02.10-3 CRYSTAL STRUCTURES OF THE OXIDIZED INTERMEDIATE OF CYTOCHROM C PEROXIDASE AND ITS DECAY PRODUCT. By S. Edwards, D. Anderson, J. Kraut and Ng.h Xuong. Departments of Chemistry, Physics and Biology, University of California, San Diego, La Jolla, California 92093.

We report the use of high-speed data collection techniques to study chemical processes in an enzyme crystal. The experimental apparatus includes the locally developed multiwire area detectors with a rotating anode x-ray source and a low-temperature device. The enzyme system under study is the oxidation and spontaneous reduction of cytochrome c peroxidase. By manipulating the experimental conditions, we have examined four structures: the parent, an intermediate that results from oxidation by the substrate, peroxide and two structures representing the decay (at different times) of the oxidized intermediate. Viewed in sequence, these structures show the dynamic events resulting from oxidation and decay and coordinated movements at the surface of the molecule which may be important for electron transfer.

Parent CCP crystals were converted to the intermediate, compound I, by soaking in a peroxide solution. X-ray data were rapidly collected at low-temperature to minimize decay (Edwards, Xuong, Hamlin and Kraut, Biochemistry, 1987). Difference fourier maps, compound I minus parent, showed many peaks and holes around the heme. The largest peak and hole indicate that the iron moves toward the distal side of the heme on oxidation ($Fe^{+3} \rightarrow Fe^{+4}$) and that six oxygen atoms move slightly toward the sulfurs of a methionine pair, which may be the second site of oxidation in compound I. Movement of the iron pulls on the proximal histidine which is located near the oxidized methionine pair. These subtle postional adjustments appear to cause a beta-turn in the same vicinity to twist. This twist may be important because it could affect the proposed docking site for cytochrome c.

In further experiments, x-ray data were collected from two crystals in which the compound I state was allowed to decay for different lengths of time at room temperature. The first one after 4 hours and the second one after one day. Solution studies suggest that the oxidizing equivalents migrate to different side chains upon decay and that the heme environment in the decay product resembles the parent. Crystallographic data confirm that the iron relaxes toward the promimate side of the heme in the decay product. However, a large hole in the differerce fourier map at the ligand binding site, where a water molecule is normally bound, suggests that some distortion of the heme vicinity persists in the decay product. The current resolution of our data 2.5 Å, will have to be extended in order to clarify the precise nature of these subtle perturbations.

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02.10-4 DYNAMICS OF NUCLEOSIDES, NUCLEOTIDES AND PEPTIDES: SOME PRELIMINARY RESULTS P. Sudarsanam, John C. Sy , N. Ramasubbu, T. Srikrishnan and R. Parthasarathy, Center for Crystallographic Research and Department of Biophysics, Roswell Park Memorial Institute, 666 Elm Street, Buffalo, NY, 14263.

In order to understand the dynamics of peptides, nucleosides and nucleotides in general and to understand the relative motion of the nucelic acid bases in particular, we analyzed the anisotropic thermal parameters of over 120 nucleosides and nucleotides using rigid-body analysis (V. Schomaker & K. N. Trueblood, Acta Cryst., B24, 63 -76, 1968). We examined in particular the librations of the bases which were modelled as rigid groups. The agreement index between the observed and calculated thermal parameters of the rigid groups ranged from 0.04 to 0.35; when this index was 0.15 or less, we examined further the directions of the axes of libration. The axis for least libration almost always lies along the normal to the bases. For pyrimidines, the major librational axis in some cases corresponded to the direction of the glycosidic bond (with value of L1 ranging upto 35°) but in most cases tilted to be along the N1-C5 direction. For the limited number of purines for which data were available (and in NAD, A2'p5'C and U3'p5'A), the major librational axis was nearly in the plane of the base and not too far from the normal to the glycosidic bond. No clear pattern of rigid body libration emerged for the limited number of peptides that we studied. Using the program CHARMM (B.R. Brooks et al., J. Comput. Chem. 4, 187-217, 1983), we also studied the normal modes of vibrations of isolated purine and pyrimidine nucleosides. The major contributor to the atomic fluctuations in these cases are the low frequency modes. The low frequency modes that correspond to the large amplitudes are "soft-modes" that change the relative conformation of the nucleosides and nucleotides about the glycosidic and C5'-O5'bonds. Work supported in part by NIH GM 24864, CA 23704, a N.Y. State Fellowship to P.S. and a N.Y. State Summer Fellowship to J.C.S.