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^{13}C Magnetic Relaxation Study of Hindered Motion of Arginine in Clupeine

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ABSTRACT: ^{13}C NMR parameters such as chemical shift, spin–lattice relaxation time (T_1), and nuclear Overhauser enhancement (*NOE*) were measured for the arginine residue of clupeine in aqueous solution. The correlation time for molecular reorientation of the backbone and side-chain carbons were estimated from T_1 and *NOE*. The correlation time for the α-carbon in the backbone was 0.57 ns at 300 K, indicating rapid segmental motion and that the clupeine molecule is thus flexible. The side chain carbons are more mobile due to the presence of internal motion than the backbone carbon. The internal motion of the side chain of arginine was found to be more restricted compared with that of lysine. This is interpreted in terms of the presence of the bulky guanidino group.

KEY WORDS Clupeine / ^{13}C NMR / Spin–Lattice Relaxation Time / Correlation Time / Segmental Motion / Internal Motion /

Clupeine, one of the typical protamines, is a strongly basic protein associated with DNA in the spermatozoa of herring. Clupeine is composed of three molecular species (YI, YII, and Z) the primary structures of which have been determined and are similar to one another.[1] These species contain 30—31 amino acids, two-thirds of which are arginine, and the short sequences of arginines are separated by neutral amino acid residues. Optical rotatory dispersion and circular dichroism studies revealed that the conformation of clupeine is a completely random coil in an aqueous solution.[2,3]

There are a few nuclear magnetic resonance (NMR) studies on protamines.[4-6] Recently, a ^{13}C NMR study on the fractionated species of clupeine has been reported by Bonora and coworkers,[6] they assigned the observed resonances to individual carbons. They also measured the line width and nuclear Overhauser enhancement, and estimated the correlation time of the tumbling motion to be around 10^{-9} s. However, no detailed studies on mobility have been made. ^{13}C NMR spectroscopy is suitable for determining the mobility of individual carbons in molecules as well as the molecular structures.

In this paper we wish to report the results of ^{13}C nuclear magnetic relaxation measurements carried out to determine the dynamic behavior of clupeine in an aqueous solution. The present work deals only with the motions of the backbone and side-chain carbons of arginine residues, examined by measuring the spin–lattice relaxation time (T_1) and the nuclear Overhauser enhancement (*NOE*).

EXPERIMENTAL

The clupeine chloride used here was purchased from Sigma Chemicals. It was not fractionated and therefore contained a mixture of the YI, YII, and Z species. Clupeine was dissolved in a 0.2 M NaCl–D_2O solution (100 mg ml^{-1}). The pH value was 4.0 at room temperature (direct meter reading). ^{13}C NMR spectra were obtained by a JEOL FX-60 Q spectrometer operating at 15.04 MHz with 16 K data memory. Chemical shift values were referred to a resonance of internal dioxane and were then converted to the δ scale using $\delta_{TMS} - \delta_{dioxane} = 67.4$ ppm. T_1 was obtained by the inversion-recovery method and *NOE* by the gated decouple method. All measurements were done at a temperature of 300 K.

RESULTS AND DISCUSSION

Figure 1 shows the ^{13}C NMR spectrum of clupeine. Large peaks could be reasonably assigned to

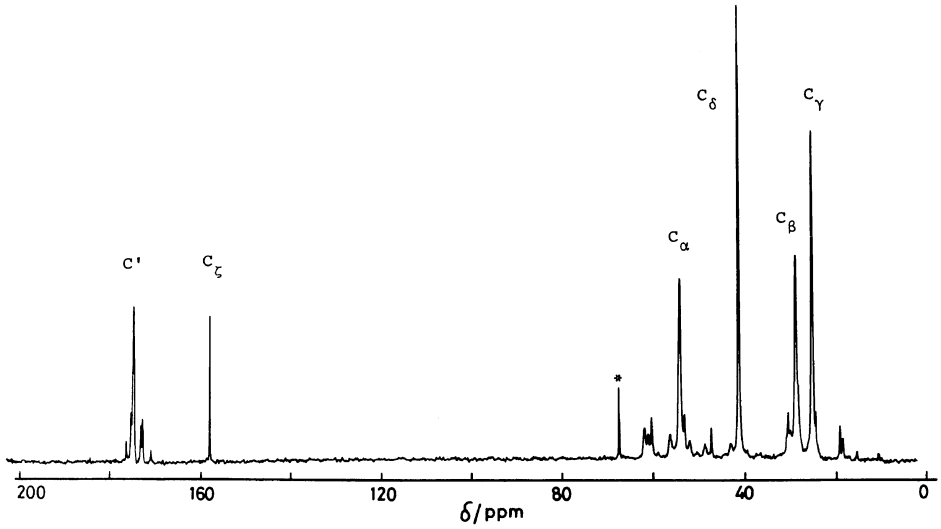

Figure 1. The ^{13}C NMR spectrum of clupeine in D_2O (0.2 M NaCl) at pH 4.0 and at 300 K. The spectrum was recorded using 8 K data points plus 8 K zero filling, 3002 Hz spectral width, pulse width 10 μs (90° flip angle), 1.5 s recycle time, 52,000 accumulations, and 0.5 Hz digital broadening. Chemical shift scale in ppm (from TMS). The asterisk indicates a resonance of dioxane as an internal reference.

resonances of the arginine residues amounting to two-thirds of all the amino acids in clupeine.[6,7] Other small peaks were assigned to other less abundant amino acids. However, since we are interested only in the arginine residues, no assignment was made to the small peaks. The results of Bonora *et al.* suggest that chemical the shift values of arginine show no differences among different arginine residues except for terminal residues nor among the species of YI, YII, and Z. Therefore, the results obtained in this work for the unfractionated clupeine are reasonable, as far as arginine residues are concerned.

Table I shows the chemical shift values of arginine in clupeine. Saito and coworkers have measured ^{13}C NMR for poly(L-arginine) in aqueous solution and reported that the chemical shifts of the backbone C_α and C′ carbons differ by 2—3 ppm in the random coil and the helix states.[8] The values of chemical shifts of C_α, C′, and other carbons of arginine of clupeine obtained in this work agree with those of poly(L-arginine) in the coil state, within 0.6 ppm. This indicates that the conformation of clupeine is a random coil in aqueous solution, which is consistent with the results of previous optical studies.[2,3]

The T_1 and *NOE* values for protonated carbons of arginine are also listed in Table I. Since the measured *NOE* values are close to the value of 2.98, T_1 for protonated carbons is determined by the ^{13}C–^1H dipolar interaction and the extreme narrowing conditions is satisfied. Under these conditions, an effective rotational correlation time, τ_{eff}, characterizing the rotational reorientation of the C–H bond ($r_{C-H}=1.09$ Å), is given by,[10]

$$\tau_{eff}=K/NT_1 \qquad (1)$$

where K is a constant equal to 4.72×10^{-11} s^2 and N is the number of hydrogens directly bonded to the observing carbons. The effective correlation time calculated from eq 1 for each carbon is shown in Table I.

A recent study by electron microscopy on clupeine YI shows a unique three-dimensional structure consisting of a loose helix of turns of various sizes.[10] If clupeine is assumed to be a rigid spherical molecule, electron micrographs shown in ref 10 indicate that the radius of the molecule will be 15—20 Å. Thus, the Stokes–Einstein relation for a rigid sphere gives the correlation time of the overall molecular reorientation, τ. A value of 4—9 ns is obtained for τ at room temperature.

The experimental τ_{eff} value of C_α carbon is 0.57 ns, and significantly smaller than the calculated τ values. This discrepancy indicates that the molecule is not rigid, but rather flexible. It has been proved for a

Table I. Chemical shift, NT_1, *NOE*, and effective correlation time (τ_{eff}) of Arg of clupeine[a]

	C_α	C_β	C_γ	C_δ	C_ζ	C'
Chemical shift[b]	54.21	29.09	25.42	41.48	157.17	173.80
NT_1[c]	83	127	208	267		
NOE	2.6	2.8	2.9	2.9		
τ_{eff}[d]	0.57	0.37	0.23	0.18		

[a] Estimated errors of chemical shift, T_1, and *NOE* measurements are ± 0.05 ppm, $\pm 10\%$, and $\pm 20\%$, respectively.
[b] In ppm from TMS.
[c] In ms.
[d] In ns.

number of polypeptides in the coil state that the extent of local segmental motions in the backbone chain are considerable.[11-17]

As one goes away from C_α to C_δ, there is a progressive decrease in τ_{eff} as seen in Table I. The decrease in the τ_{eff} of the side-chain carbons clearly shows the presence of a significant degree in internal motion. It has been found that the NT_1 value of the side-chain C_δ carbon of poly(L-lysine) in the coil state is seven times as large as that of the C_α carbon.[8,12,17] For arginine in clupeine, the ratio of the NT_1 values of C_δ and C_α carbons is only three. Such a difference between arginine and lysine suggests that the internal motion of the side chain of arginine is more restricted compared with that of lysine.

Using the isotropic rotational diffusion model of internal motion,[18] we calculate the correlation time of internal rotation about the C–C bond of the side chain, τ_{int}, from the following equation,

$$\frac{1}{NT_1}=\frac{\tau_c}{K}\left\{A+\frac{B\tau_{int}}{\tau_{int}+\tau_c}+\frac{C\tau_{int}}{\tau_{int}+4\tau_c}\right\}$$

with

$$A=(3\cos^2\theta-1)^2/4,$$
$$B=3\sin^2\theta\cos^2\theta,$$
$$C=3\sin^4\theta/4 \qquad (2)$$

where K is the same as that in eq 1, τ_c the correlation time for reorientation of the axis of internal motion, and θ the angle between the C–H bond and the axis of rotation (here, we adopt a tetrahedral angle). If the correlation time of the reorientation of the C_α–C_β bond is assumed to be equal to τ_{eff} for the C_α–H bond, τ_{int} for the C_α–C_β bond can be calculated from eq 2. Similarly, the correlation time for the reorientation of the next C–C bond is assumed to be the effective correlation time for the preceding C–H bond. In this way, we calculated τ_{int} for each bond, and the results are shown in Table II. In Table II, τ_{int} values are also listed for three homopolypeptides in the coil state;[19] poly(L-arginine),[8] poly(L-lysine),[17] and poly(D-glutamic acid).[16]

The values of τ_{int} for arginine in clupeine does not decrease monotonously, going from the backbone to the end of the side chain, and τ_{int} for the C_γ–C_δ bond is slightly greater than that for the C_β–C_γ bond. These results present a singular contrast to those of poly(L-lysine) and poly(D-glutamic acid) as seen in Table II, for which the gradual decrease in τ_{int} is obtained by going from the backbone to the end of the side chain.

Furthermore, it is of interest to notice that the τ_{int} value for the C_γ–C_δ bond in arginine of clupeine is about five times larger than that of the corresponding bond in lysine of poly(L-lysine). Similar behavior of arginine has been observed in poly(L-arginine) (see Table II). The large τ_{int} values observed in arginine are likely to be a consequence of the

Table II. Correlation time of internal motion (τ_{int})[a]

	C_α–C_β	C_β–C_γ	C_γ–C_δ	C_δ – C_ε
Arginine of clupeine[b]	2.4	1.2	1.8	—
Poly(L-arginine)[c]	2.6	2.1	2.2	—
Poly(L-lysine)[b]	1.5	1.0	0.4	0.2
Poly(D-glutamic acid)[b]	1.5	0.6	—	—

[a] In ns.
[b] At 300 K.
[c] At 301 K.

presence of the bulky guanidino group attached to the δ carbon.

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