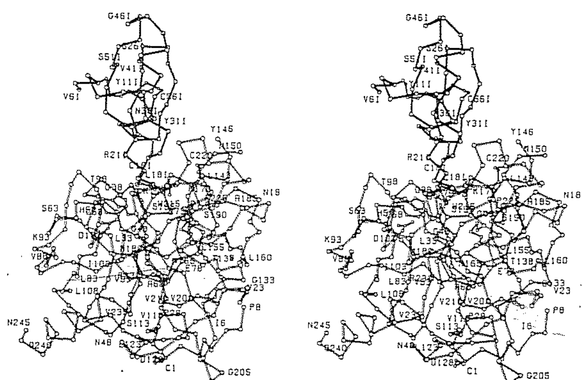

β-sheet. Along one edge of this central core runs the strand P_5-P_3' containing the reactive bond. Comparison of the structure of OMTKY3 bound to SGPB with that when bound to α-chymotrypsin shows that the strand P_5-P_3' rotates by 14.3° relative to the central core of the domain. This rotation can be modelled by a hinge-like motion about the rotation axis. This conformational flexibility of the ovomucoid domain allows the complementary segment of the inhibitor to bind to several serine proteinases in spite of differences in enzyme structure remote from S_6 to S_3'. The central α-helix and β-sheet seems to provide a relatively rigid scaffolding to support the conformationally labile reactive site. An additional conformational change in OMTKY3 or in elastase at Arg217A (or both) must occur in order for the complex between these proteins to form.

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02.10–3 DISCRETE DISORDER IN PROTEIN CRYSTALS. By Janet L. Smith, Wayne A. Hendrickson, Richard B. Honzatko and Steven Sheriff, Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D.C. 20375 USA.

Proteins in solution are widely recognized to be flexible molecules. We have recently observed the manifestations of such flexibility in the crystal structures of four proteins and have modeled several discretely disordered side chains in each. The four structures are:
Crambin with R=0.111 to 0.945A resolution (WAH with M. M. Teeter, Nature (1981) 290, 107);
Erabutoxin B with R=0.152 to 1.4A resolution (JLS with WAH, B. W. Low and P. E. Bourne; Kimball et al., Biochem. Biophys. Res. Comm. (1979) 88, 950);
Myohemerythrin with R=0.159 to 1.7/1.3A resolution (SS with WAH and JLS; Hendrickson, Klippenstein and Ward, Proc. Natl. Acad. Sci. USA (1975) 72, 2160); and
Lamprey hemoglobin with R=0.142 to 2.0A resolution (RBH with WAH; Hendrickson, Love and Karle, J. Mol. Biol. (1973) 74, 331).
Models from restrained least-squares refinement have individual thermal parameters (anisotropic for crambin) and very good stereochemistry (rms deviation from ideality in covalent bonds = 0.014-0.017A; mean magnitude of difference in B between bonded main chain atoms = 0.67-0.95A^2). We have observed in electron-density maps and included in refinement conformational heterogeneity for six side chains in crambin, ten in erabutoxin, seven in myohemerythrin and ten in lamprey hemoglobin. Most disorder mates are related by rotation of side chain torsional angles and all make sensible nonbonded or hydrogen bonded contacts. Two of the disordered side chains in crambin and one in lamprey hemoglobin are cases of heterogeneity in the amino acid sequence, and both of those in crambin exhibit further positional disorder. In most cases conformational heterogeneity is modeled only in side chains, although its effects are likely felt in the protein backbone as well. Many of the disordered side chains are associated with disorder in the solvent structure. There are also numerous pairs of water-water or water-ion sites which reproducibly refine too close to one another to be simultaneously occupied. The crambin and erabutoxin crystals both contain alternate, mutually exclusive networks of water molecules. We expect that further discrete disorder in solvent regions may be masked by the relatively high thermal parameters typically associated with solvent sites.

The effect of resolution is seen dramatically in the extent of disorder observable in these four structures. In lamprey hemoblogin (2.0A) only widely separated alternate side-chain conformers can be assigned with certainty; four of ten disordered side chains are not well resolved but were built into persistent Fo-Fc electron density. By contrast, in crambin (0.945A) features with very low occupancy can be reliably refined and some three-way disorder is observed. There is no evidence in crambin electron-density maps of continuous large-scale motion of any part of the protein. Rather we see alternate, partially occupied conformers. As we compare this to electron densities at lower resolution from which such large-scale motion could be postulated, we conclude that lack of resolution obscures much discrete disorder. The crambin results combined with the fact that discrete disorder can be stably refined at 1.4 to 2.0A resolution support the hypothesis that large atomic displacements in protein molecules generally result in discrete conformers separated by energy barriers high enough that flexible groups spend little time between stable states.

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02.10–4 CONFORMATIONAL VARIABILITY OF THE COENZYME NAD^+ IN THE FREE AND BOUND STATES: NICOTINAMIDE SANDWICHED BETWEEN ADENINE AND WATER IN THE CRYSTAL STRUCTURE OF THE FREE ACID FORM OF NAD^+.[x)] R. Parthasarathy and S.M. Fridey, Center for Crystallographic Research, Roswell Park Memorial Institute, Buffalo, NY 14263 USA.

The coenzyme NAD^+ plays a dominant role in the hydride transfer in biological redox processes. Its structure and conformation in the free state in solution as well as in the bound state complexed to enzymes have been investigated intensively over many years using a variety of spectroscopic techniques including ^{13}C and ^{31}P NMR and using theoretical calculations. X-ray crystallographic studies (at low resolution) of NAD^+ bound to several dehydrogenases demonstrated the extended form with the nucleotides exhibiting non-standard conformations (for a summary see Saenger, Reddy, Muhlegger and Weimann (1977) in Pyridine Nucleotide-Dependent Dehydrogenases, (Ed.) H. Sund, Walter de Gruyter, New York USA, pp. 222-236). A medium resolution single crystal study of Li^+-NAD^+ complex (Saenger, Reddy, Muhlegger and Weimann (1977) Nature 267, 225-229) shows an extended conformation of NAD^+, somewhat similar to that found in holoenzyme complexes. Here, we present a very accurate high resolution x-ray study of the free-acid form of NAD^+ in which the NAD^+ molecule adopts a totally different conformation. Crystals of NAD^+ tetrahydrate ($C_{21}H_{27}N_7O_{14}P_2$ $4H_2O$) are triclinic, a = 8.643(2), b = 8.857(1), c = 11.184(3)A, α = 109.74(2), β = 90.76(2), γ = 103.43(1)°, V = 779.9Å^3, Z = 1, space group P1. Using full three dimensional data to the limit of the Cu-sphere for CuKα (3460 reflections, 2624 $\geq 3\sigma$), the structure was solved using Patterson and difference-Fourier techniques and refined by full-matrix least-squares procedures to an R of 0.03. All the hydrogen atoms were located in difference electron-density maps and their parameters were refined by least-squares. The molecule exhibits

a somewhat compact shape inspite of the positive charges on the two bases; the adenine and nicotinamide rings are about 9.6Å apart. The planes through the two bases are parallel and are at 4.08Å apart. The C-NH_2 of the carboxamide group is trans to C(3)-C(4) of the nicotinamide whereas it is cis in the Li^+-complex. Both the nucleotides adopt the preferred conformation usually found for nucleotides, viz: anti, g^+ for C(4')-C(5') and C(2')-endo. Both the sugars exhibit a very pronounced bond-shortening anomeric effect (i.e. C(1')-O(4') < C(4')-O(4') by 0.078Å and 0.039Å respectively for the sugars attached to nicotinamide and adenosine. The pyrophosphate group exhibits what appears to be an inherent asymmetry in the P-O bonds of the P-O-P link; P_N-O greater than P_A-O by 0.04Å. The P_N-O-P_A angle has widened considerably to 133.3(1)°, allowing a variety of possible conformations across the two P-O bonds. Looking along the P...P virtual bond, the phosphate groups are staggered, a conformation quite different from that in the Li^+-complex. There is no intramolecular stacking, but this structure exhibits a novel intermolecular stacking giving rise to sandwiching of nicotinamide half way between adenine and water molecules 6.97Å apart. It is interesting to observe that the binding sites in different enzymes for such a chameleonic coenzyme is so similar.

x) Work supported by a grant from the National Institutes of Health, GM24864.

02.11–1 STRUCTURAL TRENDS IN N-ACYLTHIOESTERS OF RELEVANCE TO ACYLPAPAINS.

By C. P. Huber and K. I. Varughese, Div. of Biological Sciences, National Research Council of Canada, Ottawa, Canada K1A 0R6.

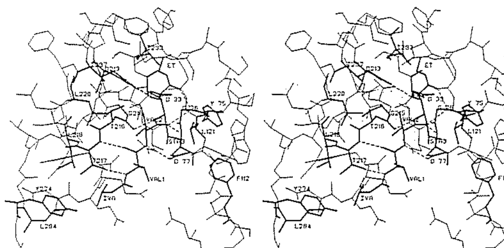
We are determining the crystal structures of a series of dithio- and thiol-esters which are closely related to the ester groups in the active site of acylpapains. The conformation of eight of these compounds is characterized by small N-C-C-S (ψ') torsional angles, with short N···S(thiol) distances in the range 2.83 - 2.93 Å, and by nearly orthogonal amide and thioester planes, leading to an N···S interaction. The C-N-C-C (ϕ') torsional angles are in the range -75.4 to -97.1° and the ψ' angles are in the range 9.5 to -23.0°. In a Ramachandran-type plot, the values of ϕ', ψ' for four N-acylglycine ethyl dithioesters lie essentially along a straight line which seems to represent the conformational pathway with maximal nitrogen-sulfur orbital interaction. Values of ϕ', ψ' for two N-acylalanine ethyl dithioesters and for two N-acylglycine ethyl thiolesters, while in the same range, show some deviations from the straight line. Resonance Raman spectroscopic studies of transient dithioacylpapains (Ozaki, Pliura, Carey and Storer, (1982), Biochemistry, 21, 3102) indicate that in the major population of the acyl-enzyme the dithioacyl group assumes a comparable conformation with N···S interaction. The fact that the conformations of the thiolesters in our series are very similar to those of the dithioesters suggests that information obtained for the dithioacylenzymes may be transferable to the natural thiol-intermediates.

02.11–2 A STEREOCHEMICAL ANALYSIS OF THE ASPARTYL PROTEINASE HYDROLYTIC MECHANISM.

Michael N.G. James and Anita R. Sielecki, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

The crystal and molecular structure of penicillopepsin, the aspartyl proteinase from *Penicillium janthinellum*, has been refined at 1.8 Å resolution to an R-factor of 0.136 for the 21,962 reflections with I≥1σ(I) (James & Sielecki, J. Mol. Biol. 163, 299 [1983]). The close proximity of the two catalytically important carboxyl groups of Asp33(32) and Asp213(215) suggests that they share a proton (d_{O-O} = 2.87 Å) in a tight hydrogen-bonded environment (residue numbers in parentheses refer to those of porcine pepsin). Hydration of this active site region suggests that a specific water molecule hydrogen-bonded to Asp33(32) plays the role of the attacking nucleophile (OH^-) in the hydrolytic mechanism. A plausible substrate binding mode to penicillopepsin has been deduced on the basis of the observed binding of a pepstatin analogue (James *et al.*, Proc. Natl. Acad. Sci. 79, 6137 [1982]). Crystallographic refinement of this molecule, Isovaleryl-valyl-valyl-statyl ethyl ester, in the complex [R = 0.131 for 21,197 reflections, I≥1σ(I)] shows the detailed binding interactions responsible for its inhibitory character (K_I = 2.4 x 10^{-8}M). This inhibitor is taken as a model for the tetrahedral intermediate in the catalytic pathway of a good substrate. The statyl residue has a secondary hydroxyl group on a tetrahedral carbon atom analogous to the C=O group of the scissile bond. The figure below shows the refined structure of the pepstatin analogue bound to penicillopepsin. The hydroxyl group is bound between the two aspartyl carboxyl groups and replaces a strongly bound solvent from the native enzyme.

In spite of the remarkable 2-fold symmetric arrangement of the active site region of the aspartyl proteases, the



interaction with substrates is decidedly asymmetric. Residues in penicillopepsin involved in hydrophobic binding of P_1 residues are tyrosine-75(75), phenylalanine-112(111) and leucine-121(120); those most important for binding P_1' residues of a substrate are: phenylalanine-190(189), isoleucine-211(213), phenylalanine-295(299), isoleucine-297(301) and isoleucine-293(297).

It is proposed that the electrophile is the shared proton between Asp213(215) and Asp33(32); the nucleophile is a water that is activated to an OH^- by the proximity of Asp33(32); the leaving group is protonated by the acidic solution in the cases of those proteases with pH optima in the range 1-4. Analysis of the stereochemistry of this proposed reaction pathway suggests a tetrahedral intermediate with opposite hand to that proposed for the serine proteinases.

Research supported by the Medical Research Council of Canada.