Table II. From these data, the standard heat of formation of C_5H_4 may be calculated at the various levels of theory. The computed MBPT(4) electronic hydrogenolysis energy of C_5H_4 at 0 K is -285.9 kcal/mol. The zero-point vibrational energy change for this reaction is 49.7 kcal/mol. The thermal vibrational energy and other thermal terms contribute -8.2 kcal/mol to the reaction enthalpy at 298 K. Thus, the computed value of ΔH^{298} for the hydrogenolysis of C_5H_4 is -244.4 kcal/mol. This value and the experimental standard heat of formation of CH_4 of -17.4 kcal/mol[17] lead to a predicted standard heat of formation of C_5H_4 of 157.4 kcal/mol. This value is more than twice the experimental heat of formation of 66.2 kcal/mol for cyclopropene[17] and is consistent with the anticipated high endothermicity of spiropentadiene.

Acknowledgment. All calculations were performed on the Cray Y-MP8/864 computer at the Ohio Supercomputer Center. We thank R. J. Bartlett and J. D. Watts for making the latest version of the ACES II program available and for assistance with its use and the Ohio Supercomputer Center for providing the computer time required for these calculations.

(17) Pedley, J. B.; Naylor, R. D.; Kirby, S. P. *Thermochemical Data for Organic Compounds*, 2nd ed.; Chapman and Hall: London, 1986.

General Approach to the Synthesis of Short α-Helical Peptides

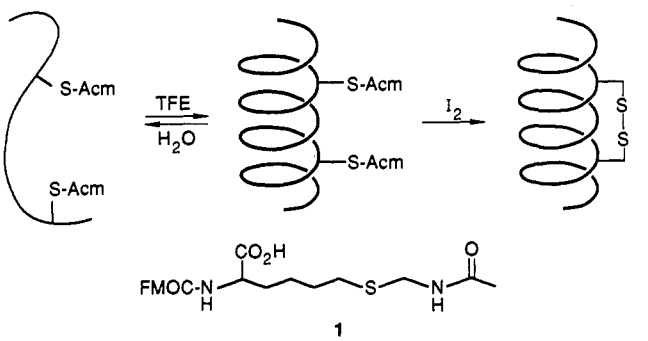
David Y. Jackson, David S. King,† Jean Chmielewski,‡ Sunil Singh,§ and Peter G. Schultz*

Department of Chemistry, University of California Berkeley, California 94720
Department of Molecular and Cell Biology University of California Berkeley, California 94720

Received August 5, 1991

The availability of stable, short α-helical peptides would provide useful frameworks for the design of biologically active small molecules as well as models for probing aspects of protein folding. Several approaches have recently been reported for stabilizing α-helical peptides including incorporation of salt bridges,[1] metal chelates,[2] or amide bonds[3] that bridge the i and $i + 4$ positions; incorporation of amino acids with high helix propensity[4] and helix caps;[5] and the formation of amphiphilic helix bundles.[6] One of the most successful strategies to date exploits the disulfide-bridged framework of the bee venom peptide, apamin, to generate chimeric α-helical peptides.[7] We now report the synthesis of short peptides containing a two-turn α-helix stabilized by a single intramolecular disulfide bond bridging the i and $i + 7$ residues (Scheme I). An eight-residue peptide containing this bridge shows high helicity in water at 0 and 60 °C.

Scheme I



1

Table I. $[\theta]_{222nm}$ for Peptides **2**–**5** (15–30 μM) in Water (0.1% TFA) at 0 and 60 °C[a]

	peptide	0 °C $-[\theta]_{222}$	0 °C f, %	60 °C $-[\theta]_{222}$	60 °C f, %
2	Ac-A-A-A-(D)1-K-A-A-A-A-K-(L)1-A-A-A-K-A-NH$_2$				
	oxidized	29 600	99	15 800	53
	protected	8400	28	5100	25
3	Ac-(D)1-K-A-A-A-A-K-(L)1-NH$_2$				
	oxidized	21 000	105	13 300	59
	protected	3100	16	2500	13
4	Ac-A-E-(D)1-A-A-A-K-F-L-(L)1-A-H-A-NH$_2$				
	oxidized	29 000	105	16 800	48
	protected	7000	25	4500	16
5	Ac-A-N-E-A-A-D-(D)1-I-A-Y-L-K-Q-(L)-T-K-NH$_2$				
	oxidized	31 100	104	14 900	49
	protected	8900	30	4500	15

[a] $-[\theta]_{222}$, mean residue ellipticity (degree·cm^2/dmol) of peptides at 222 nm. $f = [\theta]_{obsd} - [\theta]_O/([\theta]_{max} - [\theta]_O)$ = the fraction of helix. $[\theta]_{obsd}$, $[\theta]$ observed from a previous column. $[\theta]_O = 0 \pm 500$ deg·cm^2/dmol. $[\theta]_{max} = ((n - 4)/n)[\theta]_\infty$ = the maximal mean residue ellipticity value for chain length where n = the number of residues and $[\theta]_\infty = -40000$ deg·cm^2/dmol.[17]

Molecular modeling studies[8] indicated that incorporation of the deprotected forms of the D and L enantiomers of amino acid **1** at the i and $i + 7$ positions of an α-helix, respectively, could lead to intramolecular disulfide bond formation with little perturbation on helix conformation. Moreover, disulfide bonds can be formed under mild conditions, in the presence of many functional groups, and in a variety of solvents known to favor α-helix formation. The D and L forms of *N*-Fmoc-*S*-(acetamidomethyl)-2-amino-6-mercaptohexanoic acid (**1**) were synthesized via conversion of the ε-amino group of D- and L-N^α-Boc-lysine to a pyridinium salt (prepared from the corresponding pyrillium salt[9]) and subsequent displacement with 4-methoxybenzyl mercaptan. Removal of the protecting groups with TFA and reprotection with the acetamidomethyl (Acm) and Fmoc groups afforded fully protected **1**. Optical purity was confirmed by NMR analysis of the L-Ala-*O*-methyl ester derivatives.

Peptides **2**–**5** (Table I) were chosen as models to investigate the helix-stabilizing potential of an intramolecular disulfide spanning eight residues of a peptide. Peptide **2** is derived from a previously reported alanine-rich peptide[4] and contains two lysine residues that prevent intermolecular aggregation and increase water solubility. Peptide **3** is an eight amino acid truncated version of **2**, and peptide **4** incorporates essential residues of the C-peptide of ribonuclease A.[10] Peptide **5** is derived from a moth cytochrome C-peptide sequence that binds class II MHC molecules.[11] Peptides were synthesized using solid-phase methodology, protecting groups (with the exception of the Acm group) were removed with reagent K, and the deprotected peptides were purified

* Author to whom correspondence should be addressed.
† Department of Molecular and Cell Biology, University of California.
‡ Present address: Department of Chemistry, Purdue University, West Lafayette, IN 47907.
§ Present address: Affymax Research Institute, 4001 Miranda Ave., Palo Alto, CA 94304.

(1) Marquesee, S.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8898–8902.
(2) (a) Ghadiri, M. R.; Fernholz, A. K. *J. Am. Chem. Soc.* **1990**, *112*, 9633–9635. (b) Ruan, F.; Chen, Y.; Hopkins, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 9403–9404.
(3) Chorev, M.; Roubini, E.; McKee, R. L.; Gibbons, S. W.; Goldman, M. E.; Caulfield, M. P.; Rosenblatt, M. *Biochemistry* **1991**, *30*, 5968–5974.
(4) Marquesee, S.; Robbins, V. H.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5286–5290.
(5) Kemp, D. S.; Curran, J. P. *Tetrahedron Lett.* **1988**, *29*, 4935.
(6) Degrado, W. F. *Adv. Protein Chem.* **1988**, *39*, 51–124.
(7) Pease, H. B.; Storrs, R. W.; Wemmer, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 5643–5647.
(8) Minimizations were carried out using the Amber molecular mechanics program.
(9) Katritzky, A.; Yang, Y. *J. Chem. Soc., Perkin Trans. 2* **1984**, 885–889.
(10) Shoemaker, K. R.; Kim, P. S.; York, E. J.; Stewart, J. M.; Baldwin, R. L. *Nature* **1987**, *326*, 563–567.
(11) Nasseri, S. S.; McConnell, H. M. *Nature* **1989**, *337*, 274–276.

0002-7863/91/1513-9391$02.50/0

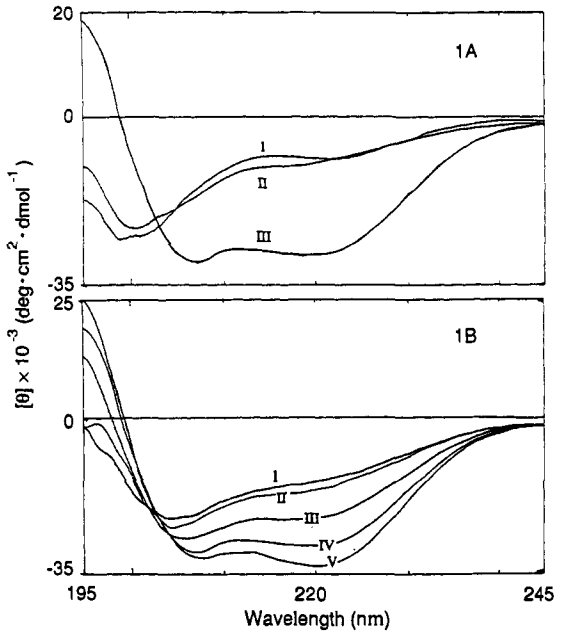


Figure 1. (A) Peptide **5** (20 μM) in H_2O (0.1% TFA) at 20 °C: I, Acm-protected; II, reduced with mercaptoethanol; III, oxidized with iodine. (B) Oxidized peptide **5** (20 μM) in H_2O (0.1% TFA): I, 80 °C; II, 60 °C; III, 40 °C; IV, 20 °C; V, 0 °C.

by reversed-phase C_{18} HPLC.[12-14] The thiol groups were then deprotected and oxidized with excess iodine in 1:1 trifluoroethanol/water, and the products were isolated by HPLC.[15]

The α-helicity of both the Acm-protected and oxidized forms of each peptide[16] was determined in water containing 0.1% trifluoroacetic acid at 0 °C by measuring ellipticity at 222 nm [the CD spectra of the reduced peptides are comparable to those of the Acm-protected peptides (Figure 1A)]. All peptides show a large increase in α-helicity upon oxidation (Table I). *Even the eight amino acid peptide* ***3***, which shows negligible helicity in the Acm-protected form, could be locked into a helical conformation using this approach. Moreover, the fact that both 16 amino acid peptides **2** and **5** show high helical content at 0 °C in their oxidized forms suggests that the disulfide bridge not only locks two turns of the helix but also efficiently propagates the helix to neighboring residues.

All four oxidized peptides retain a significant degree of α-helical character at 60 °C, whereas the Acm-protected forms are largely random coil (Table I). The temperature dependence on α-helicity was measured for peptide **5** between 0 and 80 °C (Figure 1B). A plot of $[\theta]_{222nm}$ versus temperature reveals low cooperativity in the melting behavior. This behavior, in contrast to that of typical α-helices, suggests that, upon heating, the ends of the peptide relax to a random-coil conformation while the residues within the disulfide bridge retain at least partial helicity.

In order to test the necessity of using the D enantiomer of amino acid **1** in the first position of the peptide, the corresponding L,L and L,D analogues of peptide **3** were synthesized and oxidized. The L,L analogue of **3** shows a marginal increase in helicity in the oxidized form (31% vs 11% for the oxidized and protected forms, respectively, at 0 °C) while the oxidized L,D analogue resembles a random coil/β sheet conformation.

These results demonstrate the utility of using intramolecular disulfides to stabilize helical conformations in short peptides. Disulfide bond formation can be carried out under a variety of conditions with peptides containing a wide variety of functional groups. We are currently using 2-D NMR techniques to more accurately define the structure of these peptides as well as applying this strategy to the design of α-helical peptide ligands for proteins and DNA.

Acknowledgment. We thank D. E. Wemmer and W. A. Goddard for helpful discussions. This work was supported by the Director, Office of Energy Research, Division of Material Sciences and Division of Energy Biosciences, Office of General Life Sciences, Structural Biology Division, of the U.S. Department of Energy under Contract No. DE-AC03-76-SF00098 and the Lucille Markey Charitable Trust. D.Y.J. is supported by an NIH training grant, J.C. acknowledges support by the NIH for a postdoctoral fellowship, and S.S. acknowledges the ACS for a postdoctoral fellowship.

(12) Peptides were synthesized with an ABI 431A automated synthesizer on FMOC-aminomethyl Rink resin using HBTU FMOC-amino acid activation in the presence of HOBT and diisopropylethylamine.[13] Cleavage and deprotection were accomplished with reagent K.[16] Crude peptide (80–92% purity) was purified to >98% purity by reversed-phase C_{18} HPLC (CH_3CN/H_2O/0.1% TFA). Purity and structure were confirmed by electrospray ionization mass spectrometry.

(13) Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927–30.

(14) King, D. S.; Fields, C.; Fields, G. *Int. J. Pept. Protein Res.* **1990**, *36*, 255–266.

(15) The Acm-protected peptides were dissolved in 1:1 trifluoroethanol/water (5–10 mM), and 5 molar equiv of iodine was added via addition of a 1 M stock solution in methanol. The mixture was stirred for 2 h at room temperature and concentrated. The residue was dissolved in water (0.1% TFA) and purified by reversed-phase C_{18} HPLC (CH_3CN/H_2O/0.1% TFA). Structures were confirmed by electrospray ionization mass spectrometry.

(16) Helicity was independent of peptide concentration over the range 5–50 μM.

(17) (a) Lyu, P. C.; Sherman, J. C.; Chen, A.; Kallenbach, N. R. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 5317–5320. (b) Johnson, W. C.; Tinoco, I., Jr. *J. Am. Chem. Soc.* **1972**, *94*, 4389–4390.

Conformation and Stereoselective Reduction of Hapten Side Chains in the Antibody Combining Site

Jae Il Kim, Tetsuo Nagano, Tsunehiko Higuchi, Masaaki Hirobe, Ichio Shimada, and Yoji Arata*

Faculty of Pharmaceutical Sciences
University of Tokyo, Hongo, Tokyo 113, Japan

Received July 5, 1991

We have reported multinuclear NMR analyses of the mode of interactions of ε-dansyl-L-lysine (**1**) with an anti-dansyl monoclonal antibody (**2**) raised against dansyl keyhole limpet hemocyanin.[1,2] Table I summarizes the association constants for antibody **2** with a variety of dansyl haptens with different lengths of the side chain. This result indicates that the side chain attached to the dansyl ring is recognized by the *hydrophobic subsite*[3] of antibody **2** and significantly contributes to the antigen binding. A transferred nuclear Overhauser effect (TRNOE) measurement[4] has confirmed that the lysine side chain is folded when compound **1** is bound to antibody **2**. On the basis of these results, attempts were made to use the *hydrophobic subsite* of antibody **2** as a chiral auxiliary for stereoselective organic reactions. Here we describe the result of reduction by $NaBH_4$ of 5-(dansylamino)levulinic acid (**3**) in the presence of antibody **2**. Compound **3** binds to antibody **2** with an association constant of 4.5×10^7 M^{-1}. TRNOE data have shown that the side chain of compound **3** is also folded in antibody **2**. As shown in Chart I, the reduction product was 5-[(dansylamino)methyl]-2-oxotetrahydrofuran (**5**). The *R* and *S* enantiomers of compound **5** were separated and identified on

(1) (a) Takahashi, H.; Igarashi, T.; Shimada, I.; Arata, Y. *Biochemistry* **1991**, *30*, 2840–2847. (b) Kato, K.; Matsunaga, C.; Odaka, A.; Yamato, S.; Takaha, W.; Shimada, I.; Arata, Y. *Biochemistry* **1991**, *30*, 6604–6610. (c) Takahashi, H.; Odaka, A.; Kawaminami, S.; Matsunaga, C.; Kato, K.; Shimada, I.; Arata, Y. *Biochemistry* **1991**, *30*, 6611–6619.

(2) Reidler, J.; Oi, V. T.; Carlsen, W.; Vuong, T. M.; Pecht, I.; Herzenberg, L. A.; Stryer, L. *J. Mol. Biol.* **1982**, *158*, 739–746.

(3) (a) Pecht, I.; Haselkorn, D.; Friedman, S. *FEBS Lett.* **1972**, *24*, 331–334. (b) Schweitzer-Stenner, R.; Licht, A.; Luscher, I.; Pecht, I. *Biochemistry* **1987**, *26*, 3602–3612. (c) Azuma, T.; Sakato, N.; Fujio, H. *Biochemistry* **1988**, *27*, 6116–6120.

(4) Clore, G. M.; Gronenborn, A. M. *J. Magn. Reson.* **1982**, *48*, 402–417.

0002-7863/91/1513-9392$02.50/0