tentative "X-ray sequence" has been derived from the 60% holo sMDH electron density map. The detailed map interpretation was performed using both a "Richards Box" as well as the MMS-X graphics system. Additionally several peptide fragments as sequenced by Bradshaw and coworkers have been aligned with this "X-ray sequence". This sequence alignment was facilitated by the use of a diagonal matching technique, similar to that proposed by McLachlan for amino acid sequence comparisons. It is estimated that approximately 40% of the amino acid residues in sMDH are correctly assigned at this time.

The original MIR phases were derived for the lattice of the partially substituted holo sMDH. Since apo and holo sMDH are sufficiently isomorphous at low resolution, the phases for holo sMDH can be used for the apo form (Holo: a=139.2, b=86.6, c=58.8; Apo:a=140.7, b=87.1, c=58.8; space group for both $P2_12_12$). An initial assessment of the structural differences between the two forms have been made at low resolution, and these differences along with absence of NAD will be incorporated into the initial phase calculations for apo sMDH. Through the analysis of successive F_o-F_c and $2F_o$-F_c maps, followed by structural adjustments and recalculation of phases and structure factors a high resolution electron density map for this form for the sMDH should finally emerge. This will then provide the starting point for constrained crystallographic refinement of apo sMDH. Progress on this work will be reported and if completed, the molecular structure of apo sMDH will be compared with that of the holo enzyme.

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02.1-41 CONFORMATIONAL CHANGES OF ALCOHOL DEHYDROGENASE. By Hans Eklund and Alwyn Jones, Department of Chemistry and Molecular Biology, Swedish University of Agricultural Sciences, S-750 07 Uppsala 7, Sweden.

Alcohol Dehydrogenase crystallizes in two different crystal forms in the presence and absence of the coenzyme NAD. The orthorhombic apoenzyme structure has been refined to an R-factor below 20% at 2.4 Å resolution using different refinement procedures, with group temperature factor refinement as the last step. The triclinic ternary complex structure has been independently refined by constrained-restrained least squares refinement combined with graphic display modelbuilding to an R-factor of about 25% at 2.9 Å resolution.

The results of a detailed comparison between the two structures confirm the earlier presented evidence that the main conformational change is a rigid body rotation of the two catalytic domains coupled to a change of a loop in the coenzyme binding domain. Many details of the conformational changes can now be added and described with high confidence.

Phases calculated from the refined coordinates have been used to study substrate and inhibitor binding.

We shall also discuss the benefits of carrying out the crystallographic refinement of a large protein molecule (750 amino-acid residues) at medium resolution.

02.1-42 THE MOLECULAR STRUCTURE OF L-3-HYDROXYACYL COENZYME A DEHYDROGENASE. By Hazel M. Holden and Leonard J. Banaszak, Department of Biological Chemistry, Washington University, St. Louis, MO 63110.

L-3-hydroxyacyl Coenzyme A dehydrogenase (βHADH) is a mitochondrial enzyme involved in the β-oxidation of long chain fatty acids. It is of special structural interest because its catalytic reaction involves binding sites for both CoA and NAD^+-NADH. Furthermore, the enzyme may have a substrate binding site that accommodates the long hydrocarbon chain of the acyl CoA substrate, the length of which varies as the substrate shuttles repeatedly through the β-oxidation cycle.

Isolated from pig heart, βHADH is known to be a dimer of molecular weight 67,000. The amino acid sequence has recently been established and while a small region in the active site contains several homologous residues, overall there appears to be little homology to other dehydrogenases (Bitar, et al FEBS Letters, (1980), 116, 196). An early monoclinic crystal form obtained from ammonium sulfate was extremely sensitive to unit cell and space group changes in the presence of heavy atom compounds. Crystals obtained from polyethylene glycol in the presence of 0.01 M Tris, pH 8.0, however, proved suitable for an x-ray analysis. These crystals belong to the space group $C222_1$ with a=226.5 Å, b=82.2 Å, and c=124.6 Å. Three isomorphous derivatives K_2PtCl_6, CH_3HgCl and $IrCl_3$ have been prepared using crystals first soaked in an NAD^+-containing solution. Again, it was not possible to prepare isomorphous heavy atom derivatives of the apo-enzyme, results that resemble experiences with the earlier monoclinic form. The positions of the heavy atom sites were determined by inspection of the Patterson maps and confirmed by cross difference Fourier maps. After phase refinement, electron density maps at 7.1 Å resolution (3 derivatives) and 5.25 Å resolution (2 derivatives) were calculated. The envelopes of the two dimers contained within the asymmetric unit were identified in the low resolution map. The local two-fold symmetry of the βHADH dimer does not seem to be expressed in the binding sites of all of the heavy atom derivatives. However, the results of the rotation function studies and inspection of the electron density maps has led to a tentative solution for the positions of the local two-fold rotation axes.

A difference Fourier map between the crystalline apo- and holo-enzyme has also been calculated and indicates the binding of 2 NAD^+ molecules per asymmetric unit or one site per dimer. The one site per dimer stoichiometry correlates with some of the heavy atom results. By measuring fluorescence enhancement, equilibrium studies of the binding of NADH to βHADH shows that the coenzyme binding sites in each subunit of the dimer are equivalent (McLoughlin et al, unpublished results). Hence, the one site binding of the NAD observed by X-ray studies is likely to be the result of crystal packing effects. Crystals of βHADH have also been soaked in S-acetoacetylpantetheine. Although three-dimensional X-ray data has not yet been measured, preliminary precession photographs suggest that in the presence of S-acetoacetylpantetheine, the NAD^+ is no longer bound.

Progress on obtaining a high resolution map as well as the methods used to obtain the refined positions of the heavy atom sites, local dyads, and the substrate binding sites will be presented.

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