Elliott, G. F. 1973. Donnan and osmotic effects in muscle fibers without membranes. *J. Mechanochem. Cell Motil.* 2:83–89.

Elliott, G. F., G. R. S. Naylor, and A. E. Woolgar. 1978. Measurements of the electric charge on the contractile proteins in glycerinated rabbit psoas using microelectrode and diffraction effects. *In* Ions in Macromolecular and Biological Systems. (Colston Papers No. 29.) D. H. Everett and B. Vincent, editors. John Wright & Sons Ltd., Bristol, 329–339.

Goodfellow, J. M. 1975. Studies of the cornea. Ph.D thesis. The Open University, Milton Keynes.

Loeb, G. I., and H. A. Saroff. 1964. Chloride- and hydrogen-ion binding to ribonuclease. *Biochemistry.* 3:1819–1826.

Sarkar, N. K. 1950. The effects of ions and ATP on myosin and actomyosin. *Enzymologia.* 14:237–245.

DETECTION OF INTRAMOLECULAR INTERACTIONS OF LYSYL AND N-TERMINAL AMINO GROUPS OF REDUCTIVELY METHYLATED PROTEINS BY ^{13}C NUCLEAR MAGNETIC RESONANCE

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Detailed information on the intramolecular interactions of protein amino groups has been largely unobtainable except to a limited extent from crystallographic studies. Reductive methylation with $NaCNBH_3$ and (^{13}C)-formaldehyde introduces a specific probe for ^{13}C nuclear magnetic resonance (NMR) investigation of protein amino groups (1, 2). We have shown that the chemical shifts of ^{13}C-enriched dimethylamino groups are sensitive to the

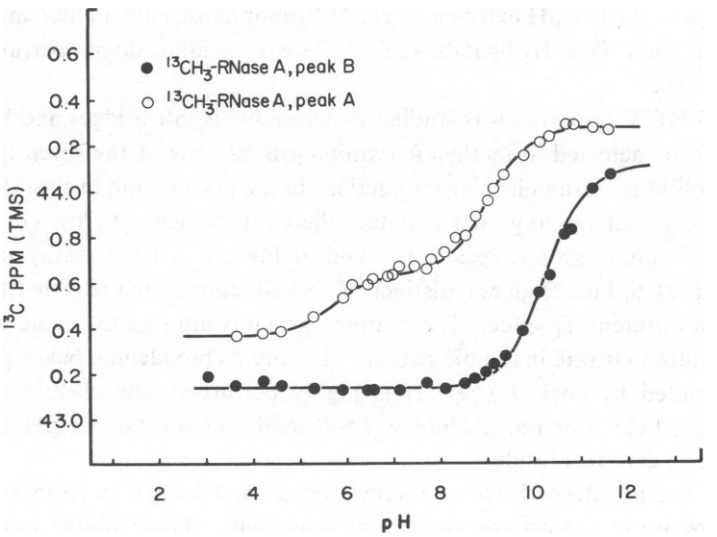

Figure 1 The ^{13}C NMR titration behavior of the [^{13}C]-ε-N,N-dimethyl lysyl residues of reductively methylated ribonuclease-A. Peak *A* is assigned to dimethyl lysine 41 and peak *B* to the remaining dimethyl bulk lysine residues. (^{13}C NMR spectra were obtained at 45.3 MHz from 7-ml samples of ~25 mg/ml methylated RNase A.)

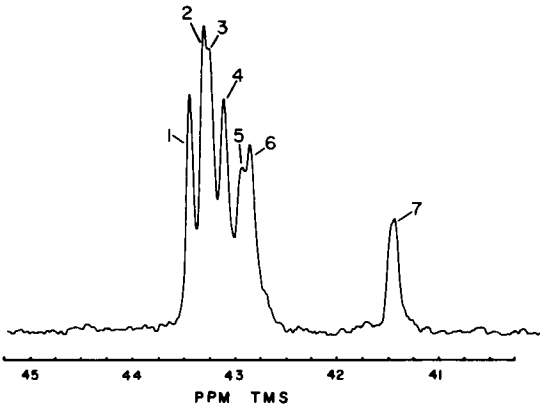

Figure 2 The 45.3-MHz ^{13}C NMR spectra of [^{13}C]-methylated lysozyme at pH 8.5. The spectrum was obtained from a 7-ml sample with a protein concentration of ~25mg/ml. Resonance assignments are discussed in the text.

environment and degree of protonation of the amino group, and we have demonstrated that the pK of the dimethylamino groups are very similar to those of unmodified amino groups.

The active site residue lysine 41 has been studied in methylated ribonuclease A. In the absence of active site ligands, the titration behavior of lysine 41 is biphasic with pK values of 5.7 and 9.0 (Fig. 1). The pK at 9.0 is due to the protonation-deprotonation of dimethyl lysine 41 (as compared with a pK of 10.2 for bulk or free dimethyl lysines), while the pK at 5.7 is likely due to a neighboring residue that upon titration affects the environment of lysine 41. The presence of active site ligands that contain a phosphate and/or a 2′ hydroxyl on the ribose ring of a nucleotide or nucleoside, increase the pK of the lysine ionization by ~0.3pH units. A more noticeable perturbation is evident for the low pH transition, where three general types of titration behavior are found. Type I ligands, such as 2′-CMP, cause both an increase in the low pH inflection point and a merger of the dimethyl lysine 41 resonance with that of the bulk dimethyl groups at the low pH extreme. Type II ligands cause only an increase in the low pH inflection point, while Type III ligands, such as 2′-deoxycytidine, do not perturb the dimethyl lysine 41 resonance.

Methylated HEW lysozyme was studied to determine if salt bridges and hydrogen bonds could form and be detected in methylated amino groups. Six of the seven lysozyme amino groups are involved in intramolecular interactions in the crystal, and our results suggest that, in solution, methylated amino groups maintain these interactions. In low salt each of the six methylated lysyl amino groups (peaks 1–5 and 7, Fig. 2) and the methylated N-terminal amino group (peak 6, Fig. 2) gives a distinct ^{13}C NMR signal; each residue titrates uniquely; and each has a different T_1 value. The α amino group is unusual by virtue of its resonance position, its failure to titrate in the pH region 5–11, and its broadening below pH 5. The lysyl residue represented by peak 7 (Fig. 2) is highly perturbed; the chemical shift is at an abnormally high field over the pH range 3.5–9, and an inflection at pH 2.5 suggests an interaction with a carboxyl residue.

Studies of the pH dependence of methylated α- and δ-chymotrypsin suggest that the α-amino groups are in at least two types of environments. These studies have established a variety of interactions and properties of protein lysyl and N-terminal amino groups, information that has previously been unobtainable experimentally.

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REFERENCES

1. Jentoft, N., and D. G. Dearborn. 1979. Labeling of proteins by reductive methylation using sodium cyanoborohydride. *J. Biol. Chem.* **254**:4359–4365.
2. Jentoft, J. E., N. Jentoft, T. A. Gerken, and D. G. Dearborn. 1979. ^{13}C NMR studies of ribonuclease A methylated with (^{13}C)formaldehyde. *J. Biol. Chem.* **254**:4366–4370.