01.2-4 X-RAY CRYSTAL STRUCTURE ANALYSIS OF 3α,20β-HYDROXYSTEROID DEHYDROGENASE FROM *STREPTOMYCES HYDROGENANS*: A PROGRESS REPORT. D. Ghosh, C. M. Weeks., W. A. Pangborn, J. S. Punzi, M. Erman and W. L. Duax, Medical Foundation of Buffalo, Buffalo, NY 14203, and P. M. D. Fitzgerald, Univ. of Alberta, Edmonton T6G 2H7 Canada.

3α,20β-HSD(EC 1.1.1.53) possesses both 3α and 20β dehydrogenase activity and accepts as substrates several androstanes and pregnanes including dihydrotestosterone, progesterone and cortisone. Crystallographic analysis has been undertaken to determine what features permit this dual functionality while maintaining fairly strict substrate specificity. Three-dimensional structure of the native molecule as well as that of the enzyme-inhibitor complex prepared by "affinity alkylation" technique are of interest in this context.

The enzyme, a tetramer of about 100,000 daltons of identical subunits, was crystallized in the space group $P4_32_12$ or its enantiomorph having cell dimensions a = 106.4Å and c = 203.4Å (Ghosh, D., Punzi, J. S. and Duax, W. L., J. of Biol. Chem. 261, 1306-1308 (1986). The complete diffraction data sets of the native and two derivative crystals, made with $K_2Pt(CN)_4$ and $KAu(CN)_2$, and a partial data set on a third, a double derivative of these two compounds, were collected at the Cornell High Energy Synchrotron Source. The native, Pt and Au derivative data films were digitized on a Optronics P-1000 film scanner using a 50 micron raster and processed using a program package developed by one of us (PMDF). There were about 223,000 total observations to 2.55Å resolution in each of the three data sets and 15 to 20 crystals were used to collect a full set. The data were processed to 3.0Å resulting in 82,807, 70,148 and 68,216 "whole" observations for the native, Pt and Au derivatives, respectively. The number of independent reflections for the three sets of data were 21,751, 35,700 and 34,919, respectively (derivatives having Friedel pairs separated). The R_{merge} values on the intensities for the three sets were 11.8, 13.7 and 14.4%, respectively. No post-refinement was performed.

The R values on the structure factor amplitudes for the native-to-Pt derivative and the native-to-Au derivative were 17 and 19%, respectively. The heavy atom positions were determined by the difference Patterson method and confirmed by direct methods using MULTAN to phase the "ΔF" structure. Meaningful phase sets were selected based on high values of the absolute figure of merit and lov values of the residual. The reflections in the starting sets were chosen by trial and error to remove weak links in the convergence map. Each derivative had two or three phase sets that were significantly better than the rest. For the Pt derivative, three of the four sites determined by the difference Patterson method were the first three peaks in the E map and the fourth was the highest in a second E map, which was origin shifted from the first. For the Au derivative, two of the four sites located from the difference Patterson map were the highest peaks in the best E map and the other two were the first two peaks in the next best map. Thus each derivative has four major binding sites, one for each subunit of the tetramer in the asymmetric unit. These derivatives do not appear to have any major sites in common. It is not yet possible to determine if either derivative also has minor sites.

Work is currently in progress towards the calculation of multiple isomorphous replacement phases.

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01.2-5 STRUCTURE ANALYSIS OF ω-AMINOACID: PYRUVATE AMINOTRANSFERASE FROM PSEUDOMONAS SP. F-126. By N. Watanabe[1], K.Sakabe[2], N.Sakabe[3], T.Higashi[4], K.Sasaki[5], S.Aibara[6], Y.Morita[6], K.Yonaha[7], S.Toyama[7] & H.Hukutani[1],1. Inst. of Physics,Univ. of Tsukuba,Ibaraki 305, 2. Dept. of Chemistry, Nagoya Univ., Nagoya 464, 3. National Lab. for High Energy Physics,Tsukuba, Ibaraki 305,4. Dept. of Pharmaceutical Science, Kyoto Univ., Kyoto 606. 5. College of Medical Technology, Nagoya Univ., Nagoya 461. 6. The Research Inst. for Food Science, Kyoto Univ. Uji 611, 7. Dept. of Agricaltural Chemistry, Ryukyu Univ., Naha, Okinawa 903, Japan.

ω-amino acid:pyruvate aminotransferase from Pseudomonas sp. F-126 is a tetrameric enzyme with 1600 amino acids including 8 residues of half-cystine/monomer. The enzymatic properties have been characterized by K. Yonaha and his colleagues (K. Yonaha, S.Toyama & H. Kagamiyama, J.B.C. 1983, 258, 2260-2265). The amino acid sequence of this enzyme has not been determined.

Crystals for X-ray diffraction analysis were grown in a solution of 0.02M potassium phosphate buffer at pH 7.66 containing 0.5 saturated ammonium sulfate. The space group is I222, and a unit cell dimensions are a=124.67, b=137.90, and c=61.45A. An asymmetric unit of the crystal contains a monomer, and the phases were calculated by MIRA method. The data collection was carried out by using a Weissenberg camera (N.Sakabe, J. Appl. Cryst. 1983, 16 542-547) with SR at the PF in Tsukuba. Two separate heavy atom derivatives have been prepared by soaking crystals in each solution containing 2mM mersalyl and 2mM K_2PtCl_4, respectively. The intensity data of the native crystal, and the mersalyl derivative were collected by using Fuji Imaging Plate(IP) at λ= 1.488 and λ= 1.004A, respectively, whereas the intensity data of Pt derivative were collected using Kodak DEF-5 film at λ= 1.488A. The merge R (F^2) was 0.065, 0.056 and 0.126 for native crystal, mersalyl and Pt derivatives, respectively. The heavy atom positions were obtained from a combination of difference Patterson and difference Fourier maps and were refined by using a least-squares technique. The final figure of merit was 0.56 with 14,674 data.

The molecular boundary of the enzyme is clear on the electron density maps. The molecular model was prepared at 6A resolution, and the shape of the molecule is shown in a Figure. The tracing of the chain is in progress using a polyalanine chain on 2.3A maps.

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