Solution Structures of FcεRI α-Chain Mimics: A β-Hairpin Peptide and Its Retroenantiomer

James M. McDonnell,[†] David Fushman,[†] Sean M. Cahill,[†] Brian J. Sutton,[‡] and David Cowburn*,[†]

Contribution from The Laboratory of Physical Biochemistry, The Rockefeller University, 1230 York Avenue, New York, New York 10021, and The Randall Institute, King's College London, 26-29 Drury Lane, London WC2B 5RL, U.K.

Received November 8, 1996[⊗]

Abstract: A central event in the development of the allergic response is the interaction between immunoglobulin E (IgE) and its cellular high-affinity receptor FcεRI. Allergen-bound IgE mediates the allergic response by binding through its Fc region to its cellular receptor on mast cells and basophils, causing the release of chemical mediators. One strategy for the treatment of allergic disorders is the use of therapeutic compounds which would inhibit the interaction between IgE and FcεRI. Using a structure-based design approach, conformationally constrained synthetic peptides were designed to mimic a biologically active β-hairpin region of the α-chain of FcεRI. Two peptide mimics of the FcεRI α-chain were previously shown to inhibit IgE−FcεRI interactions, one a peptide comprised of L-amino acids, covalently cyclized by N- and C-terminal cysteine residues, and the other its retroenantiomer. In this paper the solution structures of these compounds are derived using NMR spectroscopy. The topochemical relationship between the retroenantiomeric compounds and the structural basis of their biological activity is described.

Introduction

The synthetic recreation of protein surfaces offers a unique opportunity to evaluate the contribution of individual protein regions to macromolecular recognition events. A peptide-based approach to mimicking protein surfaces has been limited by the fact that short linear peptides rarely maintain the conformation in which they are found in the parent protein. This is particularly true for peptide models of β-sheet structure. While many examples are available for peptides that fold into α-helical structures, peptides forming monomeric β-sheet structures have been rare. Only very recently have peptide models of β-hairpin structure been described.[1−4]

The elements of affinity and specificity of molecular recognition processes depend upon the degree of complementary of the interacting surfaces. One widely used topochemical approach to studying molecular recognition is the use of retroenantiomeric peptides, modified peptides which contain D-amino acids with sequence inverted relative to an L-amino acid compound. A D-amino acid peptide with reversed peptide bond orientation will in principle yield an isomer with similar side-chain topology. Comparable biological activity has been observed for retroenantiomeric peptides in a number of biological systems,[5,6] although this approach has not been universally applicable.[7,8]

In this paper we describe the structure of two retroenantiomeric, β-hairpin-forming, peptide mimics of the high-affinity receptor for IgE. The β-hairpin structure is induced in the peptides by conformational constraints in the form of cyclization via a disulfide. The structural relationship between the retroenantiomers is described.

Results

Peptide Design. The tertiary structure of a small peptide only seldom resembles that of its parent protein,[9] so the use of short linear peptides as mimics of protein structure has only occasionally been successful. In an effort to overcome this limitation, attempts have been made to limit the conformational flexibility available to the peptide by the introduction of conformational constraints.[10] This is done commonly by incorporation of residues that display strong conformational tendencies[11] or by the covalent cyclization of the peptide backbone.

A β-hairpin region comprised of the C−C′ strands of the second extracellular domain of the α-chain of FcεRI (FcεRI α2) has been shown to be important in its interaction with IgE.[12] We have synthesized a set of cyclic peptides designed to mimic this C−C′ β-hairpin structure found in the FcεRI α-chain. Two peptides were initially prepared, one comprised of L-amino acids and another with D-amino acids with sequence reverse (retro-inverso) relative to the L analog. The D-peptide was designed to be a retroenantiomer of the L-amino acid peptide, presenting

* To whom correspondence should be addressed. Phone: (212) 327-8270. Fax: (212) 327-7566. E-mail: cowburn@rockvax.rockefeller.edu.
† The Rockefeller University.
‡ King's College London.
⊗ Abstract published in *Advance ACS Abstracts,* June 1, 1997.

(1) Searle, M. S.; Zerella, R.; Williams, D. H.; Packman, L. C. *Protein Eng.* **1996**, *9*, 559−565.
(2) Ramirez-Alvarado, M.; Blanco, F. J.; Serrano, L. *Nat. Struct. Biol.* **1996**, *3*, 604−612.
(3) Friedrichs, M. S.; Stouch, T. R.; Bruccoleri, R. E.; Mueller, L.; Constantine, K. L. *J. Am. Chem. Soc.* **1995**, *117*, 10855−10864.
(4) Tsang, K.; Diaz, H.; Graciani, N.; Kelly, J. *J. Am. Chem. Soc.* **1994**, *116*, 3988−4005.
(5) Guichard,G.; Benkirane, N.; Zeder-Lutz, G.; Van Regenmortel, M. H. V.; Briand, J. P.; Muller, S. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 9765−9769.
(6) Jameson, B. J.; McDonnell, J. M.; Marini, J. C.; Korngold, R. *Nature* **1994**, *368*, 744−746.
(7) Wermuth, J.; Goodman, S.; Kessler, H. in *Peptides 1991, Proceedings of the 23rd European Peptide Symposium*; Maia, H. S. C., Ed.; ESCOM: Leiden, The Netherlands, 1995; pp 648−649.
(8) Friedinger, R.; Veber, D. *J. Am. Chem. Soc.* **1979**, *79*, 6129−6130.
(9) DeGrado, W. *Adv. Protein Chem.* **1988**, *39*, 51−124.
(10) Rizo, J.; Gierasch, L. *Annu. Rev. Biochem.* **1992**, *61*, 387−418.
(11) Ajo, D.; Grannozi, G.; Tondello, E.; Del Prada, A. *Biopolymers* **1980**, *19*, 469−475.
(12) Sutton, B. J.; Gould, H. J. *Nature* **1993**, *366*, 421−428.

S0002-7863(96)03884-X CCC: $14.00

a similar topological surface as the L-peptide, while making use of the preferred pharmacological properties of D-amino acids. The underlying principles of the retro-inverso approach have been evident for many years.[13−15] In support of this theory, L- and retro-D-peptides have been shown to possess similar biological activities in a number of cases.[5,16] However this has not proven to be universally true.[8,17] The L- and retro-D-amino acid FcεRI peptide mimics were cyclized by an intramolecular disulfide bond formed between amino and carboxyl terminal cysteine residues. The remaining peptide sequence found in the mimics is native to the C−C′ region of FcεRI α2. Both the L and retro-D conformationally constrained FcεRI mimics inhibited IgE−FcεRI interactions at low micromolar concentrations while uncylized or scrambled sequence controls were inactive.[18] The CD spectra of the peptides suggested the formation of β-character and showed the expected reciprocal chirality.[18] We have used nuclear magnetic resonance spectroscopy to characterize the solution structures of L- and retro-D-FcεRI mimics, to evaluate the structural basis of the biological activity of the peptides, and to analyze the structural relationships between retroenantiomers.

Given the tendency of β-sheet peptides to aggregate, we thought it important to verify that the cyclo(L-262) and cyclo(rD-262) (rD = retro-D) peptides were indeed monomeric species. No concentration effect was seen in the CD spectra of the peptides in the concentration range 10 μM to 1 mM. No significant changes in chemical shift (<0.01 ppm) or line widths were observed in 1-D ^{1}H-NMR spectra from 100 μM to 10 mM. These results suggest that in the concentration range tested the peptide is monomeric. As a further test to confirm the monomeric status of peptide samples, translational diffusion coefficients were measured and hydrodynamic calculations performed for the cyclo(rD-262) peptide. Translational diffusion coefficients were measured using pulse-field-gradient NMR methods. Two different approaches were used, varying either gradient strength or gradient pulse length (see the Materials and Methods). The two approaches gave translational diffusion coefficients of $(2.21 \pm 0.06) \times 10^{-6}$ and $(2.19 \pm 0.04) \times 10^{-6}$ cm^2/s. To interpret the significance of these experimental values, hydrodynamic calculations were performed on NMR-derived structural models of cyclo(rD-262). The hydrodynamic calculations were initially performed using BRKTOS[19] (a program which converts coordinates to a sphere model) and subsequently were carried out using an all atom bead method[20] employing a MATLAB adaptation of the program HYDRO.[21] Hydration of the peptide structure was performed by solvating the peptide in a 10 Å layered water box in InsightII (Biosym, San Diego) and equilibrating according to standard protocols. Only water molecules within a given distance from the peptide were then included in the hydration shell of the structure and incorporated in the hydrodynamic calculations. The calculated translational diffusion coefficients for the cyclo(rD-262) structures were 2.33×10^{-6}, 2.20×10^{-6}, and 2.13×10^{-6} cm^2/s, for hydration shells of 3.5, 3.75, and 4.0 Å, respectively. This level of hydration is consistent with NMR-observed and hydrodynamics-calculated values for translational diffusion coefficients for the protein ubiquitin.[22] All experimental evidence fully supports the assumption of monomeric peptide structure.

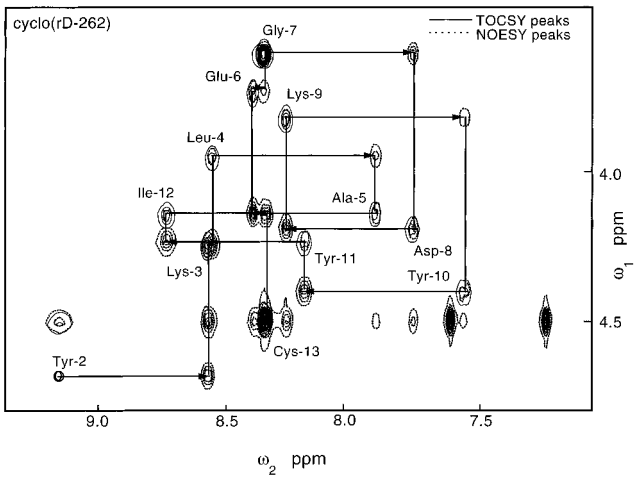

Figure 1. An illustration of the TOCSY-NOESY walk used for the sequential assignment of the cyclo(rD-262) peptide. Sequential $d_{\alpha N}(i, i+1)$ connectivities are indicated for residues 2−13.

NMR. Identification of amino acid spin system type was accomplished by analysis of TOCSY spectra. Sequential resonance assignments were performed using connectivities between H^N_i and H^α_{i+1} protons in an overlay of TOCSY and NOESY spectra.[23] Figure 1 illustrates an example TOCSY-NOESY map for the cyclo(rD-262) peptide. The unambiguous connecting walk permits sequential residue assignments. Analysis of the H^N-H^α fingerprint region also reveals that interresidue $H^N_i-H^\alpha_{i+1}$ NOEs are stronger than intraresidue H^N-H^α NOEs for the following residues: for cyclo(L-262), Ile2, Tyr3, Tyr4, Lys5, Glu8, Leu10, Lys11, Tyr12; for cyclo(rD-262), Tyr2, Lys3, Leu4, Ala5, Asp8, Lys9, Tyr10, Tyr11, Ile12, Cys13. This information suggests these residues exist in an extended conformation,[24] consistent with a β-strand conformation for these residues.

Coupling constants were calculated from 1-D proton spectra and 2-D DQF-COSY spectra, and were in good agreement with each other. Figure 2 illustrates the 1-D proton spectrum and 2-D DQF-COSY spectrum for the cyclo(rD-262) peptide, from which $^3J(H^N-H^\alpha)$ coupling constants were calculated. In both the L- and retro-D-peptides those residues designed to form β-strands show large values for $^3J(H^N-H^\alpha)$ consistent with the putative β-structure, while those residues in the predicted β-turn show smaller coupling constants.

Protection from hydrogen−deuterium exchange suggests that several amides are either buried or involved in hydrogen bond formation.[23] Both cyclo(L-262) and cyclo(rD-262) demonstrate significant protection levels for five to six of the amide protons (Figure 3). It is unusual for small peptide β-hairpin models to show measurable levels of amide protection from deuterium exchange, and the presence of amide protection in these peptides suggests stable structure. The exchange rates, corrected for temperature, pH, and nearest neighbor effects, for the cyclo(L-262) peptide are significantly faster than their equivalents in

(13) Shemyakin, M.; Ovchinnikov, Y.; Ivanov, V. *Angew. Chem., Int. Ed. Engl.* **1967**, *8*, 492−499.
(14) Goodman, M.; Chorev, M. *Acc. Chem. Res.* **1979**, *12*, 1−7.
(15) Guptasarma, P. *FEBS* **1992**, *310*, 205−210.
(16) Merrifield, R.; Juvvadi, P.; Andreu, D.; Ubach, J.; Boman, A.; Boman, H. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 3449−3453.
(17) Momany, F.; Aubuchon, J. *Biopolymers* **1978**, *17*, 2609−2615.
(18) McDonnell, J. M.; Beavil, A. J.; Mackay, G. A.; Jameson, B. A.; Korngold, R.; Gould, H. J.; Sutton, B. J. *Nat. Struct. Biol.* **1996**, *3*, 419−426.
(19) Perkins, S. J.; Smith, K. F.; Kilpatrick, J. M.; Volanakis, J. E.; Sim, R. B. *Biochem. J.* **1993**, *295*, 87−99.
(20) de la Torre, J. G.; Bloomfield, V. A. *Q. Rev. Biophys.* **1981**, *14*, 81−139.
(21) de la Torre, J. G.; Navarro, S.; Martinez, M. C. L.; Diaz, F. G.; Cascales, J. J. L. *Biophys. J.* **1994**, *67*, 530−531.
(22) Tjandra, N.; Feller, S. E.; Pastor, R.; Bax, A. *J. Am Chem. Soc.* **1995**, *117*, 12562−12566.
(23) Wüthrich, K. *NMR of proteins and nucleic acids*; John Wiley and Sons: New York, 1986.
(24) Billeter, M.; Braun, W.; Wüthrich, K. *J. Mol. Biol.* **1982**, *15*, 321−346.

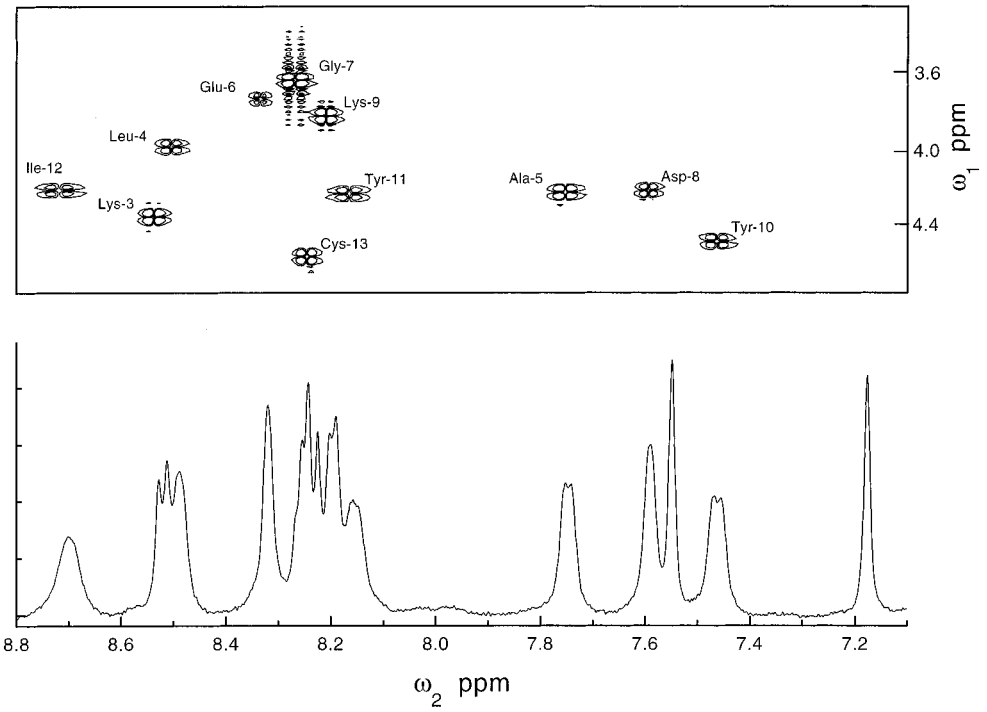

Figure 2. Both 1-D (bottom) and 2-D DQF-COSY (top) experiments were used to derive $^3J(H^N-H^\alpha)$ coupling constants. The cyclo(rD-262) spectra are shown as an example. The sharp single peaks at 7.55 and 7.18 ppm represent the C-terminal amide peaks.

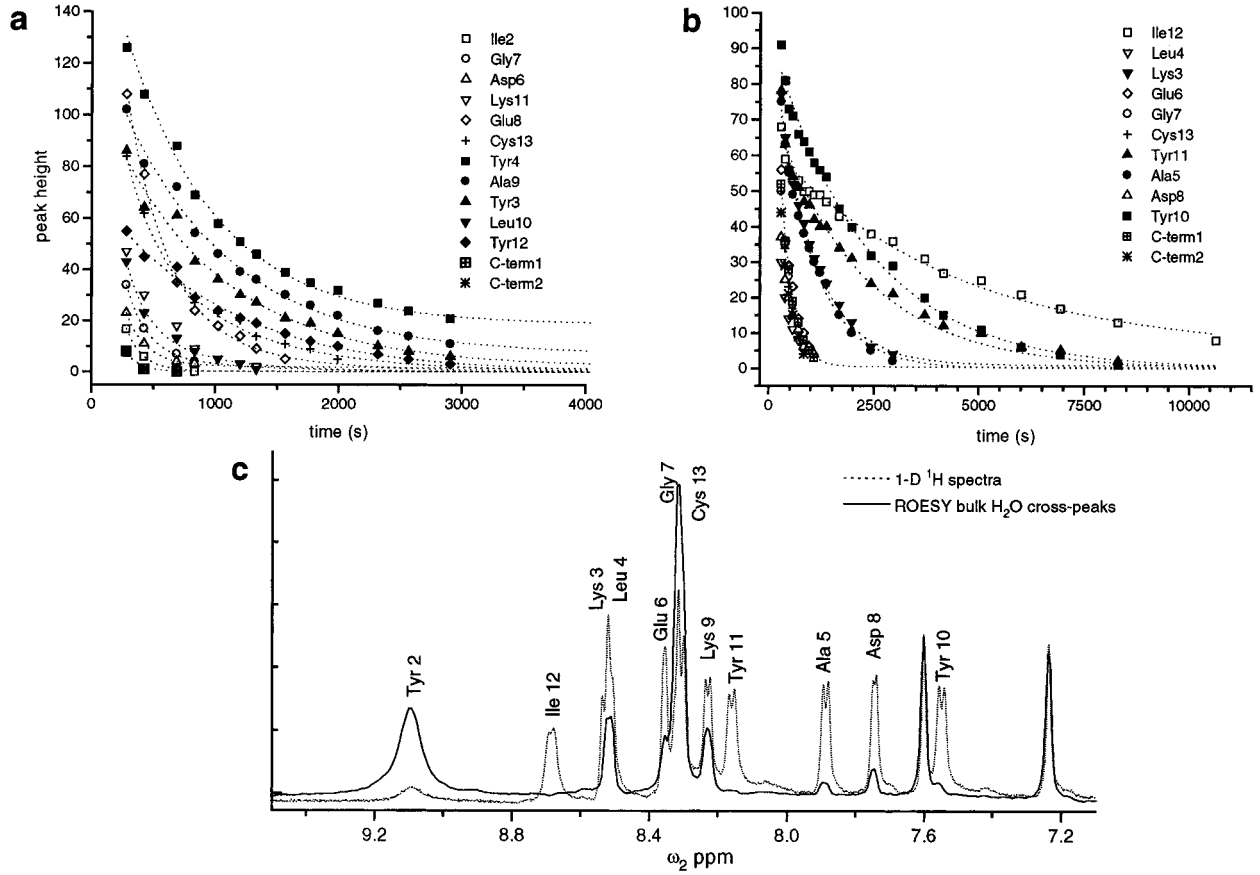

Figure 3. Hydrogen-bonded amides are suggested from the results of hydrogen−deuterium exchange experiments for cyclo(L-262) (a) and cyclo(rD-262) (b) and supported by solvent mapping analysis studies (illustrated in (c)). No amide signals were observed for residues Lys5 in cyclo(L-262) and Tyr2 and Lys9 in cyclo(rD-262) in the HD exchange experiments, presumably due to fast exchange, and consequently are not shown in (a) or (b). Solvent mapping studies (c) were used only qualitatively to confirm direct exchange experiments.

the cyclo(rD-262) peptide, suggesting a larger population of hydrogen-bonded conformers in the retro-D-peptide than in the L-peptide. This is also supported by the generally smaller $^3J(H^N-H^\alpha)$ coupling constants observed for the β-residues of the L-peptide compared to its retroenantiomer (Figure 4). In addition to the direct exchange experiments, a qualitative comparison of exchange can be made by analysis of interactions of amide protons with bulk water.[25] Figure 3c demonstrates a

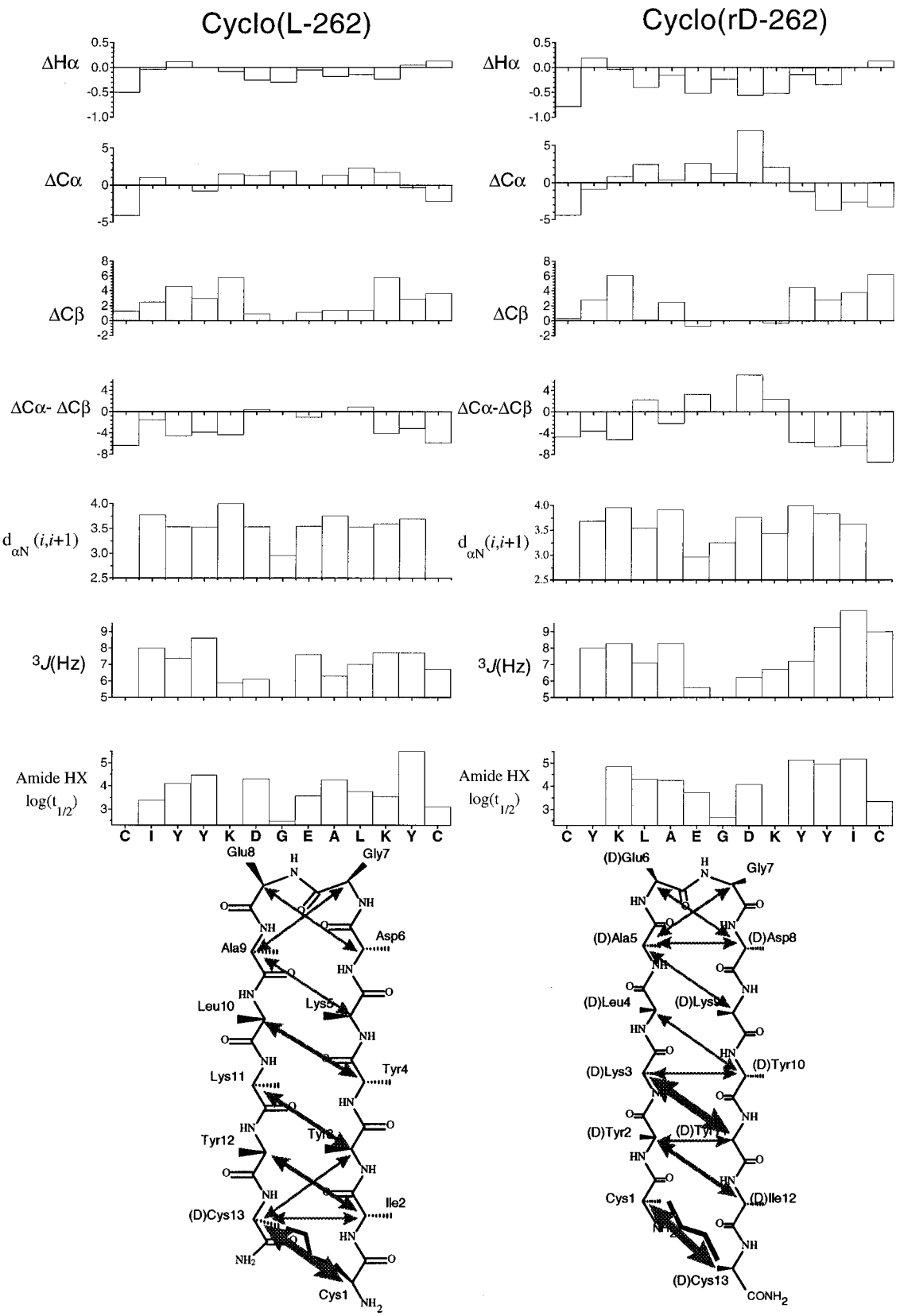

Figure 4. Summary of NMR chemical shifts, amide coupling constants, amide exchange rates, and observed cross strand NOEs. $\Delta C\alpha - \Delta C\beta$ is a qualitative indicator of secondary structure in proteins with positive values being associated with the α-helix and negative values correlated with β-strands. For $d_\alpha(i, i+1)$ NOE results are reported on a log scale; values are normalized between spectra from XEASY analysis, with a maximum value of 10 000. Amide exchange rates were calculated from a single exponential decay fit of the raw exchange rates (Figure 3) and reported here normalized for primary structure effects;[44] $t_{1/2}$ times are on a log scale. In the summary of cross strand NOEs the thickness of the arrow is proportional to the number of observed interresidue NOEs (1−3).

cross section of a ROESY spectrum indicating peptide−water cross peaks, the intensity of which is relative to the chemical exchange rate of the amide proton. For comparison a one-dimensional spectrum is also displayed. As is also shown in the hydrogen−deuterium exchange experiments (Figure 3a), the solvent interaction analysis indicates some amide peaks that are

well protected (for example, Ile12), while others show intermediate protection factors (Ala9) or very rapid exchange rates (Tyr2). This implies that some portions of the structure are more stable than others.

A summary of chemical shifts, coupling constants, amide protection factors, and sequential and cross-strand NOEs is shown in Figure 4. Elements of secondary structure are suggested by several NMR criteria. Two β-strands are predicted on the basis of strong $d_\alpha(i, i+1)$ NOEs, large $^3J(H^N-H^\alpha)$ values, and negative chemical shift values for $\Delta C^\alpha - \Delta C^\beta$. The observed set of long-range NOEs demonstrate the formation of a compact stable structure stabilized by interstrand interactions between both backbone and side-chain atoms and are consistent with the β-hairpin structure found in the native protein.

Structure Calculations. NOE-derived distance constraints, $^3J(H^N-H^\alpha)$ couplings constants, and hydrogen bonds implied by amide protection studies were input as restraints in the distance geometry program DIANA with the REDAC procedure.[26] DIANA uses no assumptions about protein energetics other than van der Waals repulsions, and standard bond lengths and angles; calculated structures are unrefined, and structures have only been adjusted by rotation and translation for comparison purposes. The structures calculated for cyclo(L-262) and cyclo(rD-262) are shown in Figure 5. Parts a and c of Figure 5 demonstrate the superposition of backbone and carbonyl oxygen atoms of the 20 lowest target function calculated structures for cyclo(L-262) and cyclo(rD-262), respectively.

For the family of structures the root mean squared (rms) deviations calculated for backbone atoms are 1.58 Å for cyclo(L-262) and 1.08 Å for cyclo(rD-262) (see Table 1). Global rms deviations are illustrated in Figure 5b,d, where the width of the ribbon is proportional to the rms deviation of the backbone atoms. The rms deviations are larger for cyclo(L-262) than for its retroenantiomer cyclo(rD-262) and imply greater conformational flexibility. These values result from the observed smaller coupling constants, which effect a greater available range of backbone dihedral angles, and from generally weaker cross strand NOEs, giving larger upper limit distance constraints input into DIANA. The larger rms deviations are also anticipated from the lower protection factors observed for amide protons in cyclo(L-262).

Cyclo(L-262) forms a type III′ β-turn[27] for residues Asp6−Ala9 and shows a high amide protection factor for Ala9, involved in the $i, i+3$ hydrogen bond. In contrast the cyclo(rD-262) peptide shows its highest amide protection factors at the base of the β-hairpin, suggesting that for some reason the retro-D structure forms, or maintains, the hydrophobic cluster found at the base of the β-hairpin better than the L-peptide. However, the turn in cyclo(rD-262) is not as well defined spectroscopically; in contrast to cyclo(L-262), the two α hydrogens of Gly7 are either averaged or equivalent and the β-methylene hydrogens of Glu6 and Asp8 are not spectroscopically distinct. The calculated structures of cyclo(rD-262) result in two distinct turn structures; 85% of calculated structures demonstrate dihedral angles roughly analogous to the type III′ turn observed for the L-peptide, but ca. 15% of the structures consistently demonstrate the inverse type III turn. Experimental data do not allow us to determine if these represent subpopulations or merely result from ambiguities resulting from the poorly defined hydrogen atoms in the turn of cyclo(rD-262), likely arising from interconverting conformers.

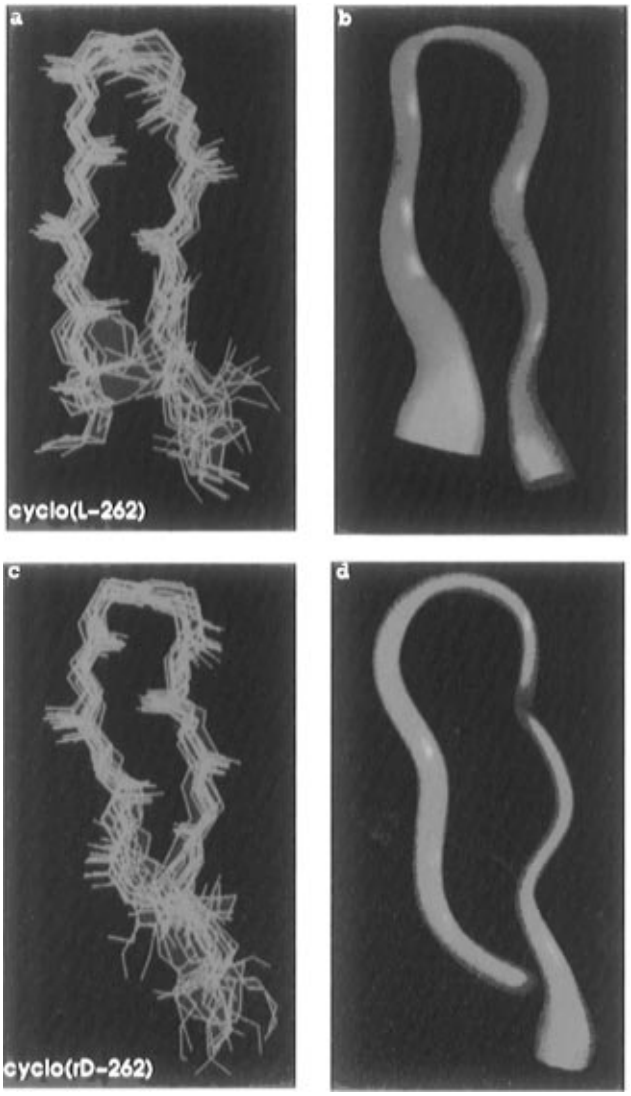

Figure 5. Superimposition of the 20 lowest target function calculated structures for cyclo(L-262) (a) and cyclo(rD-262) (c). (b) and (d) illustrate the ribbon representation of the structures where ribbon widths are proportional to the global backbone rms deviations (see also Table 1).

Discussion

The cyclo(L-262) and cyclo(rD-262) peptides exhibit a structure similar to that predicted for the C−C′ region in the homology-based model of the FcεRI α-chain.[18] A detailed structural comparison between the peptide structures and the protein region will be of great interest when the crystal structure for the FcεRI α-chain becomes available. The formation of structure in the peptides is dependent on covalent cyclization using the intramolecular disulfide. Peptides demonstrate random coil structure without this disulfide bond.[18] Peptide models of stable β-hairpins are rare and only recently has some insight been gained into the formation of these structures.[1−4,28] The peptide mimics of the FcεRI α2 C−C′ region are unusually well structured β-hairpins; this is illustrated by their long H−D exchange half-lives. Using some protein structures solved in this laboratory as a standard for comparison, cyclo(rD-262) show smaller amide protection values than the very stable βB−βC

(25) Bruschweiler, R.; Morikis, D.; Wright, P. *J. Biomol. NMR* **1995**, *5*, 353−356.
(26) Güntert, P.; Wüthrich, K. *J. Biomol. NMR* **1991**, *2*, 517−530
(27) Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, *3*, 1−110.
(28) Blanco, F.; Jimenez, M.; Pineda, A.; Rico, M.; Santoro, J.; Nieto, J. *Biochemistry* **1994**, *33*, 6004−6014.

Table 1. Statistics for NMR Calculations

	cyclo(L-262)	cyclo(rD-262)
no. of residues	13	13
no. of distance restraints		
NOEs		
intraresidue	84	77
sequential	47	45
$i, i+2$	8	6
cross strand	14	15
total no. of NOEs	155	146
no. of dihedral angle restraints	11	11
no. of hydrogen bond restraints	4	5
no. of stereospecific β-methylenes	5	4
rms deviations (Å) for		
calculated structures		
all residues (1−13)		
backbone atoms	1.58 ± 0.77	1.08 ± 0.34
heavy atoms	2.88 ± 0.96	2.50 ± 0.67
without termini (2−12)		
backbone atoms	0.98 ± 0.41	0.72 ± 0.27
heavy atoms	2.42 ± 0.79	2.27 ± 0.69
β-turn only (6−9 or 5−8)		
backbone atoms	0.22 ± 0.16	0.08 ± 0.05
heavy atoms	1.08 ± 0.33	0.91 ± 0.33

hairpin from Abl SH2,[29] but greater amide protection values than are observed for the relatively flexible $\beta4-\beta5$ hairpin structure of the Abl SH3 protein.[30] As far as we are aware, cyclo(L-262) and cyclo(rD-262) are the first synthetic β-hairpins to demonstrate significant protection in amide hydrogen−deuterium exchange experiments. The peptides described here have a number of features which may contribute to their unusual structural stability. It has been suggested that the formation of a hydrophobic cluster is a crucial nucleating event in the formation of a β-hairpin.[4] The aromatic and hydrophobic residues at the base of the β-hairpin in cyclo(L-262) (CIYY-KDGEALKYC) and cyclo(rD-262) (CYKLAEGDKYYIC) are probably important in stabilizing structure. Additionally the FcϵRI peptides have a glycine residue at position 2 of the β-turn. Glycine is the most common residue found in turns, and its flexibility effectively accommodates tight turns.[27] The peptides do exhibit deviations in behavior from ideal planar β-sheet structure, in that both demonstrate helical twist of the strands and show some bending in the plane of the β-strands. As expected cyclo(L-262) shows a right-handedness to the helical twist, while cyclo(rD-262) demonstrates a more marked left-handed twist. Variability in the amount of twist contributes to the increase in rms deviations at the termini of the peptides (see Figure 5), as a wide range of helical twist is consistent with the set of constraints used for structure calculations. These deviations from ideal behavior are expected for peptide mimics of a β-hairpin structure, as opposed to a β-hairpin in the context of a protein where the structure is tethered to the rest of the molecule covalently and by a network of interstrand interactions.

The structural relationship between peptide retroenantiomers has been much discussed (recently reviewed in ref 31). Activity studies between retroenantiomeric peptides have been analyzed in a number of different systems,[5,6,16] and conformational analyses have been carried out on small cyclic retroenantiomeric peptides.[7,32] In addition, a number of theoretical papers have speculated on the topological similarity of retroenantiomers.[8,13,15] Given the dynamic nature of these peptide structures and the fact that the side-chain conformations are generally only well defined out to the β-carbon positions, it is impossible to define a single structure for each peptide. However, from the analysis of cyclo(L-262) and cyclo(rD-262) it is clear that the retroenantiomeric peptides can present very similar, although not identical, topochemical surfaces. Figure 6 shows a GRASP surface representation of two similar conformers of cyclo(L-262) and cyclo(rD-262). This topological similarity between the two compounds is reflected in their similar affinity for IgE.[18] However, cyclo(L-262) and cyclo(rD-262) are not structurally identical on their surfaces. The issue of differing structures by retroenantiomeric peptides is well understood and has been discussed by others.[8] Some subtle structural difference results in an apparent greater flexibility of cyclo(L-262) in comparison with cyclo(rD-262), and is demonstrated most clearly in differences in amide protection factors (Figure 4). Nevertheless, this study has demonstrated that the general structural principles of the retroenantiomeric approach are valid, at least in certain systems. Further, we have shown that conformationally constrained peptide structures can be induced to mimic structures found in proteins and, in doing so, may mimic the biological activity of that protein region. The ability to synthetically recreate protein surfaces offers a unique approach for characterizing molecular recognition events. These conformationally stable mimics of the FcϵRI protein may represent a step toward the rational design of therapeutics for treating allergic disorders.

Materials and Methods

Peptide Synthesis, Refolding, Purification, and Preparation. Peptides were synthesized on an Applied Biosystems 430A peptide synthesizer using standard fmoc chemistry. Crude peptide was reduced with dithiothreitol and HPLC purified. The purified peptides were then subjected to an air oxidation protocol for intramolecular disulfide formation.[33] Peptides show greater than 95% intramolecular disulfide bonding at the end of this procedure as monitored by HPLC and MALDI-MS analysis. The calculated molecular mass for the cyclized peptides cyclo(L-262) and cyclo(rD-262) was 1566.82, while the observed masses by MALDI-MS were 1566.7 and 1567.3, respectively. No significant impurities were detectable by NMR. The cyclized product was then repurified by RP-HPLC and used for the experiments described below. The sequences of the peptides used in this study are as follows: cyclo(L-262) "L-(CIYYKDGEALKY)"D-C-amide (all L-amino acids except the C-terminal residue); cyclo(rD-262) D-C-"L-(YKLAEGDKYYIC)"-amide (all D-amino acids except the N-terminal residue). For NMR samples, lyophilized peptide was dissolved in 90% H_2O/10% D_2O, pH adjusted to 5.5.

Nuclear Magnetic Resonance. All NMR experiments were performed on a Bruker DMX-500 spectrometer. Two-dimensional spectra were recorded in pure phase absorption mode using States-TPPI phase cycling procedures. TOCSY, NOESY, and ROESY spectra were collected at 288 K for various mixing times. Water suppression was achieved either by presaturation during the relaxation delay or by using the Watergate pulse scheme.[34] For determination of coupling constants, one-dimensional spectra were collected with 16 384 data points and high digital resolution and 2-D DQF-COSY were collected with 64 scans and 8192 real data points. ^{13}C resonances were assigned from a $^{13}C-^{1}H$ HMQC experiment performed in 99.996% D_2O at natural abundance. The rate of hydrogen−deuterium exchange was analyzed using a series of one-dimensional spectra. For cyclo(rD-262) exchange was measured at 288 K, pH 5.0; 22 spectra were collected over a period of 3 h. Because of spectral overlap of amide signals at 288 K, cyclo(L-262) exchange was monitored at 298 K, pH 4.5; 14 spectra were

(29) Overduin, M.; Rios, C. B.; Mayer, B. J.; Baltimore, D.; Cowburn, D. *Cell* **1992**, *70*, 697−704.
(30) Gosser, Y. Q.; Zheng, Z.; Overduin, M.; Mayer, B. J.; Cowburn, D. *Structure* **1995**, *3*, 1075−1086.
(31) Chorev, M.; Goodman, M. *TIBTECH* **1995**, *13*, 438−445.
(32) Mammi, N. J. and Goodman, M. *Biochemistry* **1986**, *25*, 7607−7614.
(33) McDonnell, J. M.; Blank, K. J.; Rao, P. E.; Jameson, B. A. *J. Immunol.* **1992**, *149*, 1626−1630.
(34) Piotto, M.; Saudek, V.; Sklenar, V. *J. Biomol. NMR* **1992**, *2*, 661−665.

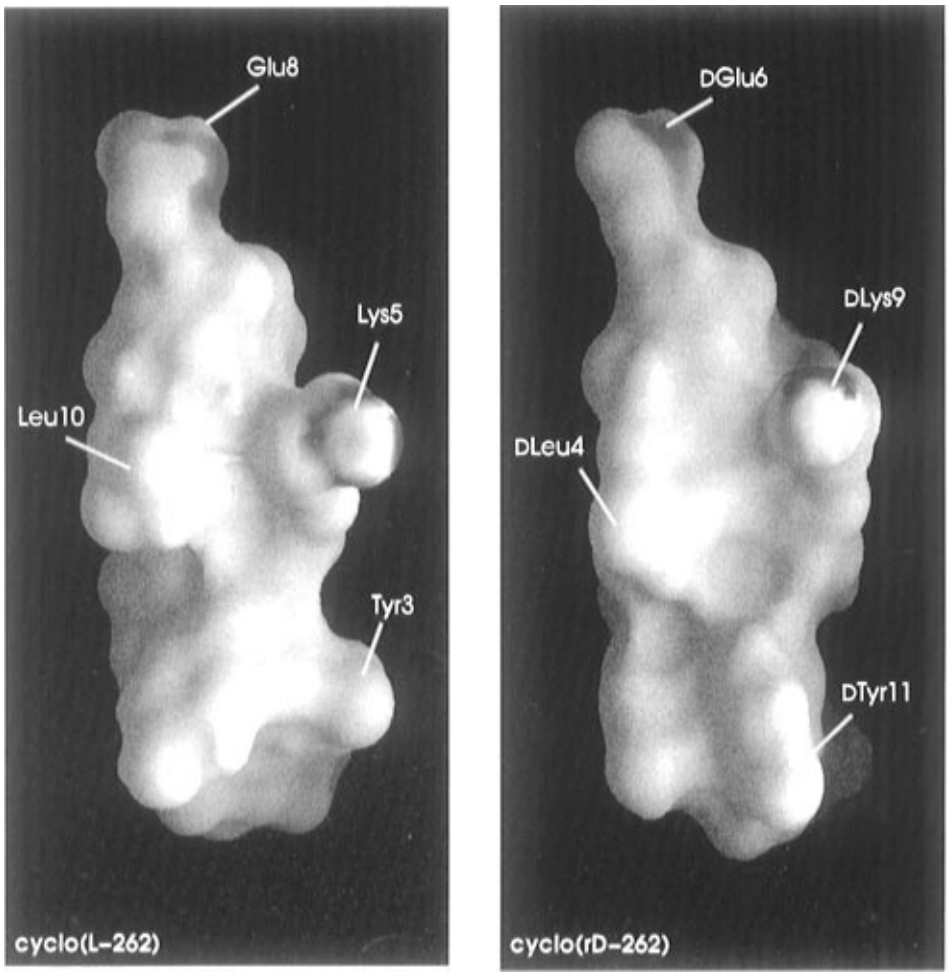

Figure 6. Similarity of topochemical features presented by the retroenantiomeric peptides. Solid surface representations demonstrate that side chains can be presented analogously in cyclo(L-262) and cyclo(rD-262) structures. The surface which is predicted to be exposed in the FcεRI α-chain protein is shown, and several prominent side chains are indicated. The figure was prepared using the program GRASP.[45]

recorded in 1 h. Signal intensity was plotted versus time and fitted to a single exponential decay in order to derive exchange rates. Solvent accessibility of amide protons was also confirmed by analyzing amide cross peaks with bulk water in ROESY experiments.[35] NOE correlations in H_2O and D_2O were assigned in XEASY[36] and peak volumes integrated using the program PEAKINT.[36] Peak volumes are converted into upper limit distance constraints using the program CALIBA,[37] where nonstereospecifically assigned methylene and methyl groups are corrected for center averaging[23] and distance constraints involving methyl groups are increased by 1.0 Å to account for their greater intensity.[38]

Measurements of the translational diffusion coefficients were performed using pulse-field-gradient NMR methods. Two measurement schemes were employed, based on variation of either gradient-pulse length,[39] in the range of 2−10 ms at a constant magnetic field gradient (21 G/cm), or the gradient strength (from 2 to 21 G/cm) in the pulse sequence incorporating bipolar gradient pulses.[40] Thirty to forty 1-D spectra, corresponding to various gradient lengths or strengths, were acquired in a pseudo-2-D fashion, and intensities of the NMR signals were fitted to standard equations[41] using a MATLAB platform employing a simplex algorithm. Peptide hydration was performed in InsightII (Biosym, San Diego) using a 10 Å layered waterbox. The heavy atoms of the peptide and water oxygen atoms were assigned radii of 1.0 and 1.6 Å, respectively.[22] The errors calculated for the derived translational diffusion coefficients were assessed as standard deviations of diffusion coefficients calculated from different peaks in the NMR spectrum.

Structure Calculations. Structures were calculated using the DIANA program.[42] Both one-dimensional ^{1}H spectra (Figure 2a) and double quantum filtered COSY spectra (Figure 2b) were used to derive $^3J(H^N-H^\alpha)$ coupling constants. For the one-dimensional spectral data, spin−spin couplings were calculated by fitting individual amide peaks to a double Lorentzian equation and measuring the separation between the two Lorentzian maxima. Because several of the peaks in the one-dimensional spectra overlap, we additionally used two-dimensional DQF-COSY experiments to unambiguously resolve cross peaks and calculate $^3J(H^N-H^\alpha)$ constants. For fits of the DQF-COSY peaks, spectra were imported into the program MATLAB and one-dimensional slices of each peak were extracted and fit to an antiphase superposition of two Lorentzians, due to the nature of antiphase doublet peaks in the DQF-COSY. Restricted values of ϕ were derived as previously described.[43] Structures were initially calculated without hydrogen bonds, and restraints for these were added only when present in a set of initial calculated structures, and when the amide exchange protection factor was significant.

(35) Otting, G.; Liepinsh, E.; Wüthrich, K. *Science* **1991**, *254*, 974−80.
(36) Eccles, C.; Güntert, P.; Billeter, M.; Wüthrich, K. *J. Biomol. NMR* **1991**, *1*, 111−130.
(37) Güntert, P.; Wüthrich, K. *J. Biomol. NMR* **1991**, *1*, 447−56.
(38) Wagner, G.; Braun, W.; Havel, T. F.; Schaumann, T.; Go, N.; Wüthrich, K. *J. Mol. Biol.* **1987**, *196*, 611−639.
(39) Dingley, A. J.; Mackay, J. P.; Chapman, B. E.; Morris, M. B.; Kuchel, P. W.; Hambly, B. D.; King, G. F. *J. Biomol. NMR* **1995**, *6*, 321−328.
(40) Wu, D.; Chen, A.; Johnson, C. S. *J. Magn. Reson., A* **1995**, *115*, 260−264.
(41) Stejskal, E. O.; Tanner, J. E. *J. Chem. Phys.* **1965**, *42*, 288−292.
(42) Güntert, P.; Braun, W.; Wüthrich, K. *J. Mol. Biol.* **1991**, *217*, 517−30.
(43) Clore, G.; Appella, E.; Yamada, M.; Matsushima, K.; Gronenborn, A. *Biochemistry* **1990**, *29*, 1689−96.
(44) Molday, R. S.: Englander, S. W.; Kallen, R. G. *Biochemistry* **1972**, *11*, 150−158.
(45) Nicolls, A.; Sharp, K. A.; Honig, B. *Proteins* **1991**, *11*, 281−296.

Acknowledgment. We thank Drs. A. Beavil and H. Gould for helpful discussion and Drs. E. Barbar and S. Sechi for critical review of the paper. We are grateful to Dr. S. Perkins for allowing use of the hydrodynamics program BRKTOS and Dr. P. Güntert for advice on the modification of the DIANA library. J.M.M. gratefully acknowledges support from a Hitchings-Elion Fellowship from the Burroughs Wellcome Fund. D.F. was supported by American Cancer Society Grant NP-922.

Supporting Information Available: A complete table of ^{1}H and ^{13}C chemical shifts for the cyclo(L-262) and cyclo(rD-262) peptides (2 pages). See any current masthead page for ordering and Internet access instructions. Coordinates for the families of NMR structures have been deposited with the Protein Data Bank.

JA963884O