CMR STUDIES OF PROTEIN MODIFICATION. PROGRESSIVE DECREASE IN CHARGE DENSITY AT THE ε-AMINO FUNCTION OF LYSINE WITH INCREASING METHYL SUBSTITUTION

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Summary: Progressive methyl substitution at the ε-amino group of lysine results in a progressive downfield shift of the signals from the ε and N-methyl carbon atoms in the Carbon-13 nmr spectra of the resulting ε-N-methylated lysine derivatives. The results are consistent with the view that the greater the number of ε,N-methyl groups on protein basic aminoacid residues, the greater the affinity of these residues for anionic species, e.g. as in histone-DNA interactions.

The occurrence of sidechain N-methylated aminoacids in the free form and in proteins is very widespread throughout nature (1), although the biological significance of methylation is, as yet, unknown. Although there is no doubt that methylation increases the hydrophobicity of thus modified amino groups (2), some argument exists, however, concerning their relative polarities (1). Within a series of structurally closely related compounds, changes in electron density have been shown to be reflected in chemical shift changes in the carbon-13 nuclear magnetic resonance (CMR) spectra (3,4). Hence it was felt that the CMR spectra of lysine and its ε,N-methyl derivatives would help elucidate the effects of progressive N-methylation on the polarity of lysine residues, and hence their relative affinities for species with negative charge, such as the phosphate anions of DNA.

Materials and Methods. Lysine, ε-N-methyllysine and ε-N, ε-N-dimethyllysine were obtained as hydrochloride salts from Cyclo Chemical, Los Angeles, California. ε-N, ε-N, ε-N-trimethyllysine hydrochloride was synthesized as previously described (5). Fourier-Transform carbon-13 (22.63 MHz) NMR spectra were obtained on 0.25

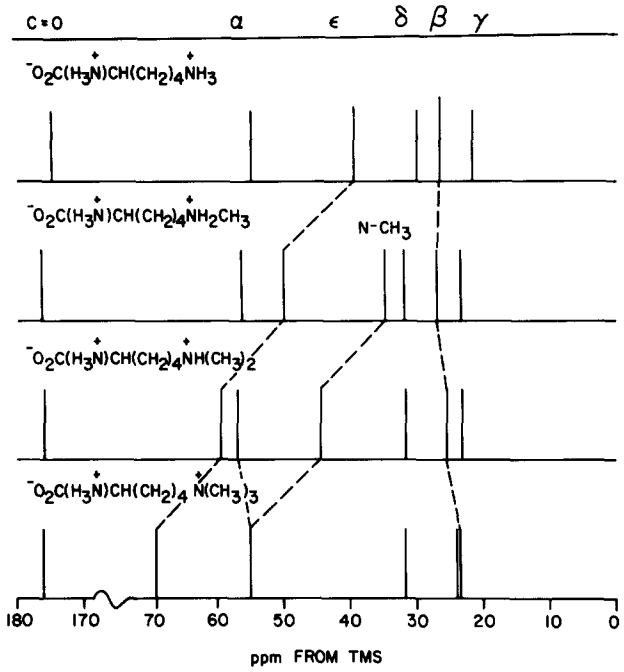

Figure 1. Graphical representation of 22.63 MHz CMR spectra of lysine and ε-N-methylated lysine derivatives in aqueous solution at pH 5. Experimental conditions are described in Materials and Methods.

M aqueous aminoacid solutions at pH 5 using a Brucker HFX-90 spectrometer interfaced to a Nicolet pulse generator and computer. Hexafluorobenzene was used as external standard, and chemical shifts were re-referenced to tetra-methylsilane (TMS) using (Shift from TMS) = (Shift from hexafluorobenzene) +129.0 ppm. The sum of 4,000 transients was recorded for proton-decoupled, and 12,000 for off-resonance decoupled spectra. Resonances were assigned using data published previously for lysine (4,6,7) and structurally related compounds (8,9) and by off-resonance decoupling experiments. Chemical shift values for lysine were in good agreement with those reported previously. The convention was followed whereby shifts to lower field are designated by a positive sign.

Results and Discussion. The 22.63 MHz proton-decoupled CMR spectra of lysine and its ε,N-methylated derivatives at pH 5 are shown in Fig. 1. In the

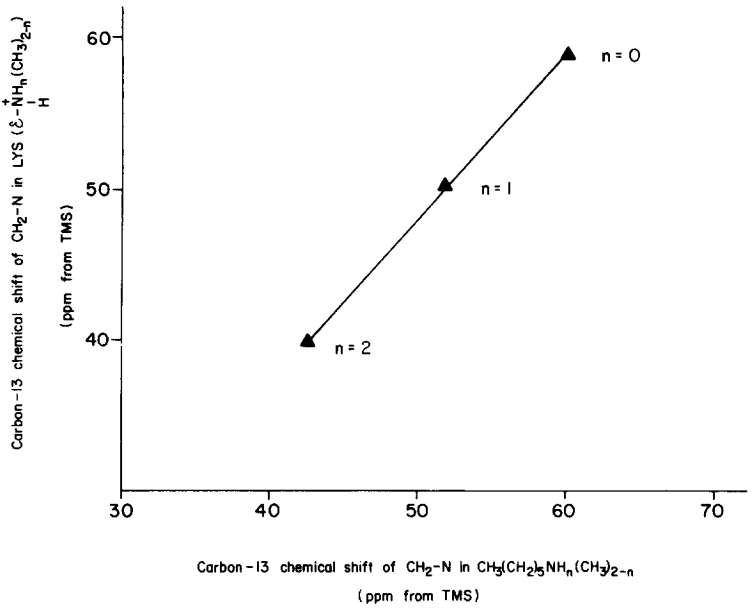

Figure 2. Relationship between CMR chemical shifts of $\underline{CH}_2$-N carbon atoms in ε-N-methylated lysine derivatives at pH 5 and N-methyl-n-hexylamines of analogous structure. Data for the hexylamine series is quoted from reference 9.

first instance, it is apparent that methyl substitution results in marked changes in the chemical shift of only the $\underline{CH}_2$-N and $\underline{CH}_3$-N signals. The relative insensitivity of the signals from the other carbon atoms suggests that direct substituent effects on the ε-amino function of protein lysine residues may be closely approximated by corresponding effects observed for the free aminoacids. Secondly, regular and almost additive low-field shifts are observed for the former signals with increasing methyl substitution at the side-chain nitrogen atom. The relative differences in chemical shift appear for the most part not to be due to differential solvation effects, since there is a strong correllation between them and the shifts of the corresponding $\underline{CH}_2$-N and $\underline{CH}_3$-N carbon atoms in the unprotonated amines derived from n-hexylamine (Fig. 2). The CMR data for the latter were recorded in solution in the nonpolar solvent, benzene (9). Furthermore the small, constant difference between the chemical shifts of corresponding

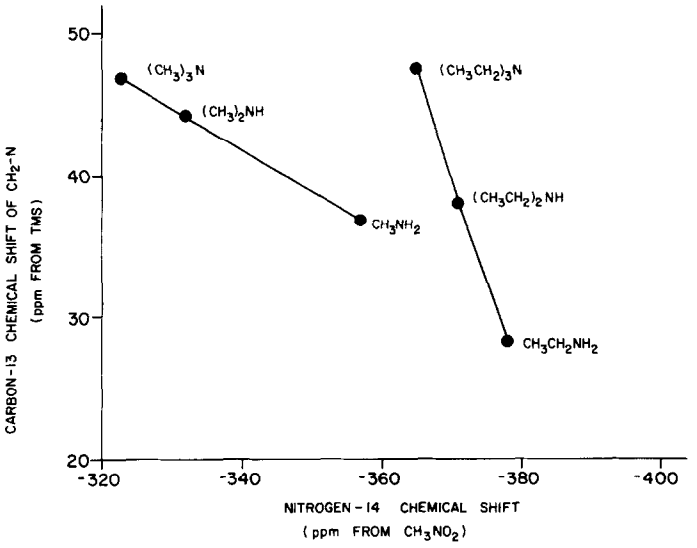

Figure 3. Relationship between Nitrogen-14 (reference 12) and $\underline{CH}_2$-N CMR chemical shifts (reference 9) in simple aliphatic amines.

carbon atoms in the lysine and unprotonated n-hexylamine series suggests that the electron densities at the nitrogen and next-neighbour carbon atoms of aliphatic primary, secondary and tertiary amines are relatively unchanged following protonation This postulate has previously been verified for methylamine using electron densities calculated by the CNDO/2 procedure (4).

On the basis of the observed "anomalous" order of basicities of simple aliphatic amines in aqueous solution ($-N(CH_3)_2$ <$-NH_2$< $-HNCH_3$), it has been proposed (1) that whereas monomethylation of lysine side-chain amino-groups increases their polarity (basic charge), and hence their affinity for anions such as DNA phosphate, dimethylation should have exactly the reverse effect. Due to their high pKa values simple aliphatic amines are almost completely protonated at physiological and lower pHs, hence a more accurate measure of their electrostatic attraction for anions should be the relative charge densities on their nitrogen and next-neighbour carbon atoms. Within a series of compounds of closely related structure, changes in charge density at a particular carbon atom have been observed to be reflected in changes in the chemical shift of the signal arising from

that atom in the CMR spectrum (3,4). Hence the regular increase in chemical shifts described above suggests that progressive ε,N-methyl substitution of lysine residues leads also to a progressive decrease in the charge density on the $\underline{CH}_2$-N and $\underline{CH}_3$-N carbon atoms. Information on the relative charge densities at the side-chain nitrogen atom would be obtained with difficulty from Nitrogen nmr spectra, since the corresponding chemical shifts, though governed by factors similar to those for the carbon nucleus, are sensitive to solvation effects (10). However, we have noted a strong correlation between the Nitrogen-14 chemical shifts in simple amines and the Carbon-13 chemical shifts of their neighbouring $\underline{CH}_2$-N carbon atoms (Fig. 3). This correllation provides evidence that the charge densities at both the side-chain nitrogen atoms and the next-neighbour carbon atoms decrease regularly with increasing methyl substitution. Thus our observations would infer that the affinity of basic amino-acid residues in proteins for biologically important anions increases regularly with the number of N-methyl groups, and not in the order previously suggested. This theory is supported by the recent evidence that histones containing greater proportions of ε-N-methylated lysine residues have a correspondingly greater affinity for DNA in the chromatin complex (11).

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REFERENCES

1. Paik, W.K. and Kim, S., Science, 174:114 (1971).
2. Tanford, C., J. Amer. Chem. Soc., 84:4240 (1962).
3. Levy, G.C. and Nelson, G.L., Carbon-13 Nuclear Magnetic Resonance for Organic Chemists. Wiley-Interscience. New York (1972).
4. Horsley, W., Sternlicht, H. and Cohen, J.S., J. Amer. Chem. Soc., 92:680 (1970).
5. Seely, J.H. and Benoiton, J.H., Canad. J. Chem., 48:1122 (1970).
6. Voelter, W., Jung, G., Breitmaier, E. and Bayer, E., Z. Naturforsch., 266: 213 (1971).
7. Christl, M., and Roberts, J.D., J. Amer. Chem. Soc., 94:4565 (1972).
8. Williams, E., Sears, B., Allerhand, A. and Cordes, E.H., J. Amer. Chem. Soc., 95:4871 (1973).
9. Eggert, H. and Djerassi, C., J. Amer. Chem. Soc., 95:3710 (1973).
10. Witanowski, M. and Januszewski, H., Canad. J. Chem., 47:1321 (1969).
11. Byvoet, P. Submitted for publication.