Biologically Active Arg-Gly-Asp Oligopeptides Assume a Type II β-Turn in Solution†

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ABSTRACT: The sequence Arg-Gly-Asp (RGD) has been found to be the consensus sequence of matrix proteins for binding cell surface receptors (integrins). Studies with synthetic peptides containing the RGD sequence show that the biological activity of these oligopeptides is removed upon a conservative substitution of Glu for Asp in the RGD sequence. Two-dimensional ^{1}H NMR methods were used to investigate the secondary structures in aqueous solution for two such oligopeptides of differing biological activity. The sequence Tyr-Gly-Arg-Gly-**Asp**-Ser-Pro, which binds to selected integrins, is found to assume a type II β-turn at both pH 4 and 7. In contrast, the sequence Tyr-Gly-Arg-Gly-**Glu**-Ser-Pro, which does not interfere with integrin-mediated cell attachment, is found to assume a type I or III β-turn at both pH 4 and 7. This comparison confirms not only that oligopeptides can assume a secondary structure in aqueous solution, but also that these structures may be important to biological functions.

Substrate-specific adhesion contributes to modulation of cell behavior (Edelman, 1983). Many cell types interact with extracellular matrix through membrane-spanning receptors called integrins (Hynes, 1987) that bind to several proteins (e.g., fibronectin, vitronectin, laminin, and collagens) (Ruoslahti et al., 1981; Hayman et al., 1983; Carlsson et al., 1981; Kleinman et al., 1981). The sequence Arg-Gly-Asp (RGD) has been shown to be the consensus sequence for matrix protein binding of these integrins. In fibronectin, this tripeptide exists within the sequence GRGDSP, which has been suggested to comprise a β-turn (Pierschbacher & Ruoslahti, 1984a). The type of β-turn is determined by the ϕ and ψ angles of residues 2 and 3 in the turn (Richardson, 1981).

Coating tissue culture surfaces with oligopeptides containing RGD supports cell adhesion. Furthermore, a solution of RGD oligopeptides was found to inhibit the attachment of cells to a surface coated with adhesive proteins possessing the RGD recognition sequence (Pierschbacher & Ruoslahti, 1984a,b). Conservative changes in the RGD sequence, such as substitution of Glu (E) for Asp (D) to produce the sequence Arg-Gly-Glu (RGE), virtually obliterate the binding properties of RGD oligopeptides (Pytela et al., 1985; Basson et al., 1990).

Two-dimensional ^{1}H NMR methods are a powerful way to investigate the conformation of moderately sized biological molecules in solution (Wüthrich, 1986), and recently a number of laboratories have used the technique to investigate RGD proteins and oligopeptides. Echistatin, with 49 residues, is the smallest known natural protein with RGD activity, and four different laboratories (Saudek et al., 1991a,b; Dalvit et al., 1991; Cooke et al., 1991; Chen et al., 1991) have used 2D ^{1}H NMR to investigate its structure. All agree that it can be described as a bundle of loops. Disulfide bonds and hydrogen bonding stabilize the bulk of the protein, but the RGD sequence is believed to be at the tip of a mobile hairpin. The hairpin is two extended antiparallel strands with no regular β-structure. Although cross-peaks corresponding to four β-turns in the bulk of echistatin are obvious in the NOESY data, there is no indication of a β-turn at the ARGD sequence.

Kristin is a 68-residue protein that inhibits platelet aggregation and contains an RGD binding site. Its structure, as determined by 2D ^{1}H NMR, is in many ways similar to that of echistatin. It is described as a tightly packed bundle of loops with six disulfide bonds and has the RGD sequence at the tip of a hairpin (Adler et al., 1991; Adler & Wagner, 1992). Lack of spectral resolution at the G of RGD contributes to uncertainty of the conformation for the site.

Several conformationally constrained RGD peptides have been investigated. Bogusky et al. (1992) used 2D ^{1}H NMR to study CRGDC constrained by a disulfide bond, which inhibits fibrinogen binding, as well as the weak inhibitor sequence with D-Asp, in water and in deuterated dimethyl sulfoxide (DMSO-d_6). There was no indication of intramolecular hydrogen bonding in DMSO-d_6 on the basis of temperature coefficients, but many NH–NH NOESY cross-peaks were observed. Distance constraints derived from the NOESY data in DMSO-d_6 were used in molecular modeling, and no single structure could explain the data. For the CRGDC peptide, two low-energy conformers in fast exchange on the NMR time scale, best described as a type VII β-turn that does not involve a hydrogen bond, were derived. The results suggest a salt bridge between the Asp carboxylate and the Arg guanidinium group.

Aumailley et al. (1991) investigated two cyclic pentapeptides containing RGD that are strong inhibitors of vitronectin and laminin fragment P1 binding, using 2D ^{1}H NMR with DMSO as the solvent. On the basis of molecular modeling, they conclude that both pentapeptides assume an all-trans con-

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formation with a β-II′ and γ-turn. They suggest that the active form has a hydrogen bond between the Gly amide and the Phe carbonyl in both cases.

Siahaan et al. (1989) studied c-(2–9) G penicillamine GRGDSPCA by 2D ^{1}H NMR in DMSO and molecular modeling. Their NOESY data clearly show NH–NH cross-peaks typical of a type I (or III) β-turn at the GRGD sequence. The molecular dynamics simulations suggest consecutive type I β-turns around G5–D6 and D6–S7. However, this peptide has a 10^4-fold higher affinity for the vitronectin receptor over the fibronectin receptor, while the peptide studied here binds strongly to fibronectin.

Linear peptides have also been studied. Two laboratories (Genest et al., 1989; Nègre et al., 1989) have studied GRGDSG hexapeptides, inhibitors of fibronectin binding, in DMSO-d_6 using ^{1}H ROSEY methods. NH–NH cross-peaks are observed between G3–D, D–S, S–G6, and G6–NHEt pairs. Both groups agree that molecular modeling based on the NMR data suggests an extended structure with a salt bridge between the Asp carboxylate and the Arg guanidinium.

Research most similar to this report involves linear peptides studied by 2D ^{1}H NMR in aqueous solution. Reed et al. (1988) investigated the hexapeptide GRGDSP at pH 7, and their NMR and molecular modeling suggest nested β-bands with a type III β-turn involving hydrogen bonding between G1 and D. Their conclusions were critically dependent on the $^3J_{CH}$ coupling constant between G3α_S and the R2 carbonyl carbon, which constrains the ϕ angle for G3. No corroborating evidence is found in their 2D ^{1}H NMR spectrum for the type III β-turn. Due to the overlap of the R2NH and G3NH resonances, an NOE between R2NH and G3NH indicative of a type I or III β-turn could not be monitored. Mikos et al. (1990) showed that the 1D results for GRGDSP are similar at pH 3.5. Hull et al. (1989) compared the corresponding nonbinding hexapeptide GKGESP and concluded that its structure in aqueous solution at pH 7 is similar to GRGDSP.

In contrast, Cachau et al. (1989) applied one- and two-dimensional ^{1}H NMR at low field and molecular modeling to conclude that the pentapeptide GRGDS is primarily a type II β-turn in the unusual RGDS position at pH 5.1.

Recently, the peptides Tyr-Gly-Arg-Gly-**Asp**-Ser-Pro (YGRGDSP) and Tyr-Gly-Arg-Gly-**Glu**-Ser-Pro (YGRGESP) were used to investigate the attachment of bovine aortic endothelial cells to fibronectin, laminin, and fibrinogen (Basson et al., 1990). The RGD-containing peptide inhibited attachments to these matrix proteins, while the RGE-containing peptide did not. These results, taken with the conservative nature of this substitution, suggest the possibility of a conformational difference between the two heptapeptides. In this work we use one- and two-dimensional ^{1}H NMR methods to investigate the structures of these two heptapeptides, both at pH 4 where the exchange rate of amide protons with H_2O is slow and at pH 7 in 50% acetone/H_2O where the mixed solvent and lower temperature slow the exchange rate. We observe amide–amide NOE's that indicate the presence of a type I (or III) β-turn in the RGE peptide, and a type II β-turn with the expected GRGD register in the RGD peptide. Furthermore, the contrast in structure between the RGD and RGE heptapeptides in aqueous solution shows that observed structures may be indicative of biological function.

MATERIALS AND METHODS

Synthetic Peptides. A Model 403A Applied Biosystems synthesizer (Applied Biosystems Inc., Foster City, CA) using the normal *tert*-butoxycarbonyl chemistry was employed to synthesize YGRGDSP and YGRGESP. Hydrogen fluoride methods with appropriate scavengers were used to deprotect and release the peptides from their solid-phase support. The peptides were purified on a reverse-phase HPLC with a Dynamax Macro C18 preparative column (Rainin Instrument Co., Inc., Woburn, MA). The gradient consisted of trifluoroacetic acid and acetonitrile, and residual trifluoroacetate was removed from the peptides by exchange for chloride salts. Amino acid analysis confirmed the composition of the peptides.

NMR Spectroscopy. The peptides were dissolved in a solvent mixture of 90% H_2O and 10% D_2O at a concentration of about 15 mg/mL. The pH was brought to 4.1 or 7.0 with sodium hydroxide. The 50% methanol/H_2O solutions were made by adding 0.5 mL of CD_3OD (MSD Isotopes, Rahway, NJ) to the pH 7 H_2O solutions. To make the 50% acetone/H_2O solutions, the 50% methanol/H_2O solutions were taken to dryness on a rotary evaporator. The resulting residue was dissolved in 0.25 mL of H_2O and 0.25 mL of acetone-d_3 (MSD Isotopes, Rahway, NJ) to obtain the 50% acetone/H_2O solutions.

The temperature dependence of the amide resonances at pH 4 was followed by acquiring one-dimensional spectra on a Bruker MSL 200-MHz NMR spectrometer using low-power presaturation of the H_2O resonance, a sweep width of 2000 Hz, 8K data points, and 64 transients. The spectra were acquired at 5-°C intervals from 5 to 50 °C. All other spectra were acquired on a Bruker AM 500-MHz NMR spectrometer.

The temperature dependence of the amide resonances at pH 7 was followed by acquiring one-dimensional spectra at 5-°C intervals from 5 to 25 °C using low-power presaturation of the H_2O resonance, a sweep width of 5000 Hz, 16K data points, and 16 transients.

All two-dimensional spectra were processed using the FTNMR or Felix programs (Hare Research, Inc., Woodinville, WA). Phase-sensitive COSY spectra are obtained from a 512 × 2048 data matrix, zero filled in the t_1 dimension to give equivalent resolution in both dimensions. The spectra were acquired at 25 °C with low-power presaturation of the H_2O resonance, a sweep width of 5000 Hz, and 32 transients per t_1 point. A sine bell filter was used in the t_2 dimension and a sine bell filter shifted by 45° was used in the t_1 dimension prior to Fourier transformation.

Phase-sensitive NOESY spectra were collected at 5 °C with low-power presaturation of the H_2O resonance, sweep width of 5000 Hz, and 64 transients per t_1 point. Between 352 and 512 FIDs of 4K data points each were acquired for each NOESY spectrum at pH 4. While mixing times of 500 ms proved most useful, spectra were collected using mixing times of 75 and 200 ms to show that the NOE cross-peaks were still building at 500 ms. A Lorentz–Gaussian function or a sine bell filter shifted by 30° was used in the t_2 dimension, and a sine bell filter shifted by 60° was used in the t_1 dimensions prior to Fourier transformation. The data were zero filled in the t_1 dimension to give equivalent resolution in both dimensions.

The NOESY sequence using solvent suppression through nonexcitation was created by substituting a "jump and return" (Plateau & Guéron, 1982) sequence for the final read pulse in the standard NOESY experiment. The spectra in 50% acetone/H_2O were collected at 5 °C using this sequence with low-power presaturation of the H_2O resonance, a sweep width of 5000 Hz, and 48 transients per t_1 point. Between 390 and 410 FIDs of 2K data points each were acquired for each spectrum. A mixing time of 500 ms and a delay of 125 μs between the two pulses of the "jump and return" sequence

Table I: Temperature Coefficients for the Amide Proton Resonances of YGRGDSP and YGRGESP at pH 4 and pH 7

	amide temperature coefficient, $-\Delta\delta/\Delta T \times 10^3$ (ppm/K)			
residue	YGRGDSP (pH 4)	YGRGDSP (pH 7)	YGRGESP (pH 4)	YGRGESP (pH 7)
2	9.2	8.0	9.7	8.0
3	9.8	*a*	9.4	*a*
4	9.9	6.0	9.4	8.6
5	5.2	2.0	5.4	2.0
6	8.2	5.4	9.4	6.6

[a] These resonances broaden and are unobservable above 10 °C.

were used. A sine bell filter shifted by 30° was used in the t_2 dimension and a sine bell filter shifted by 60° was used in the t_1 dimension prior to Fouier transformation. The data was zero filled in the t_1 dimension to give equivalent resolution in both dimensions.

RESULTS AND DISCUSSION

In general, there are three types of signatures from ^{1}H NMR spectra for the presence of β-turns (Dyson et al., 1988a). The first type is a decrease in the temperature dependence of the amide proton resonance at position 4 of the turn, indicating a 4→1 backbone amide–carbonyl hydrogen bond. The second type is the characteristic short proton–proton distances associated with a β-turn, which can be detected by NOESY spectra. The third type is the small value of the $^3J_{NH,\alpha}$ coupling constant for the residues at position 2 and/or 3 of the turn. However, these $^3J_{NH,\alpha}$ coupling constants are not much reduced from the extended chain values except for peptides in which the population of β-turn is very large (Dyson et al., 1988a). Therefore, the NOESY experiment proves to be the most decisive in recognizing the β-turn and determining the type of β-turn formed.

The presence of a β-turn in both YGRGDSP and YGRGESP is indicated by the temperature dependence of the amide proton resonances. At both pH 4 and pH 7, the amide resonances for aspartic acid in YGRGDSP and for glutamic acid in YGRGESP are both much less temeprature dependent that those of the other amides (Table I). This suggests the formation of a preferred conformation which, as suggested by sequence prediction methods (Pierschbacher & Ruoslahti, 1984a,b), could be a β-turn with the amide of the acidic residues hydrogen bonding with the carbonyl of G2. This would place the G4 residue in the third position of the β-turn, a feature that is common to β-turns. The temperature coefficients (Table I), determined from the slope of the temperature dependence of the amide proton of these acidic residues, indicate that the peptides are in a β-turn about 25% of the time at pH 4. This estimate is based on the work of Dyson et al. (1988a) using their following assumptions: (I) A temperature coefficient of -7.2×10^{-3} ppm/K in position 4 of the turn (the highest magnitude they obtained) represents the upper limit for a solvent-exposed amide proton in the unfolded peptide; (II) the temperature coefficient for a fully folded conformation in which the amide proton is hydrogen-bonded and protected from the solvent is 0 ppm/K; (III) there is a linear relationship between the population of conformers containing a β-turn and the temperature coefficient of the hydrogen-bonded amide proton at position 4 of the turn. The same determination cannot be accurately made at pH 7 since the first assumption is probably no longer valid; temperature coefficients will change with pH. There is also evidence that the amide proton of S6 in both peptides at pH 4 and 7 is involved in hydrogen bonding based on our results for line width behavior, and this was also demonstrated previously (Reed et al., 1988; Mickos et al., 1990; Hull et al., 1989).

The amide region of the NOESY spectra confirms the presence of the above-predicted secondary structures and reveals the conformational differences between the two oligopeptides. The ^{1}H resonances were assigned in the usual way using 2D NOESY and COSY spectra. We focused our attention only on the predominant resonances in both oligopeptides, which corresponded to the trans conformation of the proline residue. (Only in the case of the 50% acetone/H_2O spectrum of the RGD peptide were the resonances of the cis conformation of the proline residue of significant intensity. These are discussed below.) For both type I and type II β-turns, a strong $d_{NN}[3,4]$ NOE connectivity is expected together with a weaker $d_{\alpha N}[2,4]$ connectivity. The two types of turns can be distinguished by the nature of the NOE connectivity between the backbone protons of residues 2 and 3 in the turn; $d_{NN}[2,3]$ connectivities are expected for type I β-turns and $d_{\alpha N}[2,3]$ connectivities for type II β-turns (Wüthrich, 1986; Reed et al., 1988). However, it should be noted that for an extended chain conformation a $d_{\alpha N}[2,3]$ connectivity is also expected (Dyson et al., 1988a), so that connectivity is not a definitive secondary structure marker.

Wright and co-workers (Dyson et al., 1985; Dyson et al., 1988a,b) have pioneered the use of ^{1}H NMR techniques to show that certain oligopeptides have a preference for a recognizable secondary structure as part of the dynamically averaged structures seen in aqueous solution. Since their studies were carried out at pH 4, mainly to slow the exchange rate of amide protons with H_2O, we decided to compare the conformations of the active YGRGDSP peptide and the inactive YGRGESP peptide in aqueous solution at pH 4. From Figure 1, the NOE between G4NH and E5NH in the RGE peptide and the G4NH–D5NH NOE in the RGD peptide confirms the presence of a β-turn in both peptides at pH 4. The R3NH–G4NH cross-peak in the NOESY spectrum of the RGE peptide (Figure 1a) indicates the presence of a type I (or III) β-turn. The absence of the G4NH–R3NH cross-peak in the NOESY spectrum of the RGD peptide (Figure 1b) suggests the presence of a type II β-turn. A cross-peak between G4NH and R3α is seen for both peptides at pH 4 because 75% of the time both peptides are in an extended chain conformation.

To confirm that no change in the type of β-turn occurs when the pH is raised to neutral, NOESY experiments were performed on both the RGD and RGE peptides in aqueous solution at pH 7. A very weak G4NH–D5NH NOE in the RGD peptide and a very weak G4NH–E5NH NOE in the RGE peptide confirm the presence of a β-turn in both peptides. However, the R3NH–G4NH NOE indicative of a type I (or III) β-turn is absent in the spectra of both peptides. The other NH–NH NOEs are too weak to say with confidence that the absence of the R3NH–G4NH NOE is due to the existence of a type II β-turn in both peptides. In fact, the absence of this NOE in at least one of the spectra and the weak intensity of the G4NH–D5NH and G4NH–E5NH cross-peaks are probably due, in part, to the use of water presaturation in the NOESY experiment. From 1D experiments it was observed that the use of water presaturation causes the intensity of the R3NH, G2NH, and G4NH resonances to decrease significantly (approximately by a factor of 8) due to the exchange rate of these amide resonances with the saturated H_2O protons. To avoid this exchange problem, solvent suppression through nonexcitation (the 1–1 sequence) (Plateau & Guéron, 1982) was used in the NOESY sequence.

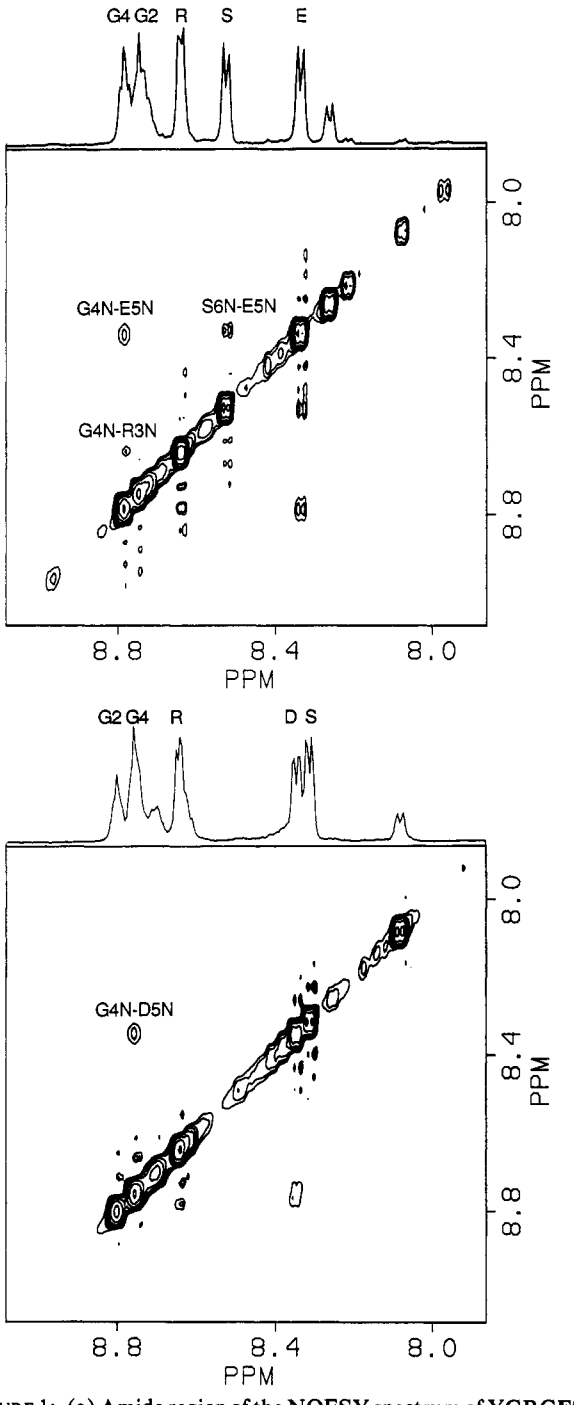

FIGURE 1: (a) Amide region of the NOESY spectrum of YGRGESP in 90% H_2O/10% D_2O at pH 4. The NOEs indicative of a type I or III β-turn, G4NH–E5NH and R3NH–G4NH, are shown. Also shown is the NOE indicative of a second β-turn, E5NH–S6NH. (b) Amide region of the NOESY spectrum of YGRGDSP in 90% H_2O/10% D_2O at pH 4. The NOE indicative of a β-turn, G4NH–D5NH, is shown. The absence of R3NH–G4NH NOE suggests the presence of a type II β-turn.

Although the 1D spectrum shows that these NH resonances are significantly more intense using this solvent suppression technique, in the 2D spectrum the diagonal peaks of these NH resonances practically disappear at mixing times of 500 (and even 200) ms, due to very effective competition with the cross relaxation rate from k_{ex}.

In order to slow down the amide exchange rates to enhance our ability to detect the R3NH–G4NH cross-peak, we investigated mixed solvent systems that would allow low-temperature measurements. The solvent system selected was 50% acetone/H_2O. Spectra were collected at 5 °C since at 0 °C the peaks began to broaden and the sample precipitated. At 5 °C the amide resonances were significantly sharper than in the aqueous solution. The NOESY sequence using solvent suppression through nonexcitation was employed to ensure that the intensity of the amide resonances, particularly R3NH and G4NH, was large enough for the presence or absence of the R3NH–G4NH NOE to be determined with confidence. The presence of a type I (or type III) β-turn a pH 7 is indicated by the R3NH–G4NH cross-peak in the NOESY spectrum of the RGE peptide (Figure 2a). The NOESY spectrum of the RGD peptide is complicated by a significant population (about 40%) of the peptide in which a *cis*-proline bond is present. However, as can be seen in Figure 2b, the G4NH resonance in both the *cis*- and *trans*-proline conformers shows no NOE to R3NH, consistent with the presence of a type II β-turn in the RGD peptide at pH 7. What may appear to be an NOE between R3NH and G4NH for the *trans*-proline conformer is dismissed on closer examination of the spectrum. This cross-peak does not match with the shift of G4NH as determined by the G4NH–D5NH NOE, and examination of the 1D spectrum (Figure 3) suggests the presence of another peak underneath the G4NH resonance.

The RGD oligomer that binds to cell integrins is drawn schematically as a type II β-turn in Figure 4. The type I β-turn for the RGE oligomer would have the amide in the center of the turn sterically reversed, with the CO group going into the page and the NH group out. Thus we have two oligopeptides, one of which binds to integrins and one of which does not. ^{1}H NMR indicates that the two oligopeptides have different secondary structures in aqueous solution.

Our results agree with those of Hull and co-workers (Hull et al., 1989) for the structure of peptides that do not bind to integrins. They find a type III (or I) β-turn in GKGESP as we find here for YGRGESP. However, we contradict this group (Reed et al. 1988) in the case of RGD peptides by showing the presence of a type II β-turn. They concluded that the very similar RGD peptide, GRGDSP, contains a type III β-turn. However, the whole basis for their claim that a type III turn was present in their RGD-containing hexapeptide came from one piece of data, the $^3J_{CH}$ coupling constant between G3α_S and the carbonyl carbon of R2. Their molecular dynamics calculations used the constraints of the ϕ and ψ angles only from R2 and G3 along with the presence of the 4→1 and 5→2 hydrogen bonds. No NOE constraints were used in their calculations. The ϕ angle constraint for R2 is consistent for a type I, II, or III β-turn; therefore, the type of turn is determined solely on the basis of the ϕ angle constraint for G3, which in turn comes from the $^3J_{CH}$ coupling constant between G3α_S and the carbonyl carbon of R2. Furthermore, the conversion of this coupling constant into a ϕ angle constraint depends on the correct stereospecific assignment of the G3α protons. If their assignments of Gα_S and Gα_R are interchanged, then the ϕ angle constraint agrees with the presence of a type II β-turn as found in our studies. Unfortunately, they have no corroborating evidence for the ϕ angle constraint either in the form of a $^3J_{NH,\alpha}$ coupling constant, since these couplings were not resolved due to the exchange rate of the amide protons with H_2O, or in the form of the NOE indicative of a type I or III β-turn due to the overlap of the R2NH and G3NH resonances. The conclusions of Reed et al. (1988), therefore, suggest to us either that the RGD peptide (in YGRGDSP) changes its type of β-turn by the addition of the N-terminal Y residue or that their interpretation of their data is incorrect.

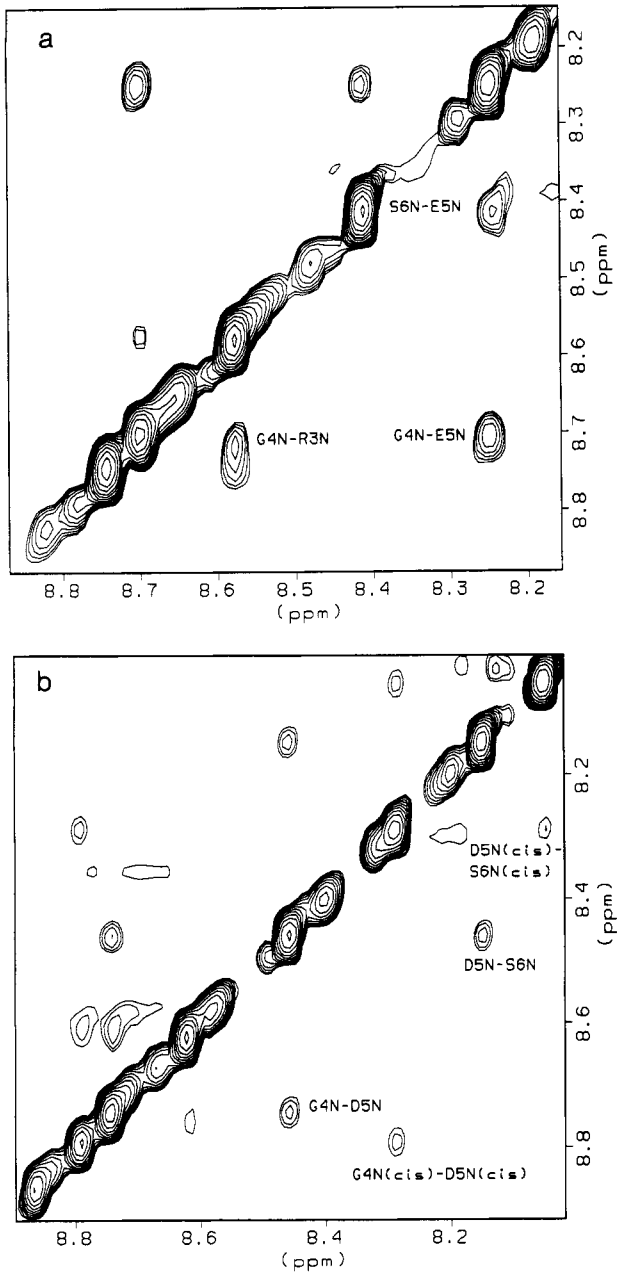

FIGURE 2: (a) Amide region of the NOESY spectrum of YGRGESP in 50% acetone/H_2O at neutral pH. The NOE indicative of a type I or III β-turn, R3NH–G4NH, is shown. Also shown is the NOE indicative of a second β-turn, E5NH-S6NH. (b) Amide region of the NOESY spectrum of YGRGDSP in 50% acetone/H_2O at neutral pH. The NOEs labeled "cis" are due to the conformation of the peptide in which a cis-proline bond is present. The other NOEs correspond to the conformation of the peptide in which a trans-proline bond is present. The NOEs indicative of a β-turn, G4NH–D5NH and G4NH(cis)–D5NH(cis), are shown. The absence of an R3NH–G4NH or an R3NH(cis)–G4NH(cis) NOE suggests the presence of a type II β-turn. Also shown are the NOEs indicative of a second β-turn, E5NH-S6NH and E5NH(cis)–S6NH(cis).

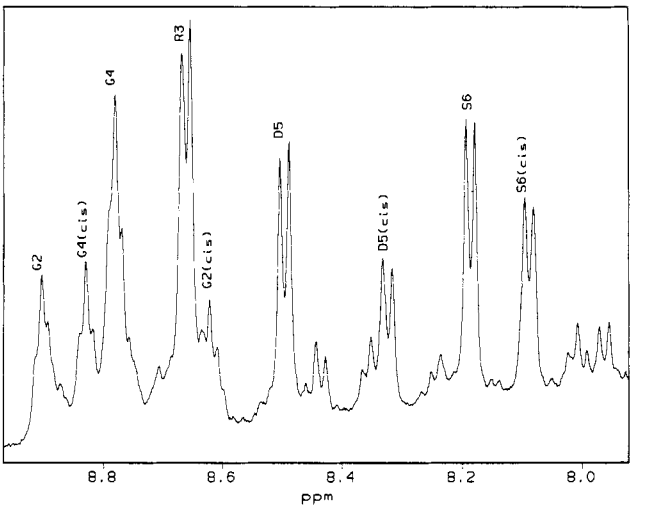

FIGURE 3: Amide region of the 500-MHz spectrum of YGRGDSP in 50% acetone/H_2O at neutral pH. The peaks labeled "cis" are due to the conformation of the peptide in which a cis-proline bond is present. The other labeled peaks correspond to the conformation of the peptide in which a trans-proline bond is present.

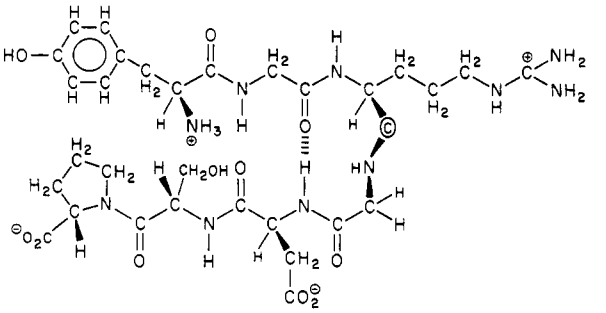

FIGURE 4: Schematic drawing of YGRGDSP as a type II β-turn.

In the NOESY spectra of both YGRGDSP and YGRGESP in H_2O at pH 4, and in 50% acetone/H_2O at neutral pH, there is evidence for the second β-turn discovered by Reed et al. (1988). This second turn involves the residues R3, G4, D5, or E5, and S6. On the basis of line width behavior there is evidence that the amide proton of S6 in both peptides is involved in hydrogen bonding, probably to the carbonyl of R3. An NOE between E5NH and S6NH in the RGE peptide and an NOE between D5NH and S6NH in the RGD peptide indicate the presence of the second turn in both peptides. However, this NOE is expected for a type I, II, or III β-turn. Therefore, the identity of the second turn cannot be determined from the NOESY spectra. It is possible that, as suggested by Reed et al. (1988) for GRGDSP, the RGE peptide contains two type III turns or a type III turn and a type I turn. For the RGD peptide a type II turn could possibly be followed by a type III′ turn [a type III′ turn is the mirror image of a type III turn (Richardson, 1981)]. This conformation was ruled out by Reed et al. (1988) on the basis of the ϕ angle constraint for G3 in GRGDSP. Since we have demonstrated the existence of the type II turn for the first turn in YGRGDSP from the NOESY spectrum, it is possible that this peptide contains the mixed II–III′ structure.

Comparing the two similar oligopeptides YGRGESP and YGRGDSP having differing biological activity allows our study to go a step beyond the studies of Wright and co-workers (Dyson et al., 1985; Dyson et al., 1988a,b). Their hypothesis is that secondary structures they find in aqueous solution are important to biological function. The structural difference that we have observed for these functionally different, but closely related, oligopeptides indicates the following: (I) oligopeptides *do* assume secondary structure in aqueous solution, (II) conformation, not surprisingly, has an important effect on biological function, and (III) transient secondary structures found in aqueous solution are important to function.

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