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.

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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, $\Delta H°$, 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 $\Delta H°$ 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

histidine residues at the protein's binding site are coupled to the binding process. The above ΔCp contribution also assumes that the pyrimidine ring of the ligand is nonprotonated when bound. A pH of 5.0 was chosen to minimize the contribution from these coupled protonic equilibria. (*b*) We estimate that the contribution due to the transfer of 3'-CMP from an aqueous environment to the protein surface will be small. Alvarez and Biltonen (3) have demonstrated that the transfer of a pyrimidine ring (thymine) from water to ethanol has a ΔCp equal to zero. We therefore expect the contribution due to the "hydrophobic" transfer of the ligand from water to the protein to be negligible. The contribution due to the desolvation of the anionic ligand phosphate group and the protonated histidine residues can be estimated to be $+37$ cal K^{-1} mol^{-1} from knowledge of the heat capacity change for the deprotonation of these groups (phosphoric acid and imidazole) and using a thermodynamic scheme in which (*1*) the groups are neutralized (in water), (*2*) the neutral reactants associate, and (*3*) there is a transfer of two protons from the phosphate group to the two histidines. (*c*) 3'-CMP exists in both a *syn* and *anti* conformation (about the glycosidic bond) in solution (4). The ligand binds to the protein in the *anti* configuration. From knowledge of the thermodynamics of the *syn*→*anti* transformation, we calculate a ΔCp contribution of -5 cal K^{-1} due to this induced conformational change in the ligand. The cytosine ring will exist to a very small extent in the imino tautomeric state. Assuming that only the amino state binds, the shift in this tautomeric equilibrium will contribute only about -1 cal K^{-1} to the observed ΔCp. (*d*) Although aggregated forms of RNAse A do exist, no concentration dependence of the ΔH of 3'-CMP binding has been observed. Thus the observed ΔCp does not include a contribution from this effect. Dimerization of 3'-CMP also is not significant at the concentration employed. (*e*) Thermal unfolding of the protein ($T_m \simeq 60°C$) will contribute less than 1 cal K^{-1} mol^{-1} to the observed ΔCp at 25°C.

From the above contributing sources we can account for a ΔCp value of about -26 cal K^{-1} mol^{-1}. This leaves a ΔCp of -150 cal K^{-1} mol^{-1} which we may attribute to a ligand induced change in the conformation of the protein. Evidence for an extensive, two state refolding transition upon ligand binding is lacking. Also the linear dependence of ΔH on temperature precludes a two state transition within this range (at least one with a significant ΔH). Most studies on the effect of nucleotide binding to RNAse A are more in line with a subtle conformational readjustment or tightening of the protein upon binding. Of particular note are the reports of Martin (5) and Petsko[1] that the temperature factor of the crystal structure of RNAse A decreases throughout upon the binding of 3'-CMP.

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