REFERENCES

1. Yang, P.-H., and J. A. Rupley. 1979. Protein-water interactions. Heat capacity of the lysozyme water system. *Biochemistry* 12:2654–2661.
2. Careri, G., A. Giansanti, and E. Gratton. 1979. Lysozyme film hydration events. An IR and gravimetric study. *Biopolymers.* 18:11187–1203.
3. Banerjee, S. K., E. Holler, G. P. Hess, and J. A. Rupley. 1975. Reaction of *N*-Acetylglucosamine oligosaccharides with lysozyme. *J. Biol. Chem.* 250:4355–4367.

SOLVENT AND TEMPERATURE EFFECTS ON CRAMBIN, A HYDROPHOBIC PROTEIN

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Crambin, a 5,000-mol. wt. water-insoluble protein found in *crambe abyssinica* seeds (1) is presently being studied by x-ray diffraction to 0.9 Å resolution (2) and ^{1}H-nuclear magnetic resonance (NMR) spectroscopy. Preliminary ^{1}H-NMR data at 250 and 600 MHz have suggested that this hydrophobic protein retains a similar globular conformation in both glacial acetic acid (AA), a Brønsted acid, and dimethylformamide (DMF), a Lewis base (3). These observations suggest that the globular conformation observed in these organic solvents is most likely the native structure present in the crystalline state. At 22–29°C, however, the DMF spectrum is significantly broadened due to aggregation, which is broken at higher temperatures. In contrast, the AA spectra are of relatively high resolution. Despite its exaggerated structural stability, requiring ~20d for the amide ^{1}H resonances to disappear in deuterated glacial AA, the protein unfolds in dimethylsulfoxide (DMSO) and in trifluoroacetic acid. We have now found that once the protein has been pretreated with AA, it can be dissolved in DMF to yield narrow-line aromatic and methyl spectra essentially identical to that of the untreated protein. The disaggregation promoted by glacial AA appears to be kinetically irreversible and to preserve the native structural features.

DMSO titration of the pretreated protein in DMF solution shows little effect below 10% DMSO/90% DMF and appearance of 100% DMSO-type spectra at a 50% solvent mixture. As DMSO is added, the transition is gradual, involving broadening (i.e., conformational exchange) and shift (to the random coil position) of tyrosyl, phenyl alanyl, and methyl peaks.

Most interestingly, the overall trend of the solvent titration spectra parallels the sequence of spectral changes caused by temperature. Heat causes unfolding, which is still incomplete up to 105°C in DMF and 85°C in AA. One of the two tyrosyl residues, Tyr_{II}, yields a "doubled" aromatic spectrum, probably because of its occurrence in a rigid conformational situation; however, its lines neither broaden nor become equivalent at the higher temperatures, suggesting a rather high interconversion barrier. Despite this, the spectra in the two solvents are indicative of increased internal mobility for side-chain groups as the temperature is raised, as can be deduced from signals broadening or sharpening according to the internal dynamics of motion as viewed by the NMR frequency window. Thus, several methyl group resonances, contributing to the broad background of the lower temperature spectra, narrow and become discernible above ~55°C, growing "out of nothing". As suggested by the high intrinsic resolution of the crystallographic x-ray diffraction pattern, and demonstrated by the NMR data, crambin is a very rigid protein. Work is in progress to assign the ^{1}H-resonances

and to correlate ^{1}H and ^{13}C NMR dynamic data with the crystallographic model. It is hoped that unravelling conformational features of this hydrophobic protein will provide clues to help us understand other membrane-bound functional proteins.

REFERENCES

1. Van Etten, C. H., H. C. Nielsen, and J. E. Peters. 1965. A crystalline polypeptide from the seed of *Crambe abyssinica. Phytochemistry (Oxf.)* 4:467–473.
2. Teeter, M. M., and W. A. Hendrickson. 1979. Highly ordered crystals of the plant seed protein crambin. *J. Mol. Biol.* 127:219–223.
3. Llinás, M. A. De Marco, and J. T. J. Lecomte. 1980. Proton magentic resonance study of crambin, a hyperstable hydrophobic protein, at 250 MHz and 600 MHz. *Biochemistry.* 19:1140–1145.

HEAT CAPACITY CHANGES FOR THE BINDING OF 3'-CYTIDINE MONOPHOSPHATE TO RIBONUCLEASE A

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In many studies in recent years it has been demonstrated that the binding of a specific ligand to a protein results in a decrease in the heat capacity of the system (1). A number of factors may contribute to the observed negative heat capacity changes (ΔCp), but the most interesting, in terms of our understanding of the functional properties of proteins, is the possibility that the ΔCp values may be due to some sort of conformational change in the protein induced by the binding of the ligand. However, before one can make this interpretation, the other potential contributions to the ΔCp values must be evaluated, if possible. Here we report our determination of the ΔCp for the binding of 3'-cytidine monophosphate (3'-CMP) to ribonuclease A (RNAse A) at pH 5.0. The pH dependence of the thermodynamics of the binding of 3'-CMP to RNAse A has been thoroughly studied (2). These studies along with other available information allow us to estimate various contributions to the ΔCp for ligand binding.

The temperature dependence of the apparent enthalpy change, ΔH°, for the binding of 3'-CMP to RNAse A at pH 5.0 (acetate buffer) and ionic strengths 0.05, 0.2, and 1.0 M were obtained using a batch microcalorimeter (LKB Instruments Co., Rockville, Md.) as described elsewhere (2). From the dependence of ΔH° on temperature (measurements at six temperatures from 18°–44°C) ΔCp values of -200, -175, and -150 cal K^{-1} mol^{-1}, respectively, were obtained. The plots were linear in each case.

The following is a consideration of various factors that are expected to contribute to the ΔCp for 3'-CMP binding (focusing on the ΔCp = -175 cal K^{-1} mol^{-1} for ionic strength 0.2 M). (*a*) A contribution of -56 cal K^{-1} mol^{-1} due to linked protonic equilibria can be calculated based on previous studies of the pH dependence of the binding of 3'-CMP. The dissociation of a proton from the phosphate group of 3'-CMP and the association of protons to the two