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Nuclear Magnetic Resonance Studies on the Structure of the Tetrapeptide Tuftsin, L-Threonyl-L-lysyl-L-prolyl-L-arginine, and Its Pentapeptide Analogue L-Threonyl-L-lysyl-L-prolyl-L-prolyl-L-arginine†

Michael Blumenstein, Porter P. Layne, and Victor A. Najjar*

ABSTRACT: Nuclear magnetic resonance spectroscopy has been used to investigate the solution conformation of tuftsin, threonyllysylprolylarginine, as well as a pentapeptide inhibitor of tuftsin, threonyllysylprolylprolylarginine. Both proton and carbon-13 studies were performed. In water, neither peptide gives evidence of a preferred conformation. In dimethyl-d_6 sulfoxide, tuftsin appears to prefer a particular conformation, but the inhibitor does not. The conformation of tuftsin is one in which the amide NH proton of arginine is solvent shielded. The conformation does not, however, appear to be such that a normal 4 → 1 β turn exists.

Tuftsin, the phagocytosis stimulating peptide (Najjar & Nishioka, 1970), has been fully characterized chemically (Nishioka et al., 1972, 1973a,b) and biologically (Constantopoulos & Najjar, 1972, 1973a–c; Constantopoulos et al., 1973; Najjar, 1974, 1977). It is an integral part of the CH3 segment of leukokinin, a cytophilic γ-globulin (Fidalgo & Najjar, 1967), residues 289–292 (Edelman et al., 1969), which binds to and stimulates the phagocytic activity of the polymorphonuclear cell and the macrophage (Constantopoulos & Najjar, 1972). This stimulatory effect resides totally in the tuftsin peptide which is active only as the free tetrapeptide (Najjar, 1974). It is released from the carrier molecule by two types of enzymes: one in the spleen and the other on the outer surface of the phagocyte membrane (Najjar, 1974). The splenic enzyme tuftsin endocarboxypeptidase splits the carboxy-terminal arginine from its adjacent glutamyl residue. This enzyme has not been purified or characterized. In the absence of the spleen, the arginyl–glutamyl bond remains intact and the carrier molecule, while still capable of binding to the membrane receptors, is incapable of activating the phagocyte. The

† From the Department of Biochemistry and Pharmacology (M.B.) and Division of Protein Chemistry (P.P.L. and V.A.N.), Tufts University School of Medicine, Boston, Massachusetts 02111. *Received May 16, 1979; revised manuscript received August 31, 1979.* This work was supported by the Department of Health, Education, and Welfare, U.S. Public Health Service Grant 5 R01-AI09116, National Science Foundation Grant PCM78-18506, and the National Foundation of March of Dimes (1-556). The NMR facility of the Francis Bitter Magnet Laboratory at the Massachusetts Institute of Technology, which includes the Bruker HX-270 spectrometer, is supported by the National Institutes of Health (RR 00995) and the National Science Foundation (Contract C670). The Bruker WP-60 NMR spectrometer was obtained in part with a National Science Foundation instrument grant (PCM 77-00884).

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phagocyte membrane enzyme leukokininase cleaves tuftsin at its amino-terminal threonine at the lysyl–threonyl bond. The enzyme is strictly an outer membrane enzyme that does not lend itself to solubilization. It has been partly purified and characterized (Najjar & Constantopoulos, 1972; Nishioka et al., 1973a).

The tetrapeptide tuftsin has been shown to exhibit several overall functions that stem basically from its direct activity on the membrane of the phagocyte. The stimulation of the phagocytic activity of either the granulocytes or the macrophages (Constantopoulos & Najjar, 1972) of the reticuloendothelial system is the first property that led originally to its discovery, isolation, and synthesis (Nishioka et al., 1973a,b). The K_m of stimulation was remarkably similar for either cell, ~100 nM (Constantopoulos & Najjar, 1972; Fridkin et al., 1977). Other activities have since been described. It affects the cyclic adenylate level of the phagocytic granulocyte little or none at all (Constantopoulos & Najjar, 1973c). However, it definitely augments the level of cyclic guanylate in both granulocytes and macrophages (Stabinsky et al., 1979). It stimulates the motility of both the granulocyte and macrophage (Nishioka et al., 1973b; Nishioka, 1979a). It augments considerably the immunogenic function of the macrophage at an optimum concentration of 16 nM (Tzehoval et al., 1978) and increases antibody formation over threefold (Florentin et al., 1978). It promotes the bacterial killing property (Martinez et al., 1977) and the tumoricidal activity of both phagocytic cells (Nishioka, 1979b). It is chemotactic in the glass microtube assay of migration, as well as in the modified Boyden chamber (Nishioka et al., 1973b; Nishioka, 1979a). It has been estimated that there are 5×10^4 and 10×10^4 binding sites per granulocyte and monocyte, respectively (Fridkin et al., 1977). Sialic acid is a major component of the site of action of tuftsin (Constantopoulos & Najjar, 1973a). There have been human cases of congenital tuftsin deficiency described both in the United States (Najjar, 1977, 1979a,b) and Japan (Inada et al., 1977). These cases possess a mutant peptide that actually inhibits the two biological activities of tuftsin that have been studied, phagocytosis and migration (Najjar, 1974). This mutation results in a defective ability of the human body to combat infections (Najjar & Constantopoulos, 1972; Najjar, 1974, 1977).

Several analogues of tuftsin have been synthesized, which indicates clearly that one cannot tamper with the structure. Substitution of any residue with analogous or nonrelated ones results in weaker activity, inactivity, or outright inhibition (Nishioka et al., 1973b; Fridkin et al., 1977; Hisatsune et al., 1978; Konopińska et al., 1978, 1979; Konopinska, 1978; Najjar & Schmidt, 1979).

In view of these considerations, a study of the conformation of tuftsin, Thr-Lys-Pro-Arg, under various conditions along with that of the inhibitor pentapeptide analogue, Thr-Lys-Pro-Pro-Arg, is warranted. It has been proposed that the structure of tuftsin could assume a $4 \rightarrow 1$ β turn (Konopińska et al., 1976) commonly observed with tetrapeptides containing proline (Kopple et al., 1975). Both ^1H and ^{13}C nuclear magnetic resonance spectroscopy have proven to be extremely useful in the determination of secondary structures of small peptides in solution [for reviews, see Hruby (1974) and Deslauriers & Smith (1975)]. We have applied these techniques to obtain structural information on tuftsin and the tuftsin inhibitor analogue.

Materials and Methods

L-Amino acids, protected at amino, hydroxy, and guanido functions, were obtained from Bachem. Organic solvents, trifluoroacetic acid (F_3AcOH),[1] and other chemicals were of reagent grade. Deuterium oxide (99.8 and 100%) and dimethyl-d_6 sulfoxide (99.5 and 99.96%) were obtained from Aldrich.

Synthesis of Tuftsin. Tuftsin and its inhibitor analogue were synthesized by the solid-phase method as described previously (Nishioka et al., 1973b). Briefly, N^{α}-Boc-N^{G}-NO_2-L-Arg was esterified to the chloromethylated resin, copolystyrene divinylbenzene, in ethanol and triethylamine at 80 °C for 24 h. The Boc group was then cleaved with 50% F_3AcOH in methylene chloride. After neutralization, N^{α}-Boc-L-Pro was coupled to the arginine resin ester with DCC. The deprotection and coupling steps were repeated with N^{α}-Boc-N^{ϵ}-Z-L-Lys and finally with N^{α}-Boc-*O*-Bzl-L-Thr. The deprotected polypeptide was then cleaved off the resin with HBr in F_3AcOH, and the remaining protecting NO_2 group was removed by catalytic hydrogenation. The polypeptide was then purified by gradient elution on an aminex column at 60 °C under a pressure of 60 psi and obtained as the triacetate, M_r 677.

Nuclear Magnetic Resonance Spectroscopy. Carbon-13 spectra were acquired at 67.9 (Bruker HX-270) and 15.1 (Bruker WP-60) MHz. Proton spectra were acquired at 270 MHz (HX-270). Sample concentrations for ^{13}C were ~0.04 M, and for ^1H the concentrations were usually ~0.01 M. T_1 values were obtained by using a $180°-\tau-90°-T$ pulse sequence (Vold et al., 1968). Spectra in Me_2SO-d_6 as well as all T_1 spectra were acquired at 37 °C. Unless otherwise noted, other spectra were acquired at ambient temperature (27 to 28 °C). All spectra were acquired in the Fourier transform mode by using quadrature detection. Proton spectra in H_2O were acquired by using a presaturation pulse located at the resonance frequency of the water protons (Campbell et al., 1977). This technique was also used (with a variable length presaturation pulse) to measure saturation transfer in Me_2SO-d_6–5% H_2O.

Results

The ^{13}C NMR spectra of tuftsin and tuftsin inhibitor in H_2O are shown in Figure 1. Assignments could be made by comparison with known positions of resonances due to the component amino acids. Titration curves of these two peptides, as a function of pH, are displayed in Figures 2 and 3. For each peptide the pK_a of the α-amino group was found to be 7.1, the α-carboxyl group 3.0, and the ϵ-amino group 10.0. A comparison of the data for these compounds indicates that (a) the corresponding carbons in the two peptides have shifts which are almost identical and (b) upon changing pH, the only resonances that experience measurable shifts are those within a few bonds of the titrating group. Consequently, there are no long-range or through-space effects. There is no doubling of resonance observed due to conformers with cis X–Pro bonds (Dorman & Bovey, 1973).

The ^{13}C spectra of tuftsin and tuftsin inhibitor in Me_2SO-d_6 were first recorded at 15.1 MHz. The resonances obtained were considerably broader than they were in H_2O, and the overall resolution was poor. The spectra were therefore retaken at 67.9 MHz, and high-quality spectra were obtained (Figure 4). The chemical shifts of tuftsin and tuftsin inhibitor are listed in Table I.

There were larger ^{13}C shift differences between the two compounds in Me_2SO than were observed in D_2O. The α carbon of proline adjacent to the C-terminal residue moves

[1] Abbreviations used: F_3AcOH, trifluoroacetic acid; DCC, *N*,*N*^{1}-dicyclohexylcarbodiimide; NMR, nuclear magnetic resonance; tuftsin, Thr-Lys-Pro-Arg; tuftsin inhibitor, Thr-Lys-Pro-Pro-Arg; Me_2SO-d_6, deuterated dimethyl sulfoxide; Z, carbobenzoxy.

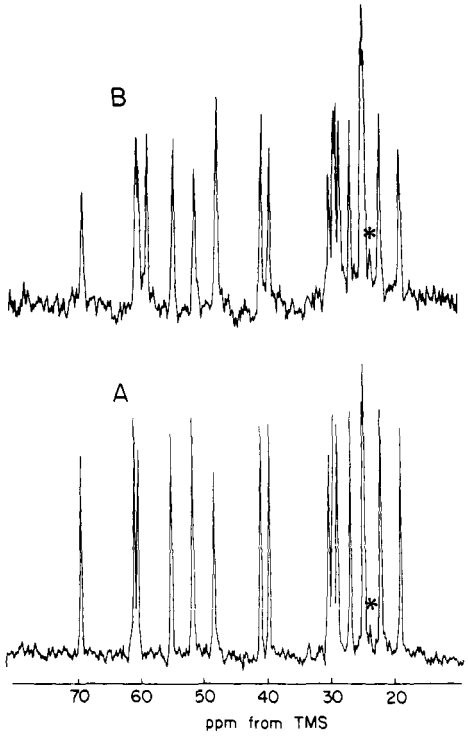

FIGURE 1: ^{13}C spectra of the aliphatic carbons of tuftsin (A) and tuftsin inhibitor (B) at 0.035 M in H_2O–10% D_2O, pH 8.7, 25 °C. Spectra were acquired at 15.1 MHz (WP-60 spectrometer) by using 90° pulses (21 μs) and a 1-s repetition rate. For each spectrum 70 000 pulses were acquired. The small peak observed at ~23 ppm (indicated by an asterisk) in each spectrum is due to the methyl carbon of acetate. Its intensity is low due to its very long relaxation time and low nuclear Overhauser enhancement.

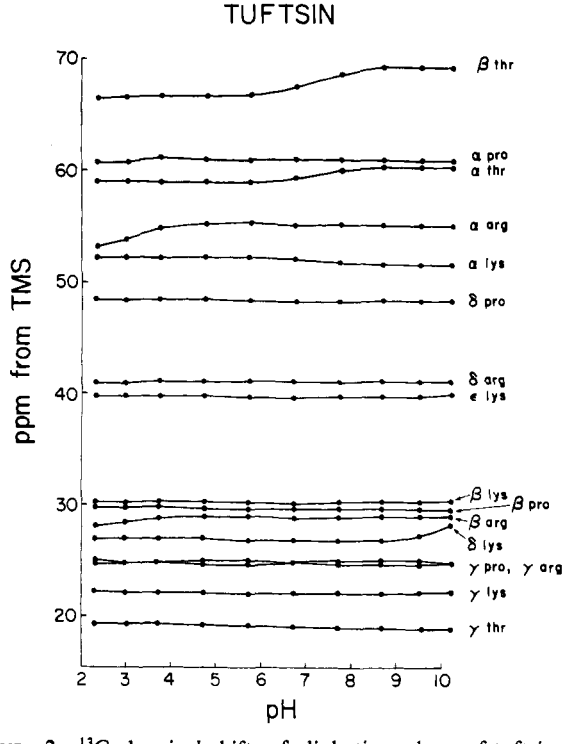

FIGURE 2: ^{13}C chemical shifts of aliphatic carbons of tuftsin as a function of pH. Solution conditions and spectral parameters are as described in Figure 1. 10 000–30 000 pulses were acquired for each spectrum.

upfield by 0.9 ppm in the pentapeptide. The assignment of this carbon was based on its shorter relaxation time as compared to the α carbon of threonine. There are upfield shifts

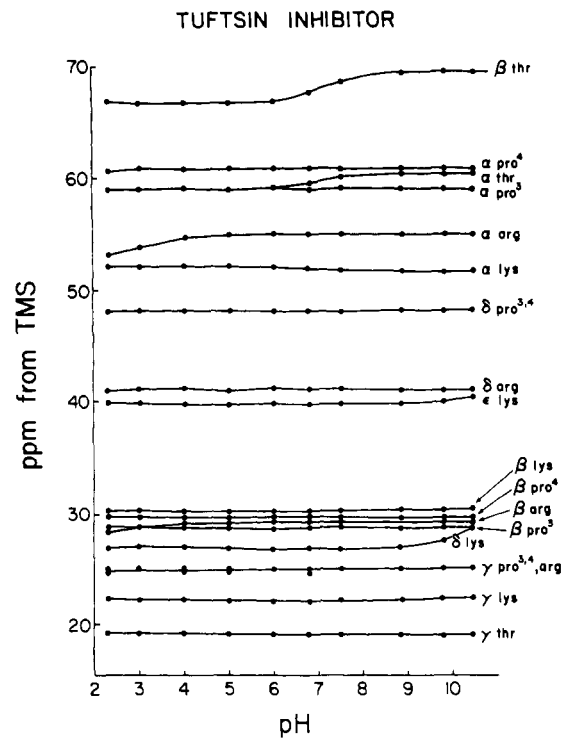

FIGURE 3: ^{13}C chemical shifts of tuftsin inhibitor as a function of pH.

Table I: ^{13}C Chemical Shifts of Tuftsin and Tuftsin Inhibitor (ppm) from Me_4Si[a]

	H_2O (pH 8.6)		Me_2SO-d_6	
	tuftsin	inhibitor	tuftsin	inhibitor
β Thr	69.4	69.5	68.4	68.3
α Pro[b]	61.1	61.0	61.4	60.5
α Thr	60.5	60.6	61.1	61.0
α Pro[c]		59.2		58.4
α Arg	55.2	55.1	54.3	54.3
α Lys	51.8	51.7	50.7	50.5
δ Pro[b]	48.4	48.2	47.8	47.7
δ Pro[c]		48.2		47.5
δ Arg	41.1	41.2	41.2	41.2
ε Lys	39.8	39.9	39.5	39.5
β Lys	30.4	30.3	{32.4	{31.9
β Pro[b]	29.7	29.6	{30.4	{30.4
β Arg	29.1	29.3	{29.9	{29.3
β Pro[c]		28.7		29.3
δ Lys	27.0	27.0	28.6	28.9
γ Pro[b] }	25.0	25.1	25.8	25.9
γ Arg }	24.9	24.9	25.2	25.4
γ Pro[c]		25.1		25.4
γ Lys	22.2	22.2	22.7	22.3
γ Thr	19.1	19.1	20.9	20.9
(O=)C Arg	178.7	178.6	{175.0	{175.4
(O=)C Thr	175.1	175.3	{174.6	{174.4
(O=)C Lys	173.6	173.7	{171.6	{171.3
(O=)C Pro	172.3	172.5	{170.7	{170.7
(O=)C Pro		171.8		{170.5
guanido Arg	157.2	157.2	158.4	158.4

[a] Braces indicate values not assignable to specific residues. [b] Designates Pro[3] of tuftsin and the corresponding Pro[4] of the inhibitor. [c] Designates Pro[3] of inhibitor.

of ~0.5 ppm in the β-carbon region, and the γ carbon of lysine moves 0.4 ppm upfield. Also, the chemical-shift difference between the carbonyl carbons of arginine and threonine increases from 0.4 to 1.0 ppm. Thus, ^{13}C chemical-shift measurements in Me_2SO indicate some conformational differences between tuftsin and tuftsin inhibitor in this solvent. The observed changes are more likely due to conformational effects than to the different primary structures of the peptides, since

Table II: Relaxation Time (NT_1) Values in Seconds of Aliphatic Carbon Atoms in Tuftsin and Tuftsin Inhibitor[a]

	H_2O (pH 5)				Me_2SO-d_6			
	tuftsin		inhibitor		tuftsin		inhibitor	
	15 MHz	68 MHz	15 MHz	68 MHz	15 MHz	68 MHz	15 MHz	68 MHz
β Thr	0.51	0.73	~0.50	0.71	0.15	0.28	0.14	0.30
α Pro[b]	0.32	0.53	0.26	0.46	} 0.08	0.19	} 0.08	0.17
α Thr	0.49	0.64	} 0.39	} 0.44		0.27		0.27
α Pro[c]							0.05	0.16
α Arg	0.41	0.70	0.42	0.58	0.06	0.16	0.05	0.18
α Lys	0.32	0.47	0.29	0.45	0.05	0.19	0.05	0.18
δ Pro[b]	0.38	0.50	} ~0.25	0.61	0.06	0.26	} 0.04	0.24
δ Pro[c]				0.61				0.28
δ Arg	0.88	1.10	0.66	1.10	NO	0.24	NO	~0.20
ε Lys	1.30	2.10	1.40	2.00	NO	0.44	NO	~0.30
β Lys	0.60	0.72	0.32	0.58	}	0.28	0.06	0.26
β Pro[b]	0.44	0.62	} 0.36	0.73	0.06	0.26	NO	0.23
β Arg	0.44	0.82		0.62	}	0.28	} 0.08	} 0.28
β Pro[c]			0.30–0.50	0.67				
δ Lys	0.96	1.40	1.10	1.30	0.20	0.34		0.26
γ Pro[b]	} 0.72	1.20	}	0.98 or 0.84	} 0.06	0.26	}	} 0.34
γ Arg		1.10	} 0.50	1.30		0.30	} 0.06	} 0.20
γ Pro			}	0.98 or 0.84				
γ Lys	0.54	0.94	0.52	0.98	0.12	0.28	0.10	0.36
γ Thr	1.50	2.90	1.60	1.60	0.66	1.40	0.54	1.00

[a] Braces indicate residues giving rise to closely spaced peaks whose T_1 values cannot be individually determined. NO, not observable. [b] Designates Pro[3] of tuftsin and corresponding Pro[4] of inhibitor. [c] Designates Pro[3] of inhibitor.

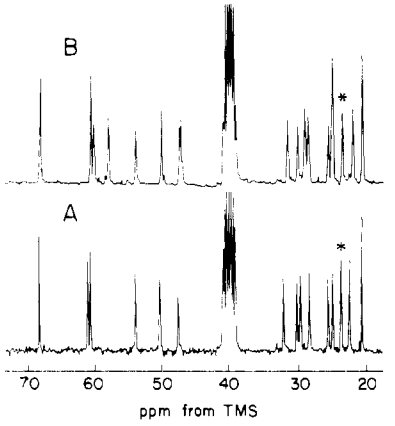

FIGURE 4: ^{13}C spectra of tuftsin (A) and tuftsin inhibitor (B) at 0.03–0.04 M in Me_2SO-d_6, 37 °C. Spectra were acquired at 67.9 MHz (HX-270 spectrometer) by using 90° pulses (21.5 μs) and a 1-s repetition rate. For (A) 7000 pulses were acquired, and for (B) 40 000 pulses. The acetate peak appearing at 23 ppm (indicated by an asterisk) is much larger than the peak observed in water since the relaxation time of the acetate is greatly reduced in Me_2SO. The large peaks centered at 40 ppm are due to Me_2SO. On an expanded scale, the peaks due to the ε carbon of lysine and the δ carbon of arginine can be observed in this region.

these differences are not observed in H_2O.

Relaxation time values for the aliphatic carbons of tuftsin and tuftsin inhibitor are listed in Table II. In water, relaxation time values, NT_1, generally increase for carbon atoms sequentially down the side chains away from the backbone. In Me_2SO this effect is not as pronounced, with only the γ-methyl group of threonine having a very long NT_1 value. In addition, all T_1 values are reduced in Me_2SO, a finding consistent with the increase in the observed line widths. The α carbon of the N-terminal threonine has a longer T_1 than other α carbons in both solvents. In water, but not in Me_2SO, the α carbon of the C-terminal arginine has a T_1 which is longer than that of the two internal α carbons. The γ carbon of proline(s) has an increased T_1 value, indicative of rapid interconversion between puckered forms [London (1978) and references therein]. T_1 values are longer at 68 MHz than at 15 MHz due to the expected frequency dependence of T_1 (Doddrell et al., 1972).

Table III: Coupling Constants (J), Chemical Shifts (Δ), and Temperature Dependence of Chemical Shifts ($\delta\Delta/°C$) for Amide Protons of Tuftsin and Tuftsin Inhibitor[a]

	Lys, Me_2SO			Arg, Me_2SO			Arg, H_2O		
	J	Δ	$\delta\Delta/°C$	J	Δ	$\delta\Delta/°C$	J	Δ	$\delta\Delta/°C$
tuftsin	7.5	8.4	4	6.0	7.4	0	7.0	8.0	8
inhibitor	7.5	8.4	4	6.0	7.7	4	6.5	7.8	8

[a] Coupling constants are in hertz (SD ±0.3), chemical shifts are in parts per million from Me_4Si (±0.1), and temperature dependence ($\times 10^3$) is in parts per million/°C (±1). Spectra were taken at 270 MHz, and chemical shifts are at 23 °C.

This difference is greater in Me_2SO than in water due to the increased correlation time for the peptides in this solvent. The approximate backbone correlation time for each peptide is 10^{-10} in water and 10^{-9} in Me_2SO. All of the above statements hold for both tuftsin and tuftsin inhibitor; there appear to be no significant relaxation time differences between the two molecules.

The 270-MHz ^1H spectra of tuftsin and tuftsin inhibitor in D_2O were recorded and assigned as shown in Figure 5. Assignments were based on known positions and patterns of amino acid residues, single-frequency decoupling, and pH titrations. As was observed in the ^{13}C studies, the two compounds gave very similar spectra.

The aliphatic region of the ^1H spectra in Me_2SO-d_6 was poorly resolved (spectra not shown). Significant information could be obtained from observation of the NH resonances, which were studied in H_2O as well as Me_2SO-d_6. Two amide NH resonances were observed in the Me_2SO. The downfield resonance broadened as the temperature was raised. Both its downfield position and its broadening are consistent with an amide NH on a residue that is adjacent to the amino-terminal residue. This peak was therefore assigned to the amide of lysine. The other NH resonance would be due to arginine. These assignments were confirmed by single-frequency decoupling. In H_2O both NH protons were visible, but as the temperature was raised the lysine NH broadened beyond detection. At low temperatures other protons due to NH groups of the guanido group of arginine were also visible, but data

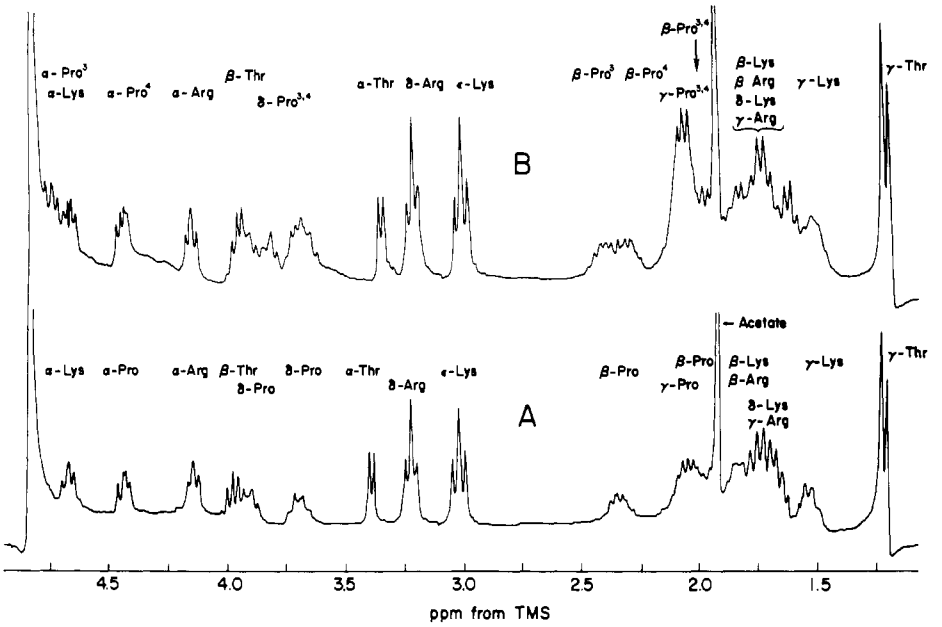

FIGURE 5: ^1H spectra at 270 MHz of tuftsin (A) and tuftsin inhibitor (B) at 0.01 M in D_2O, "pD" (direct meter reading) 7.5, 28 °C. Number of pulses, 20; repetition rate, 3.2 s.

on these protons were not quantitated.

In Table III are listed the chemical shifts, temperature dependence of the chemical shifts, and coupling constants ($J_{\alpha CH-NH}$) for the amide protons of tuftsin and tuftsin inhibitor. In Me_2SO-d_6, the NH of arginine of tuftsin displays a chemical shift which is upfield of its position in H_2O and is independent of temperature. Both observations are typical of a proton which is intramolecularly hydrogen bonded (Kopple et al., 1969) and therefore not exposed to solvent. The corresponding arginine NH of the inhibitor as well as the NH of lysine in each compound exhibits temperature-dependent shifts typical of solvent-exposed amide protons. In water the shift of the arginine NH of tuftsin is no longer temperature independent and in fact shows the same temperature dependence as the corresponding NH of the inhibitor.

We have examined the exchange rate of the lysine NH proton in Me_2SO–5% H_2O by using the saturation transfer technique developed by Redfield and co-workers (Waedler et al., 1975). A comparison of parts A and B of Figure 6 showed that the lysine NH of tuftsin experienced much greater saturation transfer than did the lysine NH of the pentapeptide inhibitor. This indicates that in tuftsin this NH proton undergoes more rapid exchange than does the corresponding proton in the analogue.

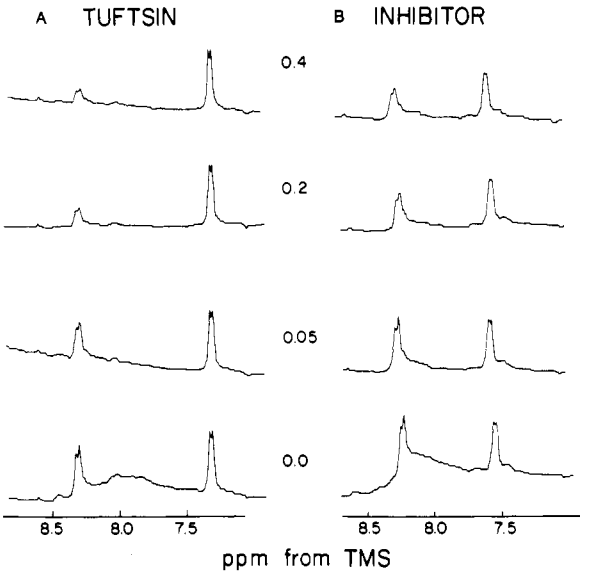

FIGURE 6: Saturation transfer experiment involving the amide NH protons and the water resonance in solutions of tuftsin and tuftsin inhibitor (0.02 M) in Me_2SO-d_6–5% H_2O, pH 7, 27 °C. The ^1H resonance due to water was specifically irradiated for varying amounts of time indicated in the center of the figure (times in seconds). Immediately thereafter, a normal observing pulse was applied. For each spectrum, the sequence was repeated 50 times, with a 2.7-s recycle time.

Discussion

The ^{13}C results in water gave little indication of conformational differences or conformational preferences in tuftsin and tuftsin inhibitor. Neither compound displayed any resonances due to a cis X–Pro bond. The pK_a values for each of the two compounds were found to be similar, and no long-range effects with changing pH were observed. This indicates an absence of conformational change following the titration of the ionizing groups. The pK_a values of the α-amino groups were somewhat lower than are usually observed in peptides, but this is consistent with the relatively low value of the corresponding pK_a of the amino acid threonine (Greenstein & Winitz, 1961). Relaxation time studies in water also follow expected patterns with increasing mobility being observed in side-chain carbon atoms furthest removed from the backbone. The α carbon of the N-terminal threonine, and to a lesser extent that of the C-terminal arginine, in both peptides showed greater mobility than the α carbon of the internal residues. The CH_3 of the threonines indeed displayed larger values of NT_1 than did other carbons. The NT_1 values of proline indicate interconversion between various puckered forms.

There was a dramatic decrease in mobility for all carbons of both compounds when the solvent was changed from H_2O to Me_2SO, a solvent with higher viscosity. This decrease was most apparent in the 15-MHz data, since for the τ_c range observed here NT_1 changes more rapidly with τ_c at this frequency than at 68 MHz (Doddrell et al., 1972). The decrease in mobility was especially large for carbons in the Lys and Arg side chains, which no longer possessed the greatly increased mobility observed in water for the other carbons. The peptides studied were triacetate salts, and in Me_2SO the three ion pairs

present at the α- and ϵ-amino groups and the guanidinium group would have much reduced mobility as compared to the dissociated and hydrated charges found in water.

There were several ^{13}C chemical-shift differences between tuftsin and tuftsin inhibitor in Me_2SO. While an exact interpretation of these differences is not possible at this time, they do indicate that overall structural differences exist between the two compounds in Me_2SO, unlike the situation in water.

In the ^1H studies, the arginine NH of tuftsin in Me_2SO displays an upfield chemical shift and the shift is independent of temperature. Both of these effects are often diagnostic of a 4 $\rightarrow$ 1 β turn (Urry et al., 1970), which in tuftsin would involve a hydrogen bond between the carbonyl oxygen of threonine and the amide NH of arginine.

The saturation transfer experiments are also consistent with a 4 $\rightarrow$ 1 hydrogen bond. The lysine NH undergoes greater saturation transfer in tuftsin than in the pentapeptide inhibitor. This indicates a more rapid exchange with water for the former compound. A hydrogen bond involving the carbonyl group of the adjacent threonine would tend to withdraw electrons from the neighboring NH of lysine, thereby leading to an increased rate of exchange of the NH protons.

While the observations mentioned above favor the existence of a 4 $\rightarrow$ 1 β turn for tuftsin in Me_2SO, as proposed by Konopińska et al. (1976), other considerations argue against it. In a β-turn structure, the coupling constant, J_{NH-CH}, of the $i + 1$ residue, in this case lysine, should be small, i.e., <4 Hz (Urry et al., 1970), while our observed coupling constant for that residue is 7.5 Hz. Theoretical calculations (Chandrasekaran et al., 1973) have shown the possible ranges of ϕ and ψ angles in residues 2 and 3 of β turns. While the favored ϕ angle of proline, about $-50°$, makes this a very likely residue in position 2 of a β turn, it virtually precludes its appearance at position 3 where angles of about -90 to $-120°$ or 40 to 60° are favored. These theoretical predictions have been borne out by the empirical studies of Chou & Fasman (1978). They find that proline occurs in position 3 of a β turn in proteins only when it has the cis conformation. Our ^{13}C results would appear to rule out the occurrence of *cis*-proline in tuftsin. Finally, an NH proton which is hydrogen bonded should show a reduced exchange rate with traces of water in the solvent, with a half-time of several minutes. The exchange rate of the arginine NH in tuftsin in Me_2SO is too fast to measure, indicating an upper limit of $\sim$10 s for the half-time.

We believe that our data are best explained by postulating a tuftsin structure in Me_2SO in which the arginine NH is shielded from solvent but is not part of a hydrogen bond. We note that in studies of the chemotactic peptide fMet-Leu-Phe, Glasel and co-workers (J. A. Glasel, personal communication) have also found an NH proton whose chemical shift is fairly insensitive to temperature but is not hydrogen bonded. Unfortunately, until more extensive experiments are carried out, we cannot define the structure of tuftsin which exists in Me_2SO.

In contrast to the results for tuftsin in Me_2SO, we find no evidence for any preferred structure for tuftsin in an aqueous environment, nor for that matter for the analogue in either solvent. It was shown in studies of antamanide that a Pro-Pro sequence could form the second and third residues in a β turn if the peptide bond between them was cis (Patel & Tonelli, 1974). It should be noted, however, that this structure was not present in the crystal (Karle et al., 1973), and it was then concluded that it was not present in solution either (Patel & Tonelli, 1974). As mentioned previously, ^{13}C NMR results indicate that neither of the two prolines in tuftsin inhibitor is in the cis configuration. It is therefore expected that the ^1H results show no evidence of a β turn. In the linear peptide bradykinin, the Pro-Pro sequence is also not part of an ordered structure (London et al., 1978).

While neither tuftsin nor its inhibitor displays any particular structure in water, it is of interest that the biologically active compound, tuftsin, does display a structural preference (as yet undefined) in Me_2SO, while the inactive but strongly inhibitory pentapeptide does not display any structure. Clearly further research into the exact structure of tuftsin in Me_2SO is indicated. Such a study would include other active, inhibitory, and inert analogues. It is possible that some correlation between biological activity and structure in Me_2SO can be made.

Recently (Sucharda-Sobezyk et al., 1979), it has been concluded that tuftsin forms a β-turn structure. This conclusion was based on studies employing analogues in which the C- and N-terminal residues, as well as the lysine and arginine side chains, were blocked. These compounds would be expected to exhibit conformations which differ significantly from that of tuftsin. Also, the structural conclusions were based wholly on the results of IR studies. As we have discussed in this paper, the evidence for a β turn may be contradictory, and we do not believe that the IR work presented by Sucharda-Sobczyk et al. can unambiguously define a β turn.

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Partial Purification and Characterization of a Ribonucleic Acid N^2-Guanine Methyltransferase Associated with Avian Myeloblastosis Virus†

Marilyn J. Taylor‡ and Raymond Gantt*

ABSTRACT: A nucleic acid methylase, N^2-guanine ribonucleic acid (RNA) methyltransferase, which is associated with type C RNA tumor viruses, has been purified from avian myeloblastosis virions by gel filtration on Sephadex G-200, followed by chromatography on hydroxylapatite. The molecular weight estimated by gel filtration is 220 000, and the methylase activity has a pH optimum of 7.6–7.9. Magnesium and ammonium ions both stimulate activity 1.5-fold at 9.5 mM and 0.36 M, respectively, but apparently neither is essential for activity. Both daunomycin and adriamycin, antineoplastic drugs, also increase activity 1.5-fold at 1 mM. The enzyme was purified 120-fold from the virions and the activity is partially stabilized by dithiothreitol, but large losses were sustained during 24-h dialysis. The purified enzyme retains 75% of its activity on storage at −25 °C for 2 months in buffer containing 50% glycerol. *Escherichia coli* tRNA^{Phe} and tRNA^{Val} are preferred substrates with methylation occurring at position 10 of *E. coli* tRNA^{Phe}.

Enzymatic methylation of nucleic acids is a widespread cellular phenomenon. One biological function of DNA methylation in some bacteria has been clearly demonstrated to confer resistance to cleavage of the DNA by specific restriction nucleases (Meselson & Yuan, 1968). RNA methylation has been implicated in cellular regulation and differentiation (Sharma et al., 1971), and 7-methylguanine at the 5′-phosphate end of mRNA has been shown (Muthukrishnan et al., 1975) to be a requirement for translation in some cases, though not all (Rose & Lodish, 1976). Further, aberrant nucleic acid methylation has been suggested as a fundamental event in some kinds of malignant transformation (Borek & Kerr, 1972).

N^2-Guanine RNA methyltransferase has been shown to be associated with the avian myeloblastosis virion (Gantt et al., 1971) and subsequently several other oncogenic RNA viruses

† From the Laboratory of Cellular and Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205. *Received May 2, 1979.* M.J.T. was a postdoctoral recipient of 5 F02 CA52447 and 1 F22 CA02366 (National Cancer Institute). The virus was provided by a contract to the Special Virus Cancer Program, National Cancer Institute.

* Address correspondence and reprint requests to this author at the National Cancer Institute, Bethesda, MD 20205.
‡ Present address: Department of Biochemistry, Howard University College of Medicine, Washington, D.C. 20059.

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