polarography. The macroscopic formal potentials of the four consecutive one electron reversible electrode reactions have been evaluated by a least squares fit to the analytical equation for the overall electrode process. The obtained values of the macroscopic formal potentials of cytochrome c_3 in 0.03 M phosphate buffer at pH 7.4 are as follows: $E_1^{0'}$ = -0.240, $E_2^{0'}$ = -0.297, $E_3^{0'}$ = -0.315 and $E_4^{0'}$ = -0.357 V. If the four redox sites are equivalent and non-interacting the spacing between macroscopic potentials are: $E_{1,2}^{0'}$ = -0.0252 $\Delta E_{2,3}^{0'}$ = -0.00208 and $\Delta E_{3,4}^{0'}$ = -0.0252 V. The observed value of $\Delta E_{2,3}^{0'}$ = -0.018 V implies that the two mid-value sites are almost equivalent and are allosterically interacting. The rest two hemes are supposed to be non-equivalent and non-interacting.

An inspection of the structure and the environment of heme groups led us to assume the model for the electrochemical behavior. (1) Heme 3 and 4 are equivalent and interacting. (2) Heme 1 and 2 are non-equivalent and non-interacting. This model enables us to deduce the intrinsic (microscopic) potentials to be -0.243, -0.316, -0.299 and -0.351 V. The plausible assignment to each heme is Heme 2, 3, 4 and 1, respectively. Heme 3 and 4 would have an attractive interaction through Phe-20. Other physico-chemical properties will be well understood in terms of the refined structure.

02.1–13 RIBONUCLEASE-A: X-RAY STUDIES OF PROTEIN-NUCLEOTIDE INTERACTIONS. By N.Borkakoti, D.S.Moss and R.A.Palmer.

The results of X-ray analyses of three ribonuclease-A-nucleotide complexes, at 2.3 Å, are reported. A modified purine mononucleotide, 8-oxo-guanosine 2'-phosphate in a syn conformation, binds at the pyrimidine-binding site of the catalytic cleft. Solvent molecules are expelled from the active site due to inhibitor binding. The positions of the side-chains of the active-site residues Gln-11, His-12 and Thr-45 are well defined and do not alter on inhibitor binding. The mobility of Lys-41 is greatly reduced in the protein-nucleotide complexes and the terminal amino group interacts directly with the 2'-phosphate group of the nucleotides. In the complex of the enzyme with a modified pyrimidine, cytidine-N(3)-oxide 2'-phosphate, His-119 is stabilised in the minor site of the native protein, while in the protein-purine derivative the imidazole group is located in the major site. Inhibitor binding induces movements in the side-chains of Lys-7 and Lys-66 which also modify the conformation of the active-site cleft of ribonuclease A.

02.1–14 CRYSTALLIZATION OF 3α,20β–HYDROXYSTEROID DEHYDROGEANSE FROM STREPTOMYCES HYDROGENANS. By Paula M. D. Fitzgerald, William L. Duax and John S. Punzi, Medical Foundation of Buffalo, Inc., Buffalo, New York, USA, 14203, and James C. Orr, Memorial University, St. Johns, Newfoundland, Canada, A1B 3V6.

3α,20β–Hydroxysteroid dehydrogenase is a NADH dependent oxidoreductase isolated from Streptomyces hydrogenans. Its steroid substrates include certain androstanes and pregnanes; both the 3- and 20-positions have been shown to be reactive. The enzyme is a tetramer with apparently identical subunits of M_r=25,000. We have obtained three crystalline modifications of this enzyme, all grown from concentrated phosphate or ammonium sulfate buffer solutions, pH between 6.2 and 8.7. Hexagonal bipyramid crystals grow when no coenzyme is added to the crystallization medium, tetragonal bipyramid crystals grow in the presence of NADH and hexagonal rods grow the the presence of NAD^+.

The hexagonal bipyramid crystals have been characterized crystallographically; they have the symmetry of space group $P6_422$ (or its enantiomorph, $P6_222$), with unit cell dimensions a=127.3Å, c=112.2Å. Volume and density considerations imply that the crystallographic asymmetric unit contains two monomers, and therefore that the tetramer possesses a twofold axis of symmetry that is coincident with a crystallographic twofold symmetry element. The fact that different crystal forms are obtained in the presence and absence of coenzyme may be taken as evidence that the molecule undergoes a conformational change upon binding of coenzyme.

This work has been supported in part by NIH Grant AM 26546 and by the James H. Cummings Foundation. X-Ray diffraction data were collected at the Cornell High Energy Synchrotron Source.

02.1–15 p-HYDROXYBENZOATE HYDROXYLASE FROM PSEUDOMONAS FLUORESCENCE. By J.M.v.d.Laan,H.Schreuder, R.K.Wieringa,W.G.H.Hol,J.Drenth,Dept. of Chemical Physics,University of Groningen,Nyenborgh 16,9747AG Groningen,The Netherlands.

p-Hydroxybenzoate hydroxylase is a yellow, NADPH dependant,flavine containing enzyme with a molecular weight of 44299 dalton. The enzyme belongs to the class of external monooxygenases. The active site catalyses two reactions :

1) $NADPH + H^+ + E\text{-}FAD.POHB \rightarrow E\text{-}FADH_2.POHB + NADP^+$

2) $O_2 + E\text{-}FADH_2.POHB \rightarrow E\text{-}FAD + 3,4\ DOHB + H_2O$

POHB is the substrate p-hydroxybenzoate and 3,4 DOHB is the product of the reaction : 3,4 dihydroxybenzoate. p-Hydroxybenzoate hydroxylase was crystallized as the oxydized enzyme-substrate complex (E-FAD.POHB).(Drenth et al. JBC (1975) 250,5268). With these crystals the three dimensional structure could be determined at a resolution of .25 nm (Wieringa et al. J.Mol.Biol (1979) 131,55).Further progress in the study of the enzymatic mechanism was seriously hampered by the bad reproducibility of the crystallisation. Improvement of the purification of the enzyme revealed a charge heterogeneity . On a DEAE-sepharose Cl-6B anion exchange column we could separate up to five fractions of p-hydroxybenzoate hydroxylase. Determination of the molecular weight of the various protein fractions from the anion exchanger with size exclusion HPLC showed that only one fraction contained pure dimers(2x44299). Crystallization experiments with the different protein fractions revealed that good crystals exclusively could be obtained from the protein fraction that contained dimers.In this way the enzyme-substrate complex could also be crystallized in the presence of NADPH,$NADP^+$ and the NADPH fragment ATP-ribose.

With crystals grown in a medium from which the substrate was deleted we were able to collect a data set up to .35 nm. We expect that this structure will indicate the conformational changes in the enzyme on substrate binding.