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Studies on the Conformation and Interactions of Elastin. Proton Magnetic Resonance of the Repeating Pentapeptide†

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ABSTRACT: Proton magnetic resonance studies have been carried out on the penta-, deca-, and pentadecapeptides of the basic sequence: L-Val-L-Pro-Gly-L-Val-Gly. Two methods—temperature dependence and methanol–trifluoroethanol solvent mixture dependence of peptide proton chemical shift—have been utilized effectively to delineate peptide protons. The two methods, the relatively high field position of the valine$_{4,9,14}$ peptide protons and the $^3J_{NH-CH}$ for the valine residues, are consistent with the presence of a β turn with the sequence L-Pro-Gly at the corners.

Elastin is the protein component of connective tissue that is largely responsible for the elastic properties of ligaments and arterial walls. Furthermore, the elastic fibers of the arterial wall are major foci for the deposition of calcium salts and lipid in the pathogenic process of arteriosclerosis (Yu and Blumenthal, 1965; Kramsch and Hollander, 1973). Elastin is an insoluble, highly cross-linked protein which until very recently has only been studied spectroscopically as fragments, called α- and β-elastin (Partridge *et al.*, 1955; Partridge and Davis, 1955), which are obtained by hot oxalic acid treatment of the purified fiber.

Recently, however, Foster *et al.* (1973) have determined the sequence of a dozen peptides obtained from tropoelastin—a soluble, noncross-linked precursor of elastin which is isolated from the aortas of copper-deficient pigs. The peptides, which represent a sum of approximately 350 residues or close to one-half of the tropoelastin molecule, exhibit repeating sequences. The repeating sequences are a tetrapeptide, -Gly-Gly-L-Val-L-Pro-; a pentapeptide, -L-Pro-Gly-L-Val-Gly-L-Val-; and a hexapeptide, L-Pro-Gly-L-Val-Gly-L-Val-L-Ala. The existence of sequences with regularly positioned glycines has been anticipated (Urry, 1972).

In the present manuscript we report proton magnetic resonance studies on repeating pentapeptides synthesized in this laboratory. The basic sequence L-Val$_1$-L-Pro$_2$-Gly$_3$-L-Val$_4$-Gly$_5$ was synthesized as were the dimer and trimer of the sequence, *i.e.*, the decapeptide and pentadecapeptide. Assignments were achieved by noting the multiplet patterns and decoupling, by noting the chemical shifts of fragments derived during the synthesis and by chemically differentiating end residues. Dependence of peptide proton chemical shifts on temperature and on methanol–trifluoroethanol solvent mixtures was utilized to delineate peptide protons as an initial step in arriving at a secondary structure. The above dependencies of chemical shift taken together with the valyl coupling constants and the relative chemical shifts of the peptide pro-

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tons provide a basis for proposing a β-turn involving the N–H of valine$_4$ and the C–O of valine$_1$.

Material and Methods

Proton magnetic resonance spectra were obtained on a Varian 220-MHz spectrometer equipped with an SS-100 computer system and a tracking frequency sweep decoupling accessory. Tetramethylsilane was used as an internal reference. The probe temperature was measured to within $\pm 2°$ using the chemical shifts of methanol or ethylene glycol samples.

Dimethyl-d_6 sulfoxide (Me_2SO-d_6, 99.5% D and 99.8% D) was obtained from Diaprep Corp., Atlanta, Ga., and from Columbia Organic Chemical Co., Columbia, S. C. Methanol-d_4, 2,2,2-trifluoroethanol-d_3, and deuterium oxide were purchased from Merck, Sharp and Dohme, Montreal, Canada. Spectroquality methanol was obtained from Matheson Coleman and Bell. Trifluoroethanol, purchased from Halocarbon Products, Hackensack, N. J., was redistilled in glass (bp 72°, 738 mm) using a Duflon Column containing glass beads (lower $^3/_4$) and glass helices (upper $^1/_4$) set to a 10% take. A small amount of $NaHCO_3$ was added to remove traces of acid. The first 10–15% of distillate was discarded as was a 20–30% pot residue.

Synthesis of N-HCO-(Val-Pro-Gly-Val-Gly)$_n$-OMe (n = 1, 2, 3) via Solid-Phase Synthesis Using the Fragment Condensation Techniques

Thin-layer chromatography was performed on silica gel G with the following solvent systems: R_F(a) is for the solvent system chloroform–methanol–acetic acid (95:15:3, v/v) and R_F(b), 1-butanol–acetic acid–pyridine–water (30:6:20:24, v/v). Materials possessing Boc-peptide groups were detected by spraying ninhydrin on the hot plates. *N*-Formyl peptide materials were detected by chlorine gas and *o*-tolidine.

I. Boc-Val-Pro-OMe. *N,N*-Dicyclohexylcarbodiimide (45 g, 0.22 mol) was added to 380 ml of a chloroform solution containing Boc-Val-OH (43 g, 0.2 mol), H-Pro-OMe·HCl (35 g) and 28 ml of triethylamine at 0°. After stirring for 5 hr at 0°, the mixture was left to stand overnight at room temperature. Approximately 0.5 ml of acetic acid was then added and the precipitate, dicyclohexylurea, was removed by filtration. The filtrate was washed successively with 1% hydrochloric acid, 4% sodium bicarbonate solution, and water, and finally dried over anhydrous sodium sulfate. Evaporation of the solution gave a residue mixture of crystals and oil. This was dissolved in a small amount of ethyl acetate and the insoluble portion was removed by filtration. Evaporation of the filtrate yielded 42 g of oil with an R_F(a) of 0.68. This product was used for the next reaction without further purification.

II. Boc-Val-Pro-OH. To a solution of Boc-Val-Pro-OMe (42 g) in methanol (200 ml) was added 1 N sodium hydroxide (150 ml) at 0°. The solution was allowed to stand for 1 hr at room temperature. After addition of water (100 ml), the solution was concentrated *in vacuo* at 15–25° to remove methanol. After washing with ether, the water layer was acidified with 2 N hydrochloride while cooling. The oily product was extracted with ethyl acetate and the extract dried over sodium sulfate. The ethyl acetate layer was filtered and evaporated. The residue crystallized by addition of ethyl acetate and petroleum ether (bp 30–60°). It was recrystallized from the same solvent: yield, 30 g (50% from Boc-Val-OH); mp 148–150°. *Anal.* Calcd for $C_{15}H_{26}N_2O_5$: C, 57.30; H, 8.33; N, 8.90. Found: C, 57.53; H, 8.36; N, 8.90.

III. Boc-Val-Gly-OMe. *N,N*-Dicyclohexylcarbodiimide (46 g, 0.22 mol) was added to 500 ml of chloroform containing Boc-Val-OH (43.4 g, 0.2 mol), H-Gly-OMe·HCl (30 g, 0.24 mol), and 28 ml of triethylamine. The procedure for the rest of the reaction was the same as described in I. The product was recrystallized from ethyl acetate and petroleum ether: yield, 46 g (85%); mp 110–112°; R_F(a) 0.81. *Anal.* Calcd for $C_{13}H_{24}N_2O_5$: C, 54.15; H, 8.38; N, 9.71. Found: C, 54.29; H, 8.48; N, 9.61.

IV. Boc-Gly-Val-Gly-OMe. Boc-Val-Gly-OMe (27.1 g, 0.1 mol) was dissolved in 20% hydrogen chloride in methanol (200 ml) at room temperature. After 1 hr the solution was evaporated and the remaining oil, H-Val-Gly-OMe·HCl, was used without purification: R_F(a) 0.15; yield, 35 g. *N,N*-Dicyclohexylcarbodiimide (22 g, 0.11 mol) was added to 100 ml of chloroform containing Boc-Gly-OH (17.5 g, 0.1 mol), H-Val-Gly-OMe·HCl obtained above, and 14 ml of triethylamine. The reaction mixture was treated as described above (I). Boc-Gly-Val-Gly-OMe was recrystallized from ethyl acetate: yield, 21 g (60%); mp 148–150°. *Anal.* Calcd for $C_{15}H_{27}N_3O_6$: C, 52.16; H, 7.88; N, 12.16. Found: C, 52.17; H, 7.92; N, 12.11.

V. Boc-Val-Pro-Gly-Val-Gly-OMe. Boc-Gly-Val-Gly-OMe (17 g, 0.05 mol) was dissolved in 50 ml of trifluoroacetic acid at room temperature. After 30 min the trifluoroacetic acid was distilled off and the remaining gel, H-Gly-Val-Gly-OMe·CF_3COOH, was dissolved in dimethyl sulfoxide (80 ml), dimethylformamide (80 ml), and 8 ml of triethylamine and the gel was used in the next step without purification. To a solution of Boc-Val-Pro-OH (16 g, 0.05 mol) in chloroform (100 ml), *N,N*-dicyclohexylcarbodiimide (12 g, 0.056 mol) in chloroform (20 ml) was added drop by drop at 0°. After 10 min the solution of the H-Gly-Val-Gly-OMe·CF_3COOH obtained above was added at 0–5°. The mixture was left to stand overnight at room temperature. Approximately 0.5 ml of acetic acid was added and the work-up was continued as described in I. Evaporation of the ethyl acetate gave a white powder from petroleum ether: yield, 16.5 g (61%); mp 92–95°; R_F(a) 0.68. *Anal.* Calcd for $C_{24}H_{42}N_5O_8$: C, 55.54; H, 7.87; N, 12.95. Found: C, 55.93; H, 7.87; N, 12.49.

VI. Boc-Val-Pro-Gly-Val-Gly-OH. To a solution of Boc-Val-Pro-Gly-Val-Gly-OMe (13.5 g, 0.025 mol) in methanol was added 1 N sodium hydroxide (25 ml) at 0°. The reaction mixture was treated as described above (II). After ethyl acetate had been distilled off, crystals were obtained. Recrystallization was from ethyl acetate: yield, 9.8 g (74.3%); R_F(a) 0.21; mp 184–185°. *Anal.* Calcd for $C_{24}H_{40}N_5O_8 \cdot H_2O$: C, 52.93; H, 7.77; N, 12.85. Found: C, 53.43; H, 7.68; N, 12.90. Amino acid analysis: Pro, 1.18; Gly, 2.0; Val, 1.10.

VII. Boc-(Val-Pro-Gly-Val-Gly)$_2$-resin. The 3 g of Boc-Gly-resin containing 0.335 mmol/g of Gly was treated for the incorporation of each Boc-peptide by the following steps: (1) the resin was washed with methylene chloride (three times); (2) cleavage of the Boc group by addition of 25 ml of 30% (v/v) trifluoroacetic acid in methylene chloride and by shaking for 30 min; (3) five washings with 20-ml portions of methylene chloride; (4) three washings with 20 ml of absolute ether; (5) three washings with 20-ml portions of methylene chloride; (6) neutralization of the trifluoroacetic acid salt with 20 ml of 10% (v/v) triethylamine in dimethylformamide for 10 min; (7) five washings with 20-ml portions of methylene chloride; (8) addition of 3 mmol of appropriate Boc-peptides (3.0-fold molar excess) in 10 ml of methylene chloride and shaking for 10 min; (9) equimolar *N,N*-dicyclohexylcarbodiimide in 6 ml of methylene chloride was added and shaking was continued overnight; (10) the coupling steps were termi-

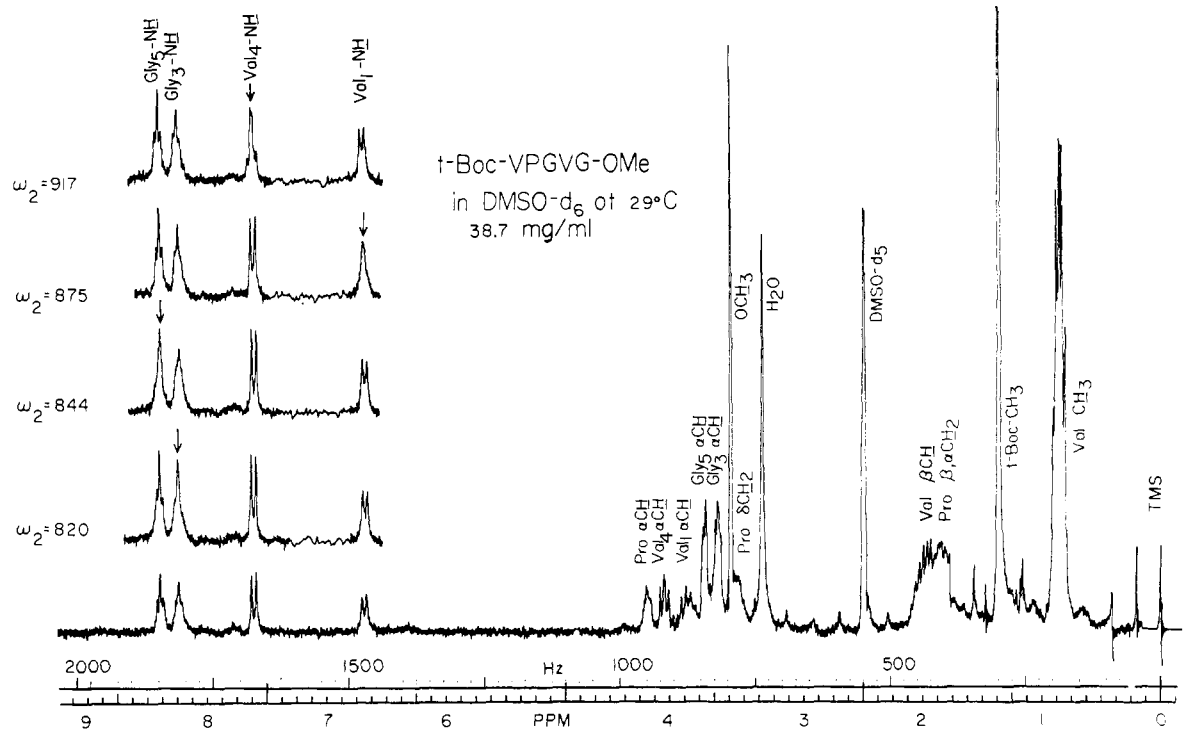

FIGURE 1: Proton magnetic resonance spectra of *t*-Boc-L-Val-L-Pro-Gly-L-Val-Gly-OMe at 220 MHz and 29° in dimethyl-d_6 sulfoxide. Tetramethylsilane (TMS) was used as an internal standard. All proton resonances are assigned. Also included are typical double-resonance experiments which demonstrate the clarity of the decoupling experiments and serve also to provide assignments for the α-CH protons.

nated by three washings with 20-ml portions of dimethylformamide.

After completion of the last coupling cycle, the Boc-decapeptide-resin was dried *in vacuo* (yield, 3.88 g).

VIII. H-(Val-Pro-Gly-Val-Gly)$_2$-OH. The above Boc-(Val-Pro-Gly-Val-Gly)$_2$-resin (1.94 g) was suspended in 20 ml of absolute trifluoroacetic acid in the cleavage vessel. Anhydrous hydrogen bromide gas was bubbled into this suspension for 90 min. The resin was filtered and washed three times with 5-ml portions of trifluoroacetic acid. The combined filtrates were evaporated *in vacuo* and the remaining oil was chromatographed on a column of Sephadex G-15 (4.5 × 90 cm) with pyridine–acetic acid buffer pH 7.0. The pure N,C free decapeptide fraction was collected and lyophilized: yield, 300 mg (71.5%); R_F(b) 0.58; mp 163–165°. *Anal.* Calcd for $C_{38}H_{64}N_{10}O_{11}\cdot H_2O$: C, 53.38; H, 7.78. N, 16.37. Found: C, 53.91; H, 7.78; N, 16.16.

IX. HCO-(Val-Pro-Gly-Val-Gly)$_2$-OH. To a solution of 100 mg of H-(Val-Pro-Gly-Val-Gly)$_2$-OH in 10 ml of 97% formic acid was added 3 ml of acetic anhydride in dropwise fashion at 0–5°. The reaction mixture was stirred for 2 hr at room temperature after the addition was complete. Five milliliters of ice water was added thereto and the solution was concentrated to dryness under vacuum. The powder obtained was used in the next step without further purification: R_F(b) 0.75; mp 114–117°.

X. HCO-(Val-Pro-Gly-Val-Gly)$_2$-OMe. The HCO-(Val-Pro-Gly-Val-Gly)$_2$-OH (86 mg) was dissolved in methanol (10 ml), and the solution was cooled in an ice bath. Ethereal diazomethane was then added until the solution turned yellow and the color remained for 15 min. Excess diazomethane was destroyed by addition of a few drops of glacial acetic acid and the solution was evaporated to dryness at 40°. The resulting powder was washed with ether to give a white solid. This solid was dissolved in methanol and purified further on a column of Sephadex LH-20 (1 × 20 cm) using methanol as eluent: yield 70 mg (80%); R_F(b) 0.85; mp 134–137°. *Anal.* Calcd for $C_{40}H_{66}N_{10}O_{12}\cdot CH_3OH$: C, 54.05; H, 7.74; N, 15.36. Found: C, 54.04; H, 7.46; N, 14.51. Amino acid analysis: Pro, 1.07; Gly, 2.0; Val, 2.04.

XI. H-(Val-Pro-Gly-Val-Gly)$_3$-OH. The polypeptide-resin, Boc-(Val-Pro-Gly-Val-Gly)$_3$-resin, was synthesized from the rest of the Boc-decapeptide-resin (1.94 g) by one more reaction cycle with Boc-Val-Pro-Gly-Val-Gly-OH, the resin being treated as had been described above (VIII): yield 450 mg (72%); R_F(b) 0.52; mp 168–170°. *Anal.* Calcd for $C_{57}H_{95}N_{15}O_{16}\cdot H_2O$: C, 54.14; H, 7.84; N, 16.60. Found: C, 54.49; H, 7.29; N, 16.31.

XII. HCO-(Val-Pro-Gly-Val-Gly)$_3$-OMe. H-(Val-Pro-Gly-Val-Gly)$_3$-OH (400 mg) was treated the same as described in IX: yield, 420 mg; R_F(b) 0.65; mp 135–137°. HCO-(Val-Pro-Gly-Val-Gly)$_3$-OH (380 mg) was treated the same as described in X: yield, 300 mg (81.5%); R_F(b) 0.82; mp 144–147°. *Anal.* Calcd for $C_{59}H_{97}N_{15}O_{17}\cdot CH_3OH$: C, 54.53; H, 7.71; N, 15.91. Found: C, 55.24; H, 7.21; N, 15.34. Amino acid analysis: Pro, 1.15; Gly, 2.0; Val, 2.1.

Results

The complete proton magnetic resonance spectrum, including double resonance followed in the peptide region, is given in Figure 1 for *t*-Boc-L-Val-L-Pro-Gly-L-Val-Gly-OMe. The peptide region contains four resonances, two doublets and two triplets for the two valine and two glycine residues, respectively. Further assignment of the resonances is achieved by delineating the terminal residues. The high-field resonance is due to the amino-terminal residue. This is readily demonstrated, for removal of the *t*-Boc-protecting group results in a loss of the resonance at 1498 Hz (20°) and appearance of a two proton resonance at 1734 Hz (20°). On removal of the methyl

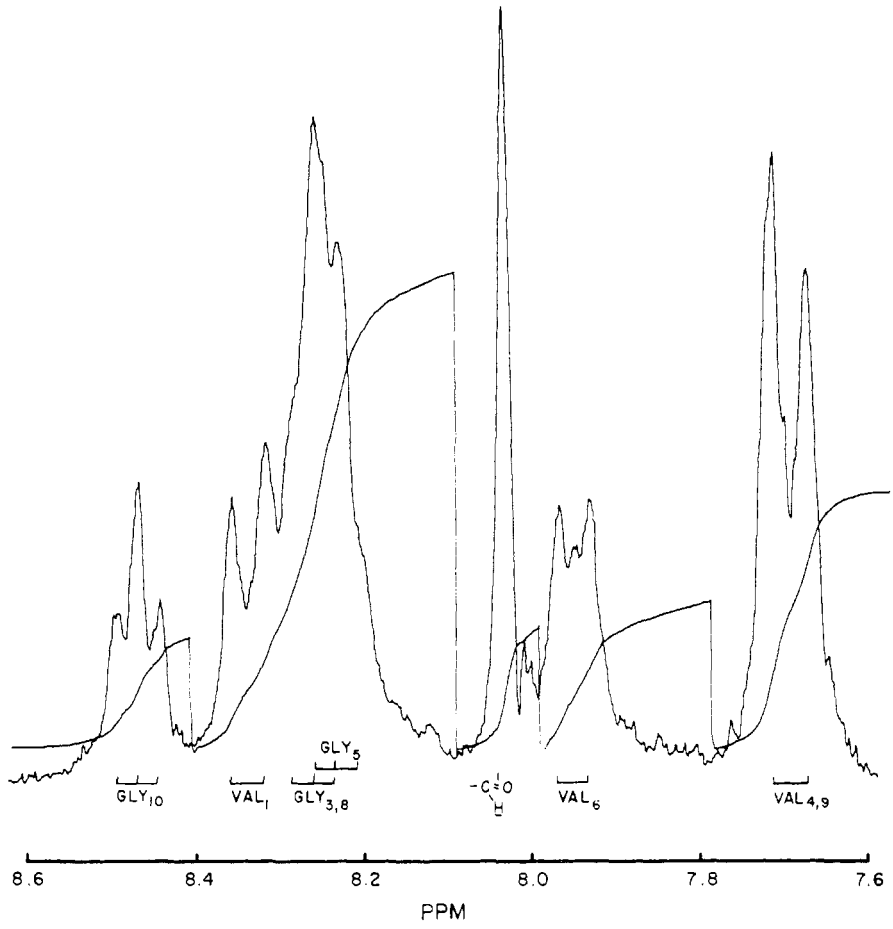

FIGURE 2: Peptide proton region at 220 MHz of *N*-HCO-(L-Val-L-Pro-Gly-L-Val-Gly)$_2$-OMe at 20° in dimethyl-d_6 sulfoxide. The chemical shifts are given in parts per million with an internal standard of tetramethylsilane at zero. See text for basis of assignments.

ester the lowest field glycine resonance shifts upfield and overlaps with the second glycine resonance. Accordingly the triplets at 1863 Hz (20°) and 1827 Hz (20°) are glycines-5 and -3, respectively; and the doublets at 1498 Hz (20°) and 1689 Hz (20°) are valines-1 and -4, respectively. Also included in Figure 1 are the decoupling experiments allowing assignment of the α protons of the glycine and valine residues. The proline assignments were assisted by the studies of McDonald and Phillips (1969). The OCH_3 of the ester and the methyls of the *t*-Boc groups were noted by their appearance on blocking of the end groups.

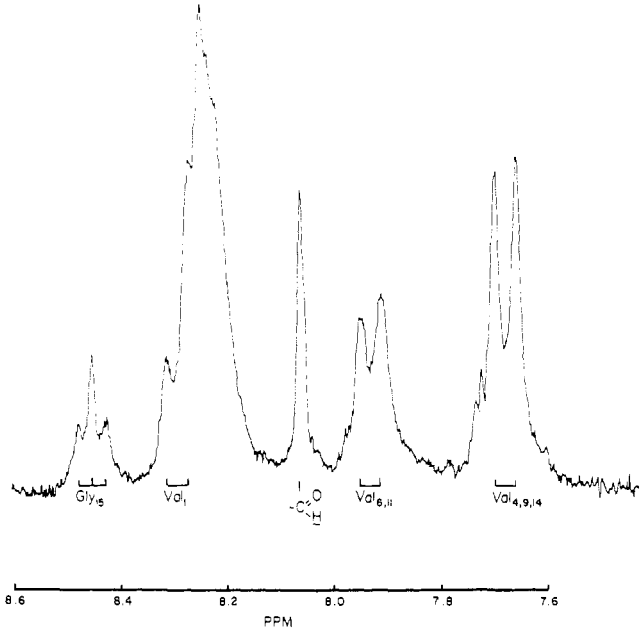

FIGURE 3: Peptide proton region at 220 MHz of the pentadecapeptide, *N*-HCO-(L-Val-L-Pro-Gly-L-Val-Gly)$_3$-OMe, in dimethyl-d_6 sulfoxide at 31°. The chemical shifts are given in parts per million with an internal standard of tetramethylsilane as the zero reference. Assignments of the valine residues and carboxy-terminal glycine are given, as well as the *N*-formyl proton.

The peptide region of the decapeptide *N*-HCO-(L-Val-L-Pro-Gly-L-Val-Gly)$_2$-OMe is given in Figure 2. The lowest field resonance is assignable to residue 10 by its appearance on formation of the methyl ester, having shifted from a higher field position in the carboxyl free peptide, and by the nearly identical position of the carboxyl-terminal residue in the esterified pentapeptide. The doublet at 8.35 ppm, 1837 Hz (20°), appears on formylation of the amino-free decapeptide having shifted from 7.88 ppm, 1734 Hz (20°). It is assigned to residue 1, the terminal valine. Also the formyl singlet at 8.04 ppm, 1769 Hz (20°), appears in its characteristic position on formylation of the amino terminus. The doublet of two protons at 7.69 ppm, 1693 Hz (20°), is within 4 Hz of the position of valine-4 in the pentapeptide and is therefore taken to be overlapping valine residues 4 and 9 resonances. The remaining doublet at 7.95 ppm, 1749 Hz (20°), is assigned to valine-6. The resonances of the remaining three glycines are overlapping near 8.25 ppm, 1815 Hz (20°).

The preceding assignments for the decapeptide are substantiated by the pattern of peptide resonances for the pentadecapeptide (see Figure 3). A single low-field triplet is ob-

TABLE I: Temperature Dependence of Peptide Proton Chemical Shift of (VPGVG) Oligomers in Me_2SO-d_6.[a]

Peptide Residue	*t*-Boc-(VPGVG)-OMe		*N*-HCO-(VPGVG)$_2$-OMe		*N*-HCO-(VPGVG)$_3$-OMe	
	Slope (Hz/10°)	0° Intercept	Slope (Hz/10°)	0° Intercept	Slope (Hz/10°)	0° Intercept
Val_1	−23.32[b]	7.03[b]	−13.2[d]	8.46[d]	−14.08[d]	8.49[d]
Val_6			−14.08	8.08	−14.3	8.13
Val_{11}					−14.3	
Gly_3	−10.34	8.39	−10.34	8.36	−11.0	8.39
Gly_8			−10.34	8.36	−11.0	
Gly_{13}					−11.0	
Val_4	−9.68	7.77	−9.68	7.78	−9.68	7.81
Val_9			−9.68	7.78	−9.68	
Val_{14}					−9.68	
Gly_5	−12.76[c]	8.58[c]	−10.34	8.33	−11.0	8.39
Gly_{10}			−12.54[c]	8.59[c]	−11.0	
Gly_{15}					−12.76[c]	8.62[c]

[a] Temperature range 20–70°. [b] This residue is displaced due to the *t*-Boc derivative. [c] This residue is the methylated carboxyl terminus. [d] This residue is the formylated amino terminus.

served at 8.51 ppm, 1872 Hz (20°), a doublet of two protons is observed at 8.00 ppm, 1759 Hz (20°), and a doublet of three protons is seen at 7.73 ppm, 1700 Hz (20°); these are the terminal glycine (residue 15), valine residues 6 and 11, and valine residues 4, 9, and 14, respectively. The terminal valine, residue 1, is at 8.36 ppm, 1839 Hz (20°). The terminal residue assignments were confirmed by comparison of the blocked, formylated, and esterified, with the free amino and carboxyl, pentadecapeptides.

The temperature dependences of peptide proton chemical shifts for the three peptides are given in Table I for dimethyl sulfoxide and in Table II for methanol. Equivalent residues (with the exception of the terminal residues) are seen to have very closely the same chemical shift (0° intercept) and temperature coefficient. This further substantiates the assignments and demonstates the equivalence of the residues in repeating units. The terminal residues, formylated and esterified, also show constancy between the different oligomers. Note the low-temperature coefficient (slope, Hz/10°) of the equivalent valine residues at positions 4, 9, and 14. In methanol, in particular, these residues have a substantially smaller temperature coefficient.

Solvent mixture studies, methanol–trifluoroethanol, are plotted in Figure 4 for the decapeptide. Whereas most of the peptide proton resonances shift dramatically upfield on addition of trifluoroethanol to a methanol solution, the peptide proton resonances of valine residues 4 and 9 show only a very small upfield shift. The pattern is the same with the pentadecapeptide; valine residues 4, 9, and 14 shift upfield by less than 20 Hz whereas the peptide proton resonances of all other residues shift upfield by from 80 to 120 Hz.

Thus two methods, temperature dependence and methanol–trifluoroethanol solvent mixture dependence of peptide proton chemical shift, delineate peptide protons of residues 4, 9, and 14 as being shielded from the solvent.

Discussion

In the previous section the peptide protons of valine residues 4, 9, and 14 were delineated from those of valine residues 1,

TABLE II: Temperature Dependence of Peptide Proton Chemical Shift of (VPGVG) Oligomers in Methanol.

Peptide Residue	*t*-Boc-(VPGVG)-OMe		*N*-HCO-(VPGVG)$_2$-OMe		*N*-HCO-(VPGVG)$_3$-OMe	
	Slope (Hz/10°)	0° Intercept	Slope (Hz/10°)	0° Intercept	Slope (Hz/10°)	0° Intercept
Val_1	−22.66[a]	6.98[a]	−11.66	8.61	−12.98	8.6
Val_6			−15.18	8.19	−17.16	8.3
Val_{11}					−17.16	
Val_3	−15.62	8.71	−13.42	8.56	−11.88	8.54
Val_8			−13.42	8.59	−11.88	
Val_{13}					−11.88	
Val_4	−9.90	8.03	−10.78	8.02	−9.9	8.06
Val_9			−9.9	8.06	−9.9	
Val_{14}					−9.9	
Gly_5	−15.62[b]	8.76[b]	−12.10	8.51	−12.10	8.7
Gly_{10}			−11.88	8.67[b]	−12.10	
Gly_{15}					−12.10[b]	

[a] This residue is displaced due to the *t*-Boc derivative. [b] This residue is the carboxyl terminus.

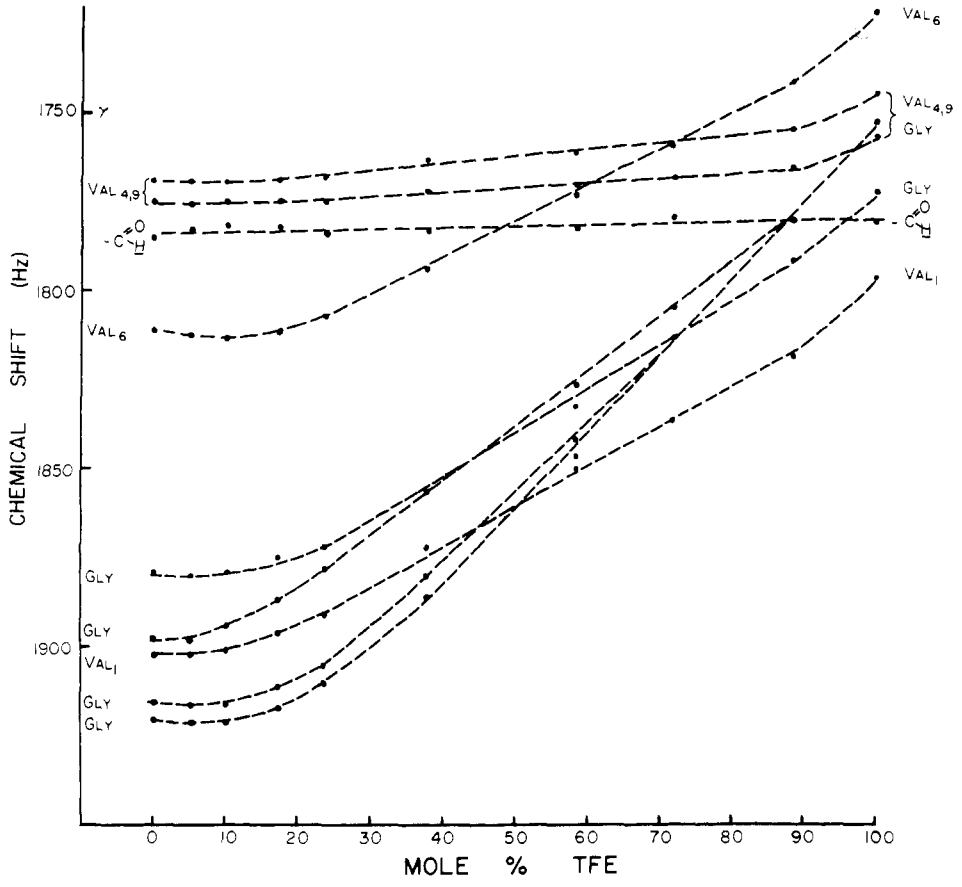

FIGURE 4: Methanol-trifluoroethanol solvent titration following the chemical shifts of the peptide protons of the decapeptide, *N*-HCO-(L-Val-L-Pro-Gly-L-Vla-Gly)$_2$-OMe. The chemical shifts, taken at 220 MHz, are given relative to a zero of a tetramethylsilane internal standard. Note that peptide protons of valine residues 4 and 9 and the formyl proton show little dependence on the solvent mixture whereas all the remaining resonances exhibit dramatic upfield shifts on addition of trifluoroethanol (TFE) to a methanol solution.

6, and 11 and from those of the glycine residues by their dependence of chemical shift on temperature and on methanol–trifluoroethanol solvent mixtures. While in the past such delineation has been interpretable in general as evidence for preferred conformation and in particular as a means of indicating secondary structure, there exists the question in any particular case whether or not simply sequence position might be responsible. This point is pertinent in the delineation that was observed for the fourth residue of the pentapeptide as seen in the monomer, dimer, and trimer. The question may be resolved by a means which would result in equivalence of chemical shift for all valines. This can be achieved by raising the temperature to 94° in dimethyl sulfoxide (see below). It is also resolved by studies of *N*-HCO-Gly-L-Val-Gly-OMe wherein the valine residue exhibits a temperature coefficient of 13.6 Hz/10° in Me_2SO-d_6. Furthermore the valine-4, -9, and -14 peptide proton resonances are at 1709 Hz whereas that of the valine of the tripeptide is at lower field, 1784 Hz. The higher field position in the pentapeptide would be consistent with β-turn formation resulting in shielding of the valyl NH by the end peptide of the β turn.

The temperature coefficients (slope, Hz/10°) listed in Table I for the pentadecapeptide were obtained by a least-squares fit of six data points taken at 20, 31, 40, 50, 60, and 68°. The fit of the 20° data point and the 68° data point, as well as all other points, was equally close, that is, well within ±2 Hz. A calculation, utilizing the temperature coefficients in Table I, extrapolates the chemical shifts at 94° to be 7.52 ppm for valine residues 6 and 11, 7.88 ppm for valine residue 1, and to be 7.40 ppm for valine residues 4, 9, and 14. Glycine residues 3, 8, 13, 5, and 16 calculate to occur at 7.92 ppm and the carboxyl glycine to be at 8.07 ppm. The peptide proton spectrum of the pentadecapeptide at 94° in Me_2SO-d_6 shows only two peptide proton peaks of equal intensity. The glycine residues occur at 7.92 ppm and the valine residues overlap at 7.45 ppm. The equivalence of the valine residues at 94° also shows that the chemical shift differences at low temperature are not due to sequence differences and that the differences observed at lower temperatures are the result of a preferred conformation in the pentamer that repeats twice in the decapeptide and three times in the pentadecapeptide.

The delineation of peptide protons indicated in Tables I and II and in Figure 4 provide information on the preferred conformation of these linear peptides. In previous studies on valinomycin (Urry and Ohnishi, 1970; Ohnishi and Urry, 1969), gramicidin S (Urry and Ohnishi, 1970; Ohnishi and Urry, 1969; Pitner and Urry, 1972), oxytocin (Urry *et al.*, 1970; Urry and Walter, 1971) and cyclic hexapeptides (Kopple *et al.*, 1969; Torchia *et al.*, 1972a,b), interpretation of the temperature dependence and methanol–trifluoroethanol solvent mixture dependence to indicate intramolecular hydrogen bonding of the delineated peptide protons has been substantiated or confirmed with X-ray crystallographic studies (Pinkerton *et al.*, 1969; Hodgkin and Oughton, 1957; Karle and Karle, 1963; Rudko *et al.*, 1971). Specifically, similar studies on oxytocin led to the proposal of a β turn involving the linear tail sequence of oxytocin. The conformation of the oxytocin carboxy-terminal tetrapeptide as determined by X-

ray crystallography (Rudko *et al.*, 1971) corresponds in detail to the previously proposed solution conformation for this sequence (Urry and Walter, 1971). The oxytocin work is particularly relevant to the present study as a proline occurs at the corner of a β turn in a manner similar to that which is obtained in the most direct interpretation of the foregoing results (see Figure 5). While the proposed conformation of the tail of oxytocin was the first time in the solution studies that proline had been placed in this position ($i + 1$ of Figure 5) of a β turn, recent work by Blout and coworkers (Pease *et al.*, 1974) finds position $i + 1$ of Figure 5 to be preferred for proline in *cyclo*(Pro-Gly-Gly)$_2$ over a conformation where the proline residue is in position $i + 2$.[1]

Solvent shielding of the peptide proton of residue 4 in the pentapeptide is most readily interpreted in terms of intramolecular hydrogen bonding. Solvent occluding this peptide proton by other means is not likely in such a small linear peptide. The effect of amino acid sequence was ruled out as above discussed. Consideration of each of the potential acyl oxygens would result in 5-, 7-, 8-, and 10- and 13-atom hydrogen-bonded rings. The five-membered ring would not provide adequate shielding over a substantial temperature range, *e.g.*, at 68°. The 13 atom hydrogen-bonded ring is the hydrogen-bonding pattern of the α helix. This results in an α-CH–NH dihedral angle with small coupling constants about 2–3 Hz. The large valine and glycine coupling constants preclude this conformation. The shielding occurs unaltered even when the carboxyl group is not esterified. This discredits the eight-membered hydrogen-bonded ring which would involve the carboxyl acyl moiety. The large coupling constants of the valine residues and the relatively high-field position of valine residues 4, 9, and 14 are consistent with the formation of a 10-atom hydrogen-bonded ring or β turn, and would not argue for the seven-membered hydrogen-bonded ring. Thus, it is not unlikely that the conformation indicated in Figure 5 represents a repeating conformational feature.

The argument for a regularly repeating β turn in the repeating pentapeptide of elastin has particular significance in considering regular conformations which could occur with this conformational feature. The β turn allows a polypeptide chain to fold back on itself and form the antiparallel-β-pleated sheet conformation. This is also referred to as the cross-β-conformation. A repeating β turn, when resulting in the formation of a cross-β-conformation, requires an even number of residues in the repeating unit. Accordingly, the repeating pentapeptide of elastin cannot form a cross-β-structure. In addition, the presence of a proline residue would prevent one of the intrachain hydrogen bonds between antiparallel chains in a cross-β-structure.

While, for the above reasons, a repeating pentapeptide could not form a cross-β-structure, the β turn of the third pentapeptide unit could turn back on top of the β turn of the first in the development of a spiral-like structure. We have termed this structure a β spiral and had first proposed it in relation to a molecular theory of voltage-dependent, ion-conducting channels (Urry, 1972). In the β spirals there can be a stacking of β turns but there need not be an even number

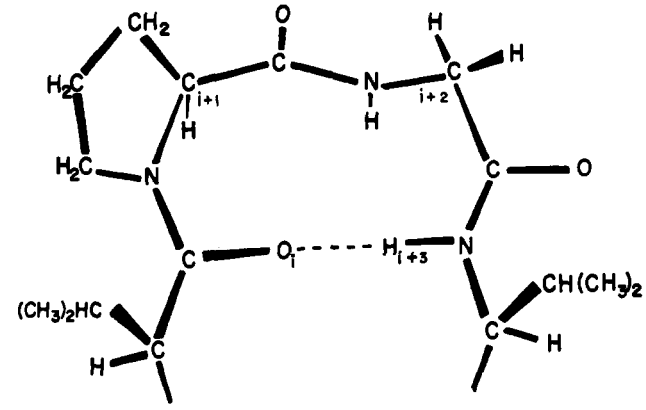

FIGURE 5: Proposed β turn for the repeating pentapeptide of elastin.

of β turns per turn of spiral. A repeating pentapeptide could have approximately two β turns per turn of spiral as would occur with the β_2[10] spiral and have hydrogen bonding between the end peptides of stacked β turns. From a side view, as contrasted with the top or spiral axis view, the structure would have a bellows or accordion-like appearance. This structure could be of interest relative to the elastic properties of elastin; however, more work is required to provide an adequate basis for such a proposal.

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[1] In the oxytocin example it should be appreciated that residues $i + 1$ and $i + 2$ are both L-amino acids giving a type I β turn. In the pentapeptide presented here and in *cyclo*(Pro-Gly-Gly)$_2$ residue $i + 2$ is glycine giving a type II β turn. The two types of β turns, of course, differ by the orientation of the end peptide. The point made above is that in all three cases—oxytocin, *cyclo*(Pro-Gly-Gly)$_2$, and the pentapeptide—residue $i + 1$ is L-proline, whereas in gramicidin S L-proline forms residue $i + 2$ of the β turn.

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Interaction of Deoxyribonucleic Acid with Histone f2b and Its Half-Molecules. Circular Dichroism Studies†

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ABSTRACT: The moderately lysine-rich histone, f2b (IIb2), from calf thymus was combined with homologous DNA by means of gradient dialysis to form reconstituted complexes at physiological salt concentration. The circular dichroic spectra of these complexes are characterized by a positive band at 272 nm which is blue-shifted and greatly enhanced relative to the corresponding band for native DNA. This result indicates that the conformational change induced in DNA upon binding of histone f2b is somewhat similar to that caused by combination with f2a1, but is very different from the structure of f1–DNA complexes. f2b was cleaved by cyanogen bromide to form half-molecules of approximately equal size, but with the N-terminal half much richer in basic amino acids than the C-terminal fragment. DNA complexes prepared with the N-terminal polypeptide exhibited circular dichroism changes similar in type to but smaller in magnitude than the distortions caused by intact f2b. The C-terminal half-molecule had a reduced binding affinity for DNA and failed to effect significant change in DNA circular dichroism upon complexation. These findings present additional evidence that the two ends of unevenly charged histone molecules interact differently with DNA. When complexes were reconstituted containing both N- and C-terminal fragments, the C-terminal half-molecule was capable of inhibiting the circular dichroism change caused by the N terminus. This antagonism is not found in the intact f2b molecule, where the C segment appears to be necessary to produce maximum circular dichroism change, indicating that the intact molecule is necessary to produce the significant histone conformation.

The histones are a set of basic proteins which are found combined with DNA in cell nuclei of higher organisms. Histones appear to stabilize the supercoiled, condensed structure of nuclear DNA (Pardon and Wilkins, 1972). They may, in addition, function as gross regulators of transcription (Elgin *et al.*, 1971), perhaps due to their effect upon the conformation of DNA. The interaction between histones and DNA is dominated by electrostatic forces; in chromatin the histones contain sufficient basic amino acid residues to neutralize nearly all the negative charges on the ionized DNA phosphates. However, if any specificity between DNA and histones exists, other interactions must play a controlling role.

This laboratory has been utilizing circular dichroism as a method of assessing conformational variations in calf thymus DNA brought about by interaction of the DNA with homologous histones. Histone–DNA complexes reconstituted with histone fractions f1 (Fasman *et al.*, 1970), f2a1 (Shih and Fasman, 1971), mixtures of f1 and f2a1 (Shih and Fasman, 1972), and f1 fragments (Fasman *et al.*, 1971) have been examined, and show that the type of circular dichroic distortion observed depends upon the nature of the histone. In the present study this series is extended to histone f2b and its half-molecules, with all complexes formed in 0.14 M NaCl.

Histone fraction f2b (IIb2) is slightly lysine-rich and contains much serine. The amino acid sequence of calf thymus f2b has been determined by Iwai *et al.* (1970). [Details of the sequencing procedure can be found in a series of papers by the same group: Ishikawa *et al.* (1972), Hayashi and Iwai (1972), and Iwai *et al.* (1972).] The protein contains 125 amino acid residues, has a mol wt of 13,800 and has a lysine : arginine ratio of 2.5. Tissue and species differences appear to be negligible (Hnilica, 1966). f2b, because of its high positive charge density, is randomly coiled in water, but acquires considerable secondary structure as the ionic strength of the medium is raised (Bradbury and Rattle, 1972; Bradbury *et al.*, 1972; D'Anna and Isenberg, 1972); concurrently there is increased aggregation of the histone (Edwards and Shooter, 1969a, 1970; Bradbury and Rattle, 1972). Histone f2b is also capable of interacting with fractions f2a1 (D'Anna and Isenberg, 1973) and f2a2 (Skandrani *et al.*, 1972; Kelley, 1973).

The distribution of the basic amino acid residues in f2b is very uneven (Iwai *et al.*, 1970). In the first 34 residues from the amino terminus there are 12 lysines and 3 arginines (occurring in clusters of 2–5), in addition to 4 prolines (which would be expected to assist the high charge density in preventing α-helical structure). In contrast, the next segment of 68 residues (35–102) contains only 4 lysines, 5 arginines, and 1 proline (with no basic clusters), and has an amino acid composition like that of a typical enzyme, with many hydrophobic residues.

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