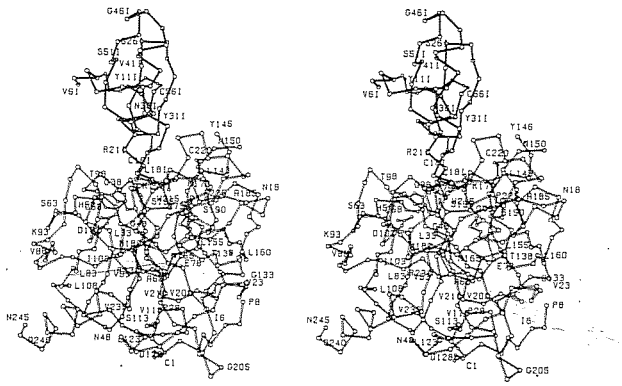

β-sheet. Along one edge of this central core runs the strand P_5-P_3' containing the reactive bond. Comparison of the structure of OMTKY3 bound to SGPB with that when bound to α-chymotrypsin shows that the strand P_5-P_3' rotates by 14.3° relative to the central core of the domain. This rotation can 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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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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02.10–3 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

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 = 11.784(3)A, α = 109.74(2), β = 90.76(2), γ = 103.43(1)°, V = 779.9A^3, Z = 1, space group P1. Using full three dimensional data to the limit of the Cu-sphere for CuKα (3460 reflections, 2624 ≥3σ), 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