Polymer Bulletin 2, 177–182 (1980)

Polymer Bulletin

^{15}N NMR Spectroscopy 26 Coil-Helix Transition of Poly-L-Ornithine

Hans R. Kricheldorf

Institut für Makromolekulare Chemie der Albert-Ludwigs-Universität, Hermann-Staudinger-Haus, Stefan-Meier-Str. 31, 7800 Freiburg i.Br., Federal Republic of Germany

SUMMARY

The coil-helix transition of poly-L-ornithine was studied in water and in a water/methanol mixture (7:3 by volume) as a function of pH. The ^{15}N NMR chemical shift of N-methylacetamide was measured under analogous conditions and compared with the chemical shifts of poly-L-ornithine to distinguish the influence of the coil-helix transition on the chemical shift from other solvent effects.

INTRODUCTION

In the course of the last fifteen years ^{1}H-NMR spectroscopy and, more recently, ^{13}C-NMR spectroscopy have been widely used to study helix-coil transitions of various polypeptides (Bovey 1974; Suzuki et al. 1977; Lader et al. 1977; Saito et al. 1979, Kricheldorf 1979 a). However, even with the application of both ^{1}H- and ^{13}C-NMR spectroscopy some problems remain unresolved. Hence, it seems to be reasonable to study the usefulness of ^{15}N-NMR spectroscopy for investigations of conformational changes of oligo-and polypeptides. A first investigation in this direction has been reported by us previously; yet, we were not able to study the complete coil-helix transition of polylysine because at pH's $\geq$ 10.5 the deprotonated polypeptide precipitated from the solution. Furthermore, we had not clarified whether the observed upfield shift of the ^{15}N-NMR peptide signal was really the consequence of a conformational change or the result of direct solvent effects. Thus, it was the purpose of this work to study the coil-helix transition of an entirely soluble polypeptide by means of natural abundance ^{15}N-NMR spectra and to elucidate the role of solvent effects.

0170-0839/80/0002/0177/$01.20

RESULTS AND DISCUSSION

Two samples of poly-L-ornithine hydrobromide with a DP in the range of 45-50 and 95-100 were synthesized according to the method reported by Katchalski et al. (1948, 1949). The measurements were carried out with 0.8 M solutions of poly-L-ornithine and at pH values $\sim$ 9.0 Titriplex V (diethylenetriaminepentaacetic acid) was added to avoid that paramagnetic cations supress the negative nuclear Overhauser effect (NOE) of the free amino groups in the side chains. That this measure was successful, even if tap-water was used, is shown in Figure 1. Because poly-L-ornithine does not form a β-structure in alkaline water, we could reach pH 12.5 without precipitation of the polypeptide.

In the pH range of 9-12.5 upfield shifts were observed for both the amide nitrogen and amino group. In the latter case the upfield shift reaches 8.7 ppm in water or 8.8 ppm in water/methanol and the inflection point of the titration curve indicates a pK_a value of 10.2. This value is, as excepted, lower than that of polylysine (10.6) and also lower than that of the δ-amino group of L-ornithine itself(10.7) (Dunn 1947). This deviation of the pK_a value of poly-L-ornithine from that of comparable compounds is not surprising, since it is well known that the basicity of amino groups in the side chains of oligopeptides and proteins depends largely on their environment. The upfied shift of the peptide nitrogen reaches ca. 1.1 ppm and is thus only a poor indicator of the conformational change. It is known for polylysine that the coil-helix transition takes place between pH 10.5 and 11.0, because a complete deprotonation of the side chains is not required. An analogous behaviour is expected for poly-L-ornithine; yet, the data in Table 1 demonstrate that the amide signal shifts slightly upfield even between pH 11.1 and 12.1. Apparently, the relatively low degree of polymerization in combination with a broad molecular weight distribution is responsible for this effect, since the helices of shorter chains are less stable than those of longer ones and thus require more complete deprotonation.

The coil-helix transition of poly-L-ornithine causes not only an upfield shift of the peptide signal but also line broadening (s. Figs. 1 A and B). This effect, likewise observed for polylysine (Hull 1979) was more pronounced in the case of $(Orn)_{95}$ than in the case of $(Orn)_{45}$. It is obvious that the reduced segmental mobility of the helical peptide chain is responsible for this line broadening. In this connection it should be mentioned that we have repeated the measurements with

$(Orn)_{95}$ in a water/methanol mixture (7:3 by volume), because methanol is a poorer solvent than water for poly-L-ornithine and thus more helix-supporting. As the data in Table 1 demonstrate, the chemical shifts in water/methanol parallel those found in water. However, the intensity of the peptide signal shows a different behaviour in that it disappears because of line broadening at pH 10.6 (and higher pH values s.Fig.1C), i.e. when the coil-helix transition is complete. Two hypotheses can account for this finding and probably both are correct; 1) the extent of helix formation is higher in water/methanol than in water; 2) the helices begin to associate after addition of methanol because poly-L-ornithine is insoluble in pure methanol. Anyway, since we have accumulated up to 50 000 transients, it is clear that a more detailed ^{15}N NMR study on the solvation and segmental mobility of coiled and helical polypeptides requires ^{15}N enriched material.

In the case of polylysine we have observed an upfield shift of the peptide signal of ca. 1.2 ppm in the pH rangeof 9.0-10.5. Because of the precipitating polypeptide we could not decide whether this shift difference represents the complete coil-helix transition or not. However, because of the 1.1 ppm upfield shift found for polyornithine we may now conclude that the 1.2 ppm shift observed for polylysine is the maximum effect. It is known that the ^{15}N-NMR chemical shift of most compounds and in particular that of amides is highly sensitive to solvent effects (Witanowski et al. 1964; Cattageno et al. 1976; Kricheldorf 1978). Hence, it must be clarified whether the observed peptide shifts are really the result of a conformational change or only a solvent effect. For this reason a mixture of N-methylacetamide and n-propylamine hydrobromide was titrated with sodium hydroxide under conditions comparable with those used for poly-L-ornithinehydrobromide. The data in Table 2 show for this titration that the chemical shift of N-methylacetamide is insensitive to a change of the pH, so that the shift differences found for poly-L-ornithine and polylysine may be considered to reflect the coil-helix transition. However, measurements of pure N-methylacetamide in neutral water, in 1 N HBr and in the presence of n-proylamine · HBr (pH 6) demonstrate that the shift of amides may be sensitive to the addition of acidic compounds, even if no protonation takes place. Obviously,the formation of stronger H-bonds attacking the carbonyl oxygen of the amide group is responsible for the downfield shifts in acidic solution.

Tab. 1 ^{15}N-NMR chemical shifts δ (ppm, upfield of external $NO_3^{\ominus}$) of poly-L-ornithine in water [a] or water + methanol [b]

Water		Water + Methanol (7 : 3)	
pH	NH - δ - NH_2	pH	NH - δ - NH_2
0.5- 0.6	-253.0; -342.4	2.0- 2.1	-253.9; -342.9
4.0- 4.1	-253.0; -342.4	5.0- 5.1	-253.9; -342.9
6.0- 6.1	-253.0; -342.4	7.0- 7.1	-253.9; -342.9
8.2- 8.3	-253.0; -342.4	8.0- 8.1	-253.9; -342.9
9.0- 9.1	-253.2; -342.8	8.9- 9.0	-253.9; -344.6
9.7- 9.8	-253.4; -345.0	9.9-10.0	-254.2; -347.6
10.5-10.6	-253.8; -348.0	10.5-10.7	-350.6
11.1-11.2	-253.9; -350.2	11.4-11.5	-351.7
12.0-12.1	-254.1; -350.9	12.1-12.2	-351.7
12.4-12.5	-254.1; -351.1		

a) Two products with DP 45-50 and 95-100 were measured
b) Only the sample with DP 95-100 was measured

Tab. 2 ^{15}N-NMR chemical shifts δ (ppm,upfield of external $NO_3^{\ominus}$) of N-methylacetamide (1.5 M) in aqueous solution at ca. 32°C

pH	Additive		pH	Additive	
1	1 N HBr	-261.8	7.5	n-Propylamine· HBr+Na OH	-262.7
7	none	-263.4			
14	1N NaOH	-263.2	9.0	"	-262.7
2	n-Propylamine· HBr+1N HBr	-262.7	10.0	"	-262.7
			11.0	"	-262.7
4	" "	-262.7	12.0	"	-262.8
6	n-Propylamine· HBr	-262.7	12.5	"	-262.8

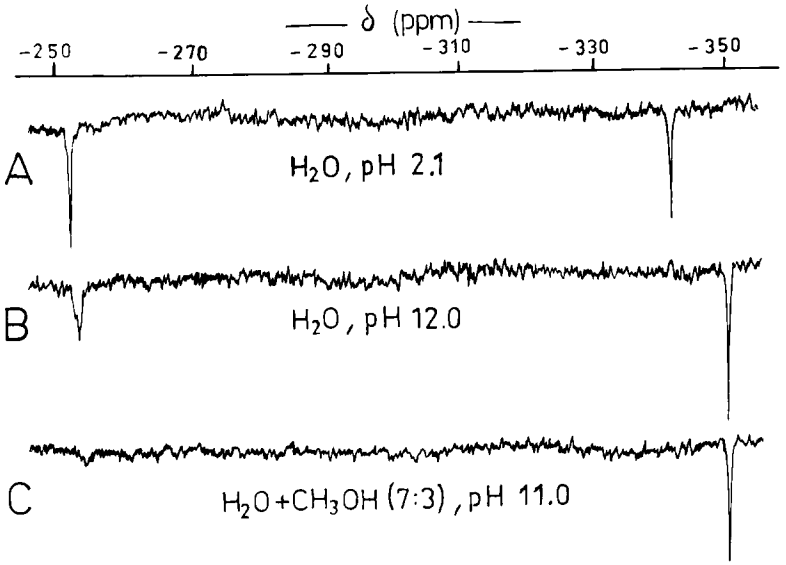

Fig. 1 9.12 MHz natural abundance ^{15}N-NMR spectra (40 000 - 50 000 transients) of poly-L-ornithine (DP $\approx$ 95)

Finally, it should be emphasized that the upfield shift of the peptide signal upon the coil-helix transition of $(Orn)_n$ and $(Lys)_n$ agrees well with the observation that helical $(Ala)_n$ absorbs ca. 1 ppm upfield of (L-Ala) blocks in a coiled poly-D,L-alanine (Kricheldorf 1979 b). Furthermore, we have observed an upfield shift for poly-γ-benzylglutamate in TFA/chloroform mixtures, when the TFA concentration decreases from 20 to 10 % (work in progress). Thus, the coil-helix transition seems to cause generally upfield shifts of the ^{15}N peptide signals, regardless of the system under investigation. This aspect is not worthy because intramolecular H-bonds in cyclopeptides cause downfield shifts of 7-10 ppm relative to analogous linear oligopeptides (Hull 1980). Why intramolecular H-bonds in helices and those in cyclopeptides cause opposite shift effects is an interesting problem which deserves further investigation.

MEASUREMENTS

All chemical shifts listed in Tables 1 and 2 were obtained from natural abundance 9.12 MHz ^{15}N-NMR spectra measured with a Bruker WH 90 PFT spectrometer. Poly-L-ornithine hydrobromide (1.9 g ∿ 10 mmol) was dissolved in water or water/methanol (7:3 by volume) so that, after adjustment of the pH by means of a "Radiometer Copengagen", the total volume of the solution was in the range of 11-12 ml. For measurements relating to Table 2, 1.0 g (15 mmol) N-methylacetamine or a mixture of 1.0 g N-methylacetamide and 1.4 (10 mmol) n-propylamine hydrobromide were dissolved in ca. 10 ml water. All measurements were carried out in 20 mm diameter sample tubes with a coaxial 5 mm tube containing D_2O for lock purposes. After the first measurement of each series this coaxial tube was replaced by another one containing a 30 % (by weight) solution of $^{15}NH_4{}^{15}NO_3$ in D_2O. The $NO_3^{\ominus}$ ion served for shift referencing; the chemical shift of the $NH_4^{\oplus}$ ion was observed at -356.5 ppm. The following acquisition parameters were used: pulse width 30 μ s (ca. 35°) for N-methylacetamide and 40 μ s (ca. 45°) for polyornithine; 2 K data points on a spectral width of 1 000 Hz, zero-filled to 4 K for Fourier transform; 4 000 - 6 000 transients in the case of N-methylacetamide and 20 000 - 50 000 transients in the case of polyornithine; exponential line broadening 1.5 Hz.

REFERENCES

1) Bovey F. A.; Macromol. Reviews 9, 1 (1974
2) Suzuki Y., Inoue Y. and Chujo R.; Biopolymers 16, 2521 (1977
3) Lader H.J., Komoroski R.A. and Mandelkern L.; Biopolymers 16, 895 (1977)
4) Saito H., Toyokazu O., Masahiko K. and Chikayoshi N.; Biopolymers 18, 1065 (1979)
5) Kricheldorf H.R.; Makromol. Chem. 180, 2387 (1979)
6) Hull, W.E. and Kricheldorf H.R.; Biopolymers 17, 2427 (1978)
7) Katchalski E., Grossfeld J. and Frankel M.; J. Am. Chem. Soc. 70, 2094 (1948)
8) Katchalski E. and Spitnik P.; Nature 164, 1092(1949)
9) Dunn M.S. and Rockland L.B.; Advances Protein. Chem. 3, 295 (1947)
10) Witanowski M., Stefaniak L. and Janusjewski H.; in "Nitrogen NMR" 1. Ed. Plenum Press, London-New York 1973, p. 164
11) Cattageno D., Hawkes G.E., and Randall E.W.; J. Chem. Soc. Perkin Trans. 2, 1527 (1976)
12) Kricheldorf H. R.; Makromol. Chem. 179, 2675 (1978)
13) Kricheldorf H.R. and Hull W.E.; Makromol. Chem. 180, 1715 (1979)
14) Hull W.E.and Kricheldorf H.R.;Biopolymers in press (Part 20)

Received December 1o, 1979