PS04.01.12 MOLECULAR REPLACEMENT AT ITS LIMITS? THE STRUCTURE DETERMINATION OF *T.cruzi* g-GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE, 12 MONOMERS IN THE ASYMMETRIC UNIT AND 41% DATA COMPLETENESS AT 3.5 Å RESOLUTION. [1]Guimarães, B.G., [1,2]Souza, D.H.F, [1]Jesus,W.D.P. and [1]Oliva, G., 1. Instituto de Física de São Carlos, USP, Cx.P.369, 13560, São Carlos, SP, Brazil; 2. Instituto de Química de São Carlos, USP.

Recent developments in the software tools available for the Molecular Replacement Technique (AMoRe, Navaza, 1994, *Acta Cryst.* A50, 157-163) have dramatically improved the efficiency and speed of the calculations, making it possible to explore a very large number of potential solutions, even in the limits of partial search models and incompleteness of diffraction data. In the search for crystallization conditions of the trypanosomal enzyme gGAPDH, a crystal form was obtained belonging to space group I4, with very large cell parameters a=b=307.93 Å, c=119.82 Å and diffracting to low resolution (3.5 Å). The crystal has 12 monomers per a.u., V_m= 3.035 $Å^3$.dalton^{-1} and 59.5% solvent. High sensitivity to radiation and lack of isomorphism between different native crystals, results in severe limitations in data collection. Data was measured at station BW6 at HASYLAB-DESY. From the seven crystals used, only two could be merged after integration. Merging of the 35139 measured reflections resulted in 29097 independent reflections (41% completeness), R_{merge}= 12.1%. The structure was solved with AMoRe, using the tetramer of *T.cruzi* gGAPDH as determined from crystal form I (space group P1). 250 peaks from the rotation function had to be included to find the correct solution, highlighting the importance of assessing a large number of possible solutions. Details of the constrained refinement will also be presented.

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PS04.01.13 THE CRYSTAL STRUCTURE OF *T.cruzi* GLYCOSOMAL GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE: IMPLICATIONS FOR THE CATALYTIC MECHANISM AND NEW POTENTIAL TARGET SITES FOR SELECTIVE INHIBITION. [1]Oliva, G.,[1,2]Souza, D.H.F, [1]Araújo, A.P.U., [1]Jesus,W.D.P. [1]Instituto de Física de São Carlos, USP, Cx.P.369, 13560, São Carlos, SP, Brazil; [2]Instituto de Química de São Carlos, USP.

Aiming at the design of specific inhibitors of the enzyme GAPDH from the parasite *Trypanosoma cruzi*, causative agent of Chagas' disease, the crystallization and structure determination of this enzyme was undertaken. We report here the structure of crystal form I, obtained from wild-type recombinant protein overexpressed in *E.coli*: protein concentration 9mg/ml in 25mM Tris pH 7.8, 0.5M Ammonium Sulphate, 2mM NAD, 1mM Azide, 1mM EDTA and 1mM DTT, and reservoir solution 18% PEG 8000, 0.2M calcium acetate and 0.1 M cacodylate, at pH 6.5. Space group P1, a=88.23 Å, b=124.49 Å, c=85.35 Å, α=101.32°, β=112.82°, γ=83.65°, two tetramers in the asymmetric unit, diffraction data collected to 2.8 Å, completeness 67%. The structure was solved by molecular replacement and refined by simulated annealing with strict 8-fold non-crystallographic symmetry. The final model included 92 water molecules per monomer and resulted in an R_{factor} of 20.1%, R_{free}=22.3%, with good geometry indicators. The structure is the first of the GAPDH family solved without any ions at the active site. This feature has resulted in a large change in the side chain conformation of active site Arg249, which salt-bridges to Asp210 in our structure. We propose that this could be important for the reaction mechanism, possibly a common feature of all other 7 known GAPDH structures. Comparison with the human enzyme structure also indicate new potential target sites for specific inhibitor design.

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PS04.01.14 STRUCTURE OF RAT LIVER ISOVALERYL-CoA DEHYDROGENASE. Karen Tiffany, Rosemary Paschke, Jerry Vockley[§], and Jung-Ja Kim , Medical College of Wisconsin, Milwaukee, WI 53226 and [§]MAYO Medical School, Rochester, MN 55905

Isovaleryl-CoA dehydrogenase (IVD) belongs to a family of acyl-CoA dehydrogenases that catalyze the α,β-dehydrogenation of acyl-CoA thioesters. Although these enzymes share similar sequences, catalytic mechanisms, and structural properties, the catalytic base is not conserved. Determination of the three-dimensional structure of IVD will allow a better understanding of the mechanism of substrate oxidation and the nature of substrate specificity and may reveal why the catalytic base of IVD is located in a different segment of the linear sequence than other acyl-CoA dehydrogenases.

Rat liver IVD, cloned and expressed in *E. coli*, was crystallized in the orthorhombic space group $P2_12_12_1$ with unit cell parameters a=94.0, b=97.7, and c=181.7. A 2.5 Å resolution data set has been collected on an R-axis image plate detector. The molecular replacement method was employed to solve the phases, using medium chain acyl-CoA dehydrogenase (MCAD) as the search model. A rotation search was carried out using the Patterson search procedure in XPLOR. The highest peak that emerged after Patterson correlation refinement was used for the translation search. Iterative steps of XPLOR programs and manual fitting are being executed to refine the model. The R-factor generated after the first rigid body refinement was 47.7%. After two rounds of positional refinement followed by simulated annealing, the current R-factor is 31.6%. Even at this stage of refinement, it can be confirmed that E254, the proposed catalytic base, is located in the active site of IVD near the C_a-C_β bond of the substrate.

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PS04.01.15 ALCOHOL DEHYDROGENASE: MORE STRUCTURES - IMPLICATIONS TO SPECIFICITY AND FUNCTION. Ramaswamy S., El Ahmed M., Jörnvall H., Plapp B.V., and Eklund H. Department of Molecular Biology, Biomedical Center, Uppsala Sweden; Department of Medical Biochemistry and Biophysics, Karolinksa Institute, Stockholm; Sweden and Department of Biochemistry, University of Iowa, Iowa City, USA.

Electron transfer pathway and the specificity of alcohol dehydrogenase (ADH) have been investigated by determining a number of structures. We have determined the structure of ADH from cod fish and several substrate and product analogue complexes of ADH from horse liver to reasonably high resolutions.

The cod fish class I ADH is closer in sequence and hence in phylogeny to the class III ADH's, which are considered the primordial ADH's. The structure was determined by molecular replacement and refined to 2.1 Ang resolution. The structure is very similar in the core regions and most of the changes are in the loops. The changes in the loops alone seem to govern the type of substrate that binds and the activity of these enzymes. The absence of the His H-bonded to the ribose suggests that this residue is probably not important for the activity of this enzyme unlike the mammalian enzymes.

Ternary complexes of Alcohol dehydrogenase with bromobenzyl alcohol, penta fluoro benzyl alcohol, hepta fluoro butanol, n-formyl piperidine, and cyclo hexyl formamide reveal the flexible nature of substrate binding to the large pocket in this enzyme. However, the pentafluoro benzyl alcohol is bound in a such a way that its modelled pro-R hydrogen is positioned towards the C4 of the NAD., poised for this proton to be transferred, suggesting that this is probably the form just before the proton is transferred. The structure of this enzyme with a potential drug, 3-butyl tmso in its two isoforms has also been determined. The results of all these studies will be presented.