO_2(NO_3)_2$ derivative (one site, 2 Å from a crystallographic 2-fold axis, occupancy 0.32), and a $K_2Pt(NO_2)_4$ derivative (three sites, occupancies 0.42, 0.15, 0.11). The U atom site was found from the Patterson series using $|F_{HLE}|^2$ as coefficients and the Pt sites from difference Fourier series. Peak heights in difference Fouriers and figures of merit for the derived phases favour the space group $P6_522$ but do not exclude $P6_122$. In the electron density map the molecular boundary can be seen, and within it there are two lobes of electron density; there are similarities in the chain folding in the two lobes, and a similarity to the patterns seen in other cytochromes. A 3 Å resolution electron density map is being prepared.

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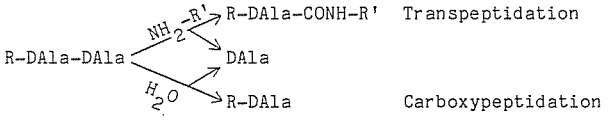
02.1-22 CRYSTAL STRUCTURE DETERMINATION OF A DD CARBOXYPEPTIDASE AT 2.5 A RESOLUTION. By O. Dideberg and P. Charlier, Institut de Physique B5, Université de Liège au Sart Tilman, B - 4000 Liège, Belgique.

The exocellular DD-carboxypeptidase of Streptomyces albus G is a metallo (Zn^{2+}) enzyme. The cofactor is required for activity on substrate analogues (e.g., Ac_2-L-Lys-D-Ala-D-Ala) and for binding of β-lactam antibiotics. We have obtained a 2.5A resolution map using the method of multiple-isomorphous replacement supplemented by anomalous-scattering information. Three heavy-atom derivatives were used; K_2AuCl_4, $K_3UO_2F_5$ and $K_2Pt(C_2O_4)_2$. For 6.700 reflections the figure of merit was 0.66. The electron density map allowed a tracing of almost all the polypeptide chain. The molecule is divided into two domains. The smallest one (71 residues) consists of three helices and random coil regions. The other domain (105 residues), where the zinc ion (catalytic site) is located, consists of two parallel strands and two α-helices, and possesses the only two disulfide bridges. No conformational similarities exist between this DD carboxypeptidase and other Zn^{++} metallopeptidases such as carboxypeptidase A and thermolysin. Ligand binding studies and high resolution map are currently under investigation, details and implications of the protein-drug interactions will be reported.

02.1-23 X-RAY CRYSTAL STRUCTURE OF A PENICILLIN TARGET: STREPTOMYCES R61 DD-TRANSPEPTIDASE-CARBOXYPEPTIDASE. By Judith A. Kelly, Paul C. Moews, James R. Knox, Biological Sciences Group and Institute of Materials Science, University of Connecticut, Storrs, Ct. 06268 U.S.A.

The DD-transpeptidase-carboxypeptidase from S. R61 is an exocellular, penicillin sensitive enzyme (MW 38,000 daltons). The reactions catalyzed are:



These reactions are critical in the growth and maintenance of the bacterial cell wall and are inhibited by beta-lactam antibiotics.

The crystal structure of this enzyme is being determined in order that we may visualize its interactions both with cell-wall substrates and with beta-lactam inhibitor molecules.

The crystals of the DD-transpeptidase are orthorhombic ($P2_12_12_1$) with unit cell dimensions a = 51.1, b = 67.5 and c = 102.9 A (Phil. Trans. R. Soc. Lond. B 289, 361 (1980); J. Molec. Biol. 124, 217 (1979)). The structure has been solved to 2.8 A resolution using three heavy atom derivatives, Na_2PtCl_6, $K_3UO_2F_5$ and CH_3HgCl. The binding site of ortho-iodophenylpenicillin has been located in a well-defined cleft in the molecule.

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