Luckow, V. A., & Summers, M. D. (1988) *Bio/Technology 6*, 47-55.
Malinowski, D. P., Sadler, J. E., & Davie, E. W. (1984) *Biochemistry 23*, 4243-4250.
McLean, J. W., Tomlinson, J. E., Kuang, W.-j., Eaton, D. L., Chen, E. Y., Gless, G. M., Scanu, A. M., & Lawn, R. M. (1987) *Nature (London) 330*, 132-137.
Moore, D. (1987) in *Current Protocols in Molecular Biology* (Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., & Struhl, K., Eds.) pp 1.7.1-1.7.4, Wiley, New York.
Powell, J. R., & Castellino, F. J. (1983) *Biochemistry 22*, 923-927.
Presper, K. A., Concha-Slebe, I., De, T., & Basu, S. (1986) *Carbohydr. Res. 155*, 73-87.
Rearick, J. L., Chapman, A., & Kornfeld, S. (1981) *J. Biol. Chem. 256*, 6255-6261.
Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463-5467.
Sottrup-Jensen, L., Claeys, H., Zajdel, M., Petersen, T. E., & Magnusson, S. (1978) *Prog. Chem. Fibrinolysis Thrombolysis 3*, 191-209.
Struhl, K. (1985) *BioTechniques 3*, 452-453.
Takada, A., & Takada, Y. (1983) *Thromb. Res. 30*, 633-642.
Tarentino, A. L., Gomez, C. M., & Plummer, T. H. (1985) *Biochemistry 24*, 4665-4671.
Vieira, J., & Messing, J. (1987) *Methods Enzymol. 153*, 3-11.
Whitefleet-Smith, J., Rosen, E., McLinden, J., Ploplis, V. A., Fraser, M. J., Tomlinson, J. E., McLean, J. W., & Castellino, F. J. (1989) *Arch. Biochem. Biophys. 271*, 390-399.
Wiman, B. (1973) *Eur. J. Biochem. 39*, 1-9.
Wiman, B. (1977) *Eur. J. Biochem. 76*, 129-137.
Wiman, B., Boman, L., & Collen, D. (1978) *Eur. J. Biochem. 87*, 143-146.
Wojchowski, D., Orkin, S. H., & Sytowski, A. J. (1987) *Biochim. Biophys. Acta 910*, 224-232.
Yamashita, K., Kamerling, J. P., & Kobata, A. (1983) *J. Biol. Chem. 258*, 3099-3106.

Peptide α-Helicity in Aqueous Trifluoroethanol: Correlations with Predicted α-Helicity and the Secondary Structure of the Corresponding Regions of Bovine Growth Hormone†

S. Russ Lehrman,* Jody L. Tuls, and Marilyn Lund
Control Biotechnology, The Upjohn Company, Kalamazoo, Michigan 49001
Received November 7, 1989; Revised Manuscript Received March 1, 1990

ABSTRACT: The relationship between trifluoroethanol (TFE) enhancement of peptide α-helicity and protein secondary structure has been studied for a series of 11 peptides which span the complete primary sequence of bovine growth hormone (bGH). Ten of these peptides become increasingly α-helical as the solution concentration of TFE is increased. The amount of α-helicity developed by these peptides plateaus above 10 mol % TFE and ranges from 0 to 71%. The increased α-helicity, as determined by CD, closely correlates with the amount of α-helix predicted for eight of the eleven peptides analyzed ($r = 0.9$). Therefore, for this group of peptides, it appears that this technique can be used as a measure of α-helical propensity. Inclusion of the remaining three peptides in this analysis significantly lowers the correlation ($r = 0.6$). The reduced correspondence between TFE-enhanced and predicted α-helicity in this latter subset of peptides may be due to their relatively high hydrophobicity. In addition, the relevance of TFE-enhanced peptide α-helicity and the secondary structure of the corresponding protein regions was explored. Although the three peptides which form the largest amount of α-helicity in the presence of 10 mol % TFE correspond to α-helical regions of the protein, the overall correlation is significantly lower than is observed for the TFE-enhanced and predicted α-helicity. These findings suggest that the propensity of specific amino acid sequences for α-helix formation influences the amount of α-helicity which forms in corresponding protein sequences, but that other factors can modify this structure.

Bovine growth hormone (bGH),[1] a protein that stimulates multiple physiological responses leading to enhanced growth and lactation in vivo, is a single-domain, 191-residue protein that folds into a four antiparallel α-helix bundle (Abdel-Meguid et al., 1987). The folding mechanism of bovine growth hormone is consistent with framework and molten globule hypotheses (Kim et al., 1982; Ptitsyn, 1987; Brems et al., 1987a; Brems & Havel, 1989). These hypotheses suggest that portions of bGH should adopt native-like secondary structure in the absence of long-range interactions within the protein. However, previous studies of a large number of bGH fragments indicate that only bGH (96-133) contains stable secondary structure in aqueous solution (Chen et al., 1977; Brems et al., 1987b,c).

The low α-helical stability of bGH fragments in aqueous solvents is not unusual. A theoretical model which predicts

† A preliminary report of this work was presented at the 11th American Peptide Symposium, July 9-14, 1989, La Jolla, CA.

[1] Abbreviations: BCA, bicinchoninic acid; bGH, bovine growth hormone; CD, circular dichroism spectroscopy; FAB-MS, fast atom bombardment mass spectrometry; Gdn·HCl, guanidine hydrochloride; HPLC, high-pressure liquid chromatography; TFE, trifluoroethanol; $[\theta]_{222}$, mean residue ellipticity at 222 nm.

0006-2960/90/0429-5590$02.50/0

that protein fragments containing less than 50 residues are not expected to form stable α-helices in aqueous solution was developed some time ago (Zimm & Bragg, 1959). One notable exception to this is the α-helical stability of an NH_2-terminal 13-residue fragment from RNase A (Bierzynski et al., 1982; Kim et al., 1982). However, the conformations of protein fragments in aqueous media may not be relevant to the secondary structure of corresponding protein regions in the native or partially folded states since the latter microenvironments are, on average, less polar. Although mixed water–alcohol solvents may more closely approximate the dielectric constant of protein interiors, their use in studying the conformation of protein fragments is not widely accepted. This is due, in part, to difficulties in defining the mechanism of α-helix formation by peptides in these solvents. There are several examples of α-helical protein regions that lack well-defined secondary structure in aqueous solution as isolated protein fragments, but that become α-helical in the presence of trifluoroethanol (TFE) or 2-chlorobutanol. These peptides include the CO_2H-terminal fragment of human C3a (Lu et al., 1984), bakers' yeast iso-1-cytochrome *c* (Moroder et al., 1975), myohemerythrin C-helix peptide (Dyson et al., 1988), and α-interferon fragments (Leist & Thomas, 1984). In this paper we consider the possibility that the enhancement of peptide α-helicity by solvents such as aqueous TFE is indicative of an intrinsic propensity for peptide α-helix formation and the secondary structure of corresponding protein regions. These studies show that, for a set of bGH fragments spanning >75% of the protein primary sequence, there is a strong, positive correlation between the TFE-enhanced and predicted peptide α-helicity, but that correlations between the TFE enhancement of peptide α-helicity and the conformation of corresponding protein regions are not as significant.

Materials and Methods

Materials. Guanidine hydrochloride (Gdn·HCl), sequanal grade, was obtained from Pierce Chemical Co. (Rockford, IL). All other reagents were analytical grade. The bGH fragments studied were synthesized by standard solid-phase methods (Applied Biosystems 430A peptide synthesizer, Foster City, CA) using BOC/benzyl orthogonal protection of amino acids. Peptides were deprotected in anhydrous HF and purified by reverse-phase HPLC on C4 and C18 preparative columns, obtained from The Separation Group (Hesperia, CA). Purified peptides were characterized by analytical reverse-phase HPLC, FAB-MS (Kratos MS-50, Kratos Instruments, Ramsey, NJ), and amino acid analysis (Beckman 7300, Beckman Instruments, Palo Alto, CA).

Peptide Characterization. **bGH(1–17) (Peptide 1)**: FAB-MS (MH^+) m/z 1763. AAA: Asx 1.1 (1), Ser 2.0 (2), Gly 1.1 (1), Ala 4.2 (4), Val 0.8 (1), Met 0.9 (1), Leu 2.9 (3), Phe 2.0 (2), Arg 1.0 (1), Pro 1.0 (1). **bGH(12–34) (2)**: FAB-MS (MH^+) m/z 2665.4. AAA: Asx 1.9 (2), Thr 1.1 (1), Glx 4.1 (4), Ala 4.9 (5), Val 1.0 (1), Leu 3.0 (3), Phe 2.0 (2), His 2.0 (2), Lys 1.0 (1), Arg 2.0 (2). **bGH(27–47) (3)**: FAB-MS (MH^+) m/z 2622. AAA: Asx 2.0 (2), Thr 1.9 (2), Ser 1.0 (1), Glx 5.1 (5), Gly 1.0 (1), Ile 1.8 (2), Tyr 2.0 (2), Phe 2.0 (2), Lys 1.0 (1), Arg 2.0 (2), Pro 1.0 (1). **[Ser^{53}]bGH(43–64) (4)**: FAB-MS (MH^+) m/z 2386. AAA: Asx 1.0 (1), Thr 3.2 (3), Ser 2.8 (3), Glx 3.3 (3), Gly 1.2 (1), Ala 2.5 (2), Val 1.2 (1), Ile 2.1 (2), Tyr 1.0 (1), Phe 2.4 (2), Lys 1.2 (1). **bGH(56–80) (5)**: FAB-MS (MH^+) m/z 2750.4. AAA: Asx 2.0 (2), Thr 1.9 (2), Ser 1.8 (2), Glx 4.9 (5), Gly 1.0 (1), Ala 2.0 (2), Ile 2.0 (2), Leu 4.2 (4), Lys 2.1 (2), Arg 1.0 (1), Pro 2.1 (2). **bGH(78–95) (6)**: FAB-MS (MH^+) m/z 2084.0. AAA: Ser 2.7 (3), Glx 2.2 (2), Gly 1.2 (1), Ile 1.8 (2), Leu 6.0 (6), Phe 0.9 (1), Arg 1.1 (1), Pro 1.1 (1), Trp not determined (1). **bGH(96–133) (7)**: FAB-MS (MH^+) m/z 4343.7. AAA: Asx 4.0 (4), Thr 2.9 (3), Ser 1.7 (2), Glx 5.2 (5), Gly 3.0 (3), Ala 1.1 (1), Val 3.1 (3), Met 1.0 (1), Ile 1.0 (1), Leu 6.0 (6), Tyr 1.0 (1), Phe 1.8 (2), Lys 2.1 (2), Arg 3.0 (3), Pro 1.1 (1). ***N*-Acetyl-bGH(130–150) (8)**: FAB-MS (MH^+) m/z 2926.4. AAA: Asx 3.0 (3), Thr 3.2 (3), Glx 2.0 (2), Gly 2.0 (2), Ala 1.0 (1), Met 0.7 (1), Ile 1.0 (1), Leu 1.1 (1), Tyr 1.0 (1), Phe 1.0 (1), Lys 2.1 (2), Arg 2.0 (2), Pro 1.0 (1). **bGH(145–162) (9)**: AAA: Asx 5.0 (5), Thr 0.9 (1), Ser 0.9 (1), Gly 1.0 (1), Ala 1.0 (1), Met 1.1 (1), Leu 4.1 (4), Tyr 1.1 (1), Phe 1.0 (1), Lys 1.0 (1), Arg 1.0 (1). **[Ser^{164}]-bGH(153–180) (10)**: FAB-MS (MH^+) m/z 3339.8. AAA: Asx 3.1 (3), Thr 2.1 (2), Ser 1.9 (2), Glx 1.1 (1), Gly 1.1 (1), Ala 1.1 (1), Val 0.9 (1), Met 0.7 (1), Leu 6.1 (6), Tyr 2.1 (2), Phe 1.0 (1), His 0.9 (1), Lys 4.0 (4), Arg 1.8 (2). **[Tyr^{179}]-bGH(179–191) (11)**: FAB-MS (MH^+) m/z 1535.6. AAA: Ser 0.7 (1), Glx 1.1 (1), Gly 1.1 (1), Ala 2.3 (2), Tyr 1.1 (1), Phe 2.2 (2), Lys 1.1 (1), Arg 2.2 (2), Cys not determined (2).

Circular Dichroism Spectra. Spectra were obtained on a Jasco J-500 C spectropolarimeter (Jasco Inc., Easton, MD). Buffer solutions were prefiltered through a 0.45-μm filter. Temperature-dependent measurements were made by using a refrigerated circulating bath with an accuracy of ±0.1 °C. Concentrations of peptide solutions were determined by the bicinchoninic (BCA) protein assay (Smith et al., 1985), using BCA reagent obtained from Pierce Chemical Co. (Rockford, IL). A quartz cell of 0.1-cm path length was used when the protein concentration was ≥0.15 mg/mL. For more dilute solutions, a 1.0-cm cell was used. Unless indicated otherwise, CD measurements were made at ambient temperature. The mean residue ellipticities at 222 nm ($[\theta]_{222}$) of these peptides were calculated by using the relationship:

$$[\theta]_{222} = 100\theta/cnl$$

where θ is the ellipticity (mdeg), c is the peptide concentration (mM), n is the number of amino acids in the peptide, and l is the path length (cm). Percent helicity for the peptides was calculated from the $[\theta]_{222}$ by assuming that a 100% helical peptide has $[\theta] = -31\,500$ deg·cm^2·dmol^{-1} (Chen et al., 1972).

Predictions of Secondary Structure. Secondary structure predictions were performed by using the Chou–Fasman method which was available as part of a computer software package (DNASTAR, Madison, WI) (Chou & Fasman, 1978). Amino acid residues which are predicted to be both α-helical and β-sheet are assumed to be α-helical since intermediate-length peptides, particularly at the concentrations used in these studies, are not expected to form the latter conformation.

Results

Selection of bGH Fragments. The 11 bGH fragments synthesized for this study are shown in Figure 1. These peptides contained between 13 and 38 residues and averaged 22 residues in length. By use of the known secondary structure of porcine growth hormone (Abdel-Meguid et al., 1987), which was assumed to be closely related to the bovine structure, these synthetic bGH peptides overlap with α-helical regions of the native protein to varying extents. For example, [Ser^{164}]-bGH(153–180) corresponds closely to the CO_2H-terminal α-helix of bGH, while *N*-acetyl-bGH(130–150) does not overlap with any of the α-helical regions of this protein (Figure 1). In addition, these sequences were predicted to possess highly variable α-helicity. Therefore, selection of these sequences permitted us to statistically correlate the TFE enhancement of peptide α-helicity with the predicted and putative secondary structure of bGH.

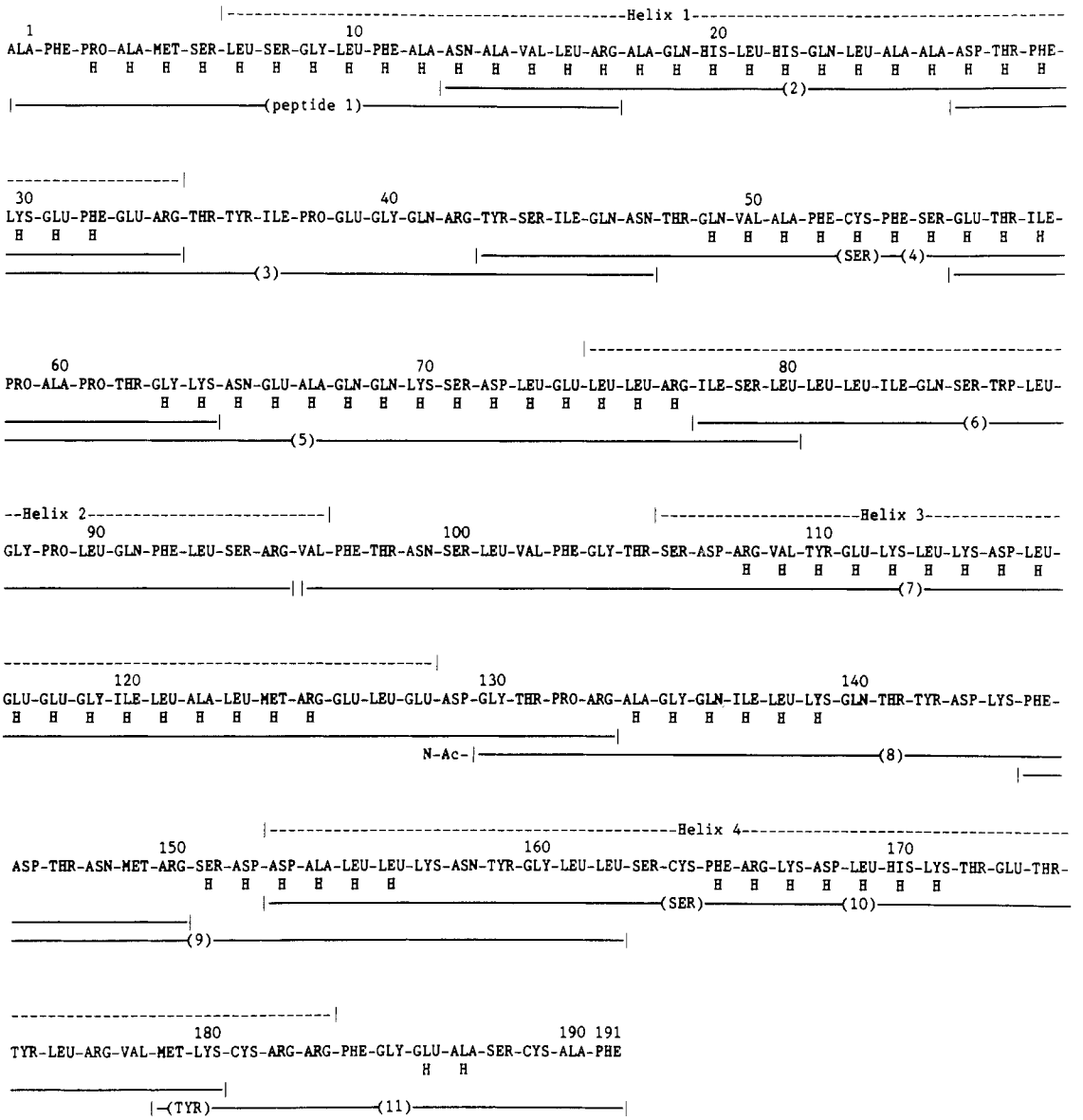

FIGURE 1: Primary sequence of bovine growth hormone. Helical regions are indicated by dashed lines above the sequence. Regions of predicted α-helicity using Chou–Fasman algorithm are noted by the letter H immediately below the primary sequence. Peptide fragments that were synthesized for use in this study are indicated by solid lines below the sequence. The synthetic peptides that correspond most closely to the four helical regions of bGH are peptides 2, 6, 7, and 10. Peptides corresponding primarily to nonhelical regions are peptides 3, 5, 8, 9, and 11.

Conformational Studies of bGH Fragments as a Function of Trifluoroethanol Concentration. The α-helicity of these synthetic peptides was determined by monitoring their $[\theta]_{222}$ (Figure 2). Full-range CD spectra (from 195 to 250 nm) were obtained for all peptides in order to confirm that other spectral features supported the presence or absence of α-helicity. In aqueous buffers, the $[\theta]_{222}$ values for nine of the peptides were greater than -2200 deg·cm^2·dmol^{-1}, and their full-range CD spectra indicated that they are nonhelical. In contrast, the $[\theta]_{222}$ values of bGH(96–133) (peptide 7) and bGH(56–80) (peptide 5) were less than -5000 deg·cm^2·dmol^{-1} and contained other spectral characteristics which indicated that these peptides are α-helical. The addition of 6 M Gdn·HCl to aqueous solutions of the latter peptides decreased their $[\theta]_{222}$ values to approximately -500 deg·cm^2·dmol^{-1}. Other studies indicated that the α-helicity of these peptides was intrinsic to their amino acid sequences, and not the result of peptide aggregation. For example, although bGH(96–133) (peptide 7) can aggregate, this occurs at concentrations above those used in this experiment (Brems et al., 1987c). In addition, the conformation of bGH(56–80) (peptide 5) did not vary as a function of peptide concentration from 0.008 to 0.3 mg/mL (data not shown).

All bGH fragments, except [Tyr^{179}]bGH(179–191) (peptide 11), became α-helical as the TFE content of solutions was increased. The latter peptide was constrained from forming an α-helix due to the presence of an intramolecular disulfide bond connecting cysteine residues between positions 3 and 11. All of the remaining peptides adopted 50% of their maximal α-helicity in about 5 mol % TFE, and greater than 90% of their maximal helicity by 10 mol % (Figure 2). The maximum α-helicity developed by these peptides varied from 0 to 71% (Table I). The peptides are listed here in order of their decreasing α-helical content in 10 mol % TFE: bGH(12–34) (2) > [Ser^{164}]bGH(153–180) (10) > bGH(96–133) (7) > bGH(56–80) (5) > bGH(1–17) (1) > bGH(78–95) (6) > *N*-acetyl-bGH(130–150) (8) > bGH(145–162) (9), [Ser^{53}]-bGH(43–64) (4) > bGH(27–47) (3) > [Tyr^{179}]bGH(179–191) (11).

Comparison of the TFE-Enhanced Conformation and

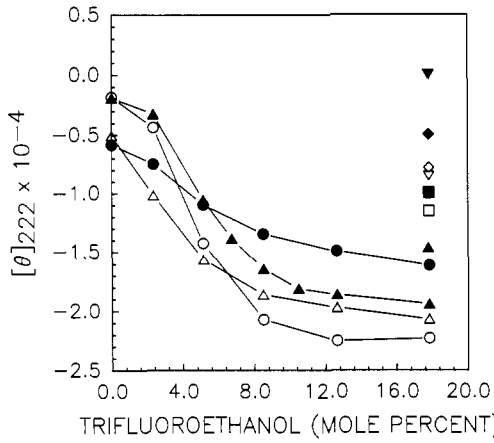

FIGURE 2: Effect of TFE on the α-helicity of synthetic bGH fragments: peptide 1 (0.007 mg/mL; filled triangle—single point); peptide 2 (0.008 mg/mL; open circles); peptide 3 (0.015 mg/mL; filled diamond); peptide 4 (0.013 mg/mL; open diamond); peptide 5 (0.018 mg/mL; filled circles); peptide 6 (0.1 mg/mL; open square); peptide 7 (0.009 mg/mL; open triangles); peptide 8 (0.026 mg/mL; filled square); peptide 9 (0.03 mg/mL; open inverted triangle); peptide 10 (0.014 mg/mL; filled triangles); and peptide 11 (0.032 mg/mL; filled inverted triangle). Spectra were obtained at ambient temperature, in 1 mM sodium phosphate, pH 7.4, and variable amounts of TFE as indicated.

Table I: Comparisons of Predicted and TFE-Enhanced Peptide α-Helicity and the Secondary Structure of the Corresponding Protein Regions

peptide	CD data[a] (%)	Chou–Fasman[b] (%)	X-ray[c] (%)
(1) bGH(1–17)	46	88	65 (1)[d]
(2) bGH(12–34)	74	91	100 (1)
(3) bGH(27–47)	16	29	38 (1)
(4) [Ser[53]]bGH(43–64)	27	45	27[e]
(5) bGH(56–80)	51	60	24 (2)
(6) bGH(78–95)	37	0	100 (2)
(7) bGH(96–133)	60	47	58 (3)
(8) *N*-acetyl-bGH(130–150)	32	29	0
(9) bGH(145–162)	27	33	61 (4)
(10) [Ser[164]]bGH(153–180)	62	25	100 (4)
(11) [Tyr[179]]bGH(179–191)	0	0	38 (4)

[a] Experimental values used for determining the percent α-helix were obtained in 10 mol % TFE. [b] Chou & Fasman, 1978. [c] Abdel-Meguid et al., 1987. [d] Numbers in parentheses indicate the helix number (as shown in Figure 1) which overlaps with each bGH fragment. [e] A short region of α-helicity appears to extend from residues 53 to 58.

Predicted α-Helicity of bGH Fragments. The amount of α-helicity that was observed in the presence of 10 mol % TFE and the predicted α-helicity for each bGH fragment are compared in Table I. The predicted α-helicity was corrected to omit helical sequences containing fewer than five amino acid residues since sequences of this length were not expected to form α-helices in solution. The predicted and TFE-enhanced α-helicity for peptides 2–5, 7–9, and 11 agreed to within 20% (Table I). For this group of peptides, the TFE-enhanced and predicted α-helicity showed a significant correlation ($r = 0.9$). Peptides 1, 6, and 10, on the other hand, had conformations in aqueous TFE that significantly diverged from the predicted α-helicity. Inclusion of these peptides in the statistical analysis reduced the overall correlation to 0.6.

The Relative Hydrophobicity of bGH Fragments. We examined the hydrophobicity of these bGH fragments to determine if this characteristic can account for the differences discussed above between peptides 1, 6, and 10 and the remaining peptides. The average hydrophobicity of each peptide was determined by using the Hopp–Woods (Hopp & Woods, 1981) or the Kyte–Doolittle (Kyte & Doolittle, 1982) methods (Table II). Both analyses indicated that peptides 1 and 6 are

Table II: Average Hydrophobicity of the bGH Fragments

peptide	Hopp and Woods[a]	Kyte and Doolittle[b]	peptide	Hopp and Woods[a]	Kyte and Doolittle[b]
1	+67	+110	7	−33	−30
2	−12	−47	8	−34	−120
3	−38	−141	9	−8	−41
4	+32	−22	10	−15	−63
5	−38	−62	11	−15	−36
6	+89	+99			

[a] Hopp & Woods, 1981. [b] Kyte & Doolittle, 1982.

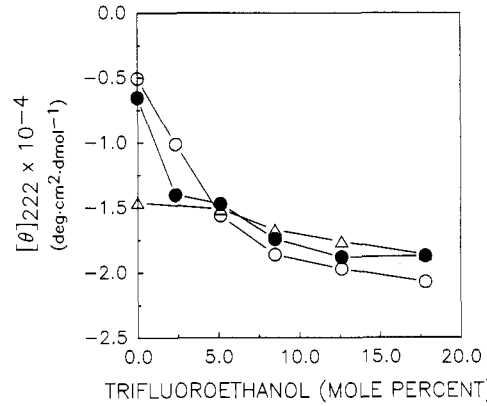

FIGURE 3: Effect of TFE on the concentration dependence of α-helicity in peptide 7. Spectra were obtained in 1 mM sodium phosphate, pH 7.4, at 0.01 mg/mL (open circles), 0.04 mg/mL (closed circles), and 0.26 mg/mL (open triangles).

significantly more hydrophobic than the other peptides included in this study. The difference in hydrophobicity between peptides 1 and 6 and the remaining peptides may be responsible for the reduced correlation between TFE-enhanced and predicted α-helicity. The reason for the weak correspondence between the TFE-enhanced and predicted α-helicity for peptide 10 does not appear to result from high hydrophobicity of this peptide.

α-Helicity of bGH(96–133) as a Function of Peptide and Trifluoroethanol Concentration. The correlation between the TFE-enhanced and predicted α-helicity of bGH fragments suggested that amino acid sequences have distinct potentials for α-helix formation. If so, factors that independently increased the amount of α-helicity of a given peptide were not expected to induce an amount of α-helicity that exceeds this potential. As shown previously, the α-helical content of bGH(96–133) is enhanced by the addition of TFE (Figure 2) or by increasing peptide concentration (Brems et al., 1987c). Therefore, the α-helicity of bGH(96–133) was determined as a function of TFE at a series of peptide concentrations. The data presented in Figure 3 indicated that the maximum $[\theta]_{222}$ of this peptide was about −19 000 deg·cm^2·dmol^{-1}, at high concentrations of either TFE or peptide. Relatively small enhancements in the α-helicity formed by this peptide were made by increasing the TFE concentration above 10 mol % at elevated peptide concentrations (0.26 mg/mL). Therefore, it appears that this peptide has a maximal potential for α-helix formation that can be attained by independently increasing peptide or TFE concentrations.

Comparison of Peptide Helicity with the Tertiary Structure of Porcine Growth Hormone. X-ray diffraction studies of porcine growth hormone (pGH) have shown that this protein contains four antiparallel α-helices which span residues 7–34, 75–96, 106–128, and 153–183 (Abdel-Meguid et al., 1987). An additional short α-helix spans residues 53–58. Because of the high sequence identity of bovine and porcine growth hormones (greater than 90%), and the high number of con-

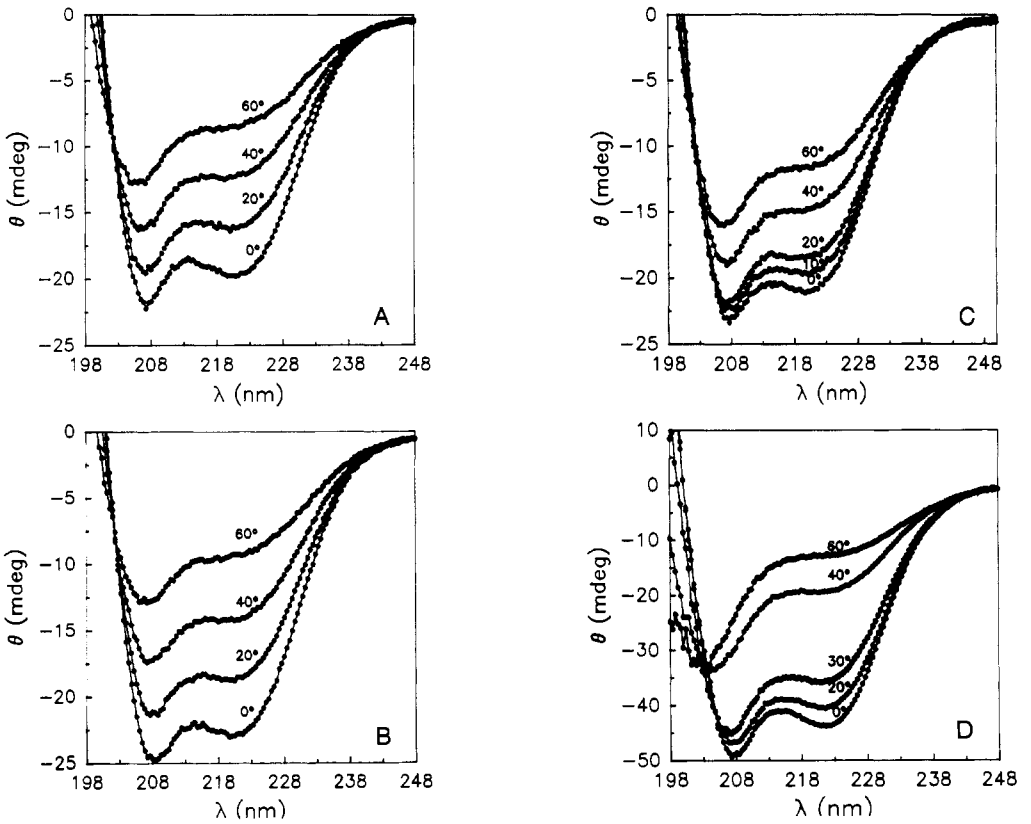

FIGURE 4: Effect of temperature on the CD spectra of the helical bGH fragments. Peptide 2 (panel A; 0.067 mg/mL), peptide 7 (panel B; 0.076 mg/mL), and peptide 10 (panel C; 0.126 mg/mL) were dissolved in 1 mM sodium phosphate, pH 7.4, containing 5 mol % TFE. Panel D shows the effect of temperature on the CD spectra of peptide 7 as in panel B (0.33 mg/mL), in the absence of TFE.

servative substitutions, it is probable that these proteins have closely related secondary and tertiary structure. We therefore compared the TFE-enhanced α-helicity of bGH fragments with the putative secondary structure of bGH (Table I). For the comparison, the percent α-helicity of the regions of bGH which corresponded to bGH fragments 1–11 were determined by dividing the number of α-helix-forming residues by the total number of residues within each protein region. Where protein-bound α-helices overlapped the peptide NH_2 and CO_2H termini of the protein fragments by five or fewer residues, the amino acid residues were not counted as α-helical since these amino acid sequences were not expected to form α-helices within the peptides. This comparison showed that the three bGH fragments which formed the most α-helix in the presence of TFE (peptides 2, 7, and 10) correspond to three of the four α-helices found in the native protein. For the complete set of bGH fragments, the correlation of α-helicity found for the bGH fragments dissolved in TFE and the protein secondary structure was weaker than had been observed in the previous comparison. Agreement between the TFE-enhanced and the bGH secondary structure is only within 20% for three of these peptides (1, 4, and 7), and the overall correlation is 0.54. When the peptides that show poor correlation with the predicted α-helicity (peptides 1, 6, and 10) were excluded from this analysis, the correlation did not improve (r = 0.52). Except for peptides 5 and 8, the TFE-enhanced α-helicity underpredicted the amount of α-helicity which was found within corresponding regions of bGH.

Temperature and pH Dependence of the TFE-Enhanced Secondary Structure. Since the bGH fragments which become most α-helical in the presence of TFE (peptides 2, 7, and 10) correspond to α-helical regions of bGH, we were interested in determining the effect of temperature and pH on their conformations. When the CD spectra of these bGH fragments

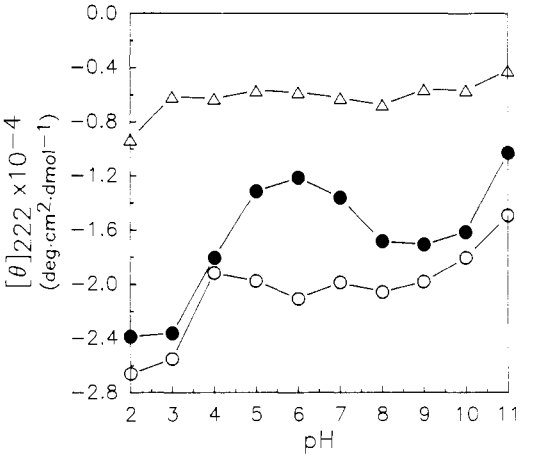

FIGURE 5: Effect of pH on the helicity of bGH peptides. Spectra were obtained at peptide 2 (closed circles), peptide 7 (open circles), or peptide 10 (open triangles) by using the solvent conditions described in Figure 4. All peptides were dissolved at a concentration of 0.01 mg/mL.

were obtained in 5 mol % TFE as a function of temperature, an isodichroic point formed at 202 nm (Figure 4, panels A–C). Therefore, α-helix formation by these peptides appeared to be fully cooperative under these conditions. The enthalpies of α-helix formation, which were determined by using the van't Hoff relationship, were −6.5, −4.5, and −5.4 kcal/mol, for peptides 2, 7, and 10, respectively.

The effect of temperature on the CD spectra of peptide 7, dissolved in aqueous buffers at concentrations where it is known to aggregate with concomitant α-helix formation, did not result in a discrete isodichroic point (Figure 4, panel D). The structural alterations that were responsible for this change in the CD spectrum of this peptide occurred between 30 and

40°. We do not understand the reason for this change in temperature behavior.

The CD spectra of these peptides, dissolved in 5 mol % TFE, were affected differently by changing the solution pH (Figure 5). Although all three peptides are more α-helical at acid pH, each peptide responded differently to this variable. For example, the α-helicity of peptides 7 and 10 were only enhanced by 40–50%, but the pH transitions were relatively sharp. For peptide 7, this transition was observed between pH 3 and 4, while for peptide 10 this transition was observed below pH 3. Although the α-helical content of peptide 2 increased by more than 200% as the pH was lowered, the transition was broad. For the latter peptide, α-helicity increased continually as the pH was lowered from 6 to 3.

Discussion

We find that most of these bGH fragments become α-helical in the presence of TFE and that the amount of α-helicity formed by each peptide is variable, reaching a maximum at about 10 mol % TFE. Since the amount of peptide α-helicity which develops in 10 mol % TFE agrees with the predicted α-helicity for eight of the eleven peptides tested to within 20%, these data suggest that TFE-enhanced α-helicity is indicative of α-helical propensity. This observed relationship is excellent, particularly since all of the bGH fragments within this series (with the exception of peptide 8) are zwitterionic, peptides 4 and 10 contain serine in place of cysteine residues at positions 53 and 164, respectively, and peptide 11 contains tyrosine in place of methionine at position 179. Eliminating charges at the NH_2 and CO_2H termini of these peptides is expected to improve the correlations. For example, preliminary studies of *N*-acetyl-bGH(12–34)-NH_2 suggest that this peptide is more α-helical than peptide 2 (data not shown), in greater agreement with the predicted α-helicity for this region. However, the α-helical content of peptides 1, 6, and 10 in aqueous TFE significantly differs from the predicted α-helicity of these peptides. Since two of these peptides are strongly hydrophobic, it appears that the relationship between TFE-enhanced α-helicity and the predicted α-helicity does not apply to hydrophobic peptides.

Another recent study has included data suggesting that the TFE enhancement of peptide α-helicity parallels predicted conformations for these peptide sequences. In this example, a RNase C analogue containing the sequence -Ala-Ala-Ala-Lys-Phe- developed 17% more α-helix in the presence of 10 mol % TFE than a peptide in which this sequence was replaced by -Ser-Ser-Ser-Arg-Tyr- (Merutka & Stellwagen, 1988). This corresponds with the higher helix-forming potential of the amino acid residues included in the former peptide.

These studies show that bGH(96–133) forms about 60% α-helix when this peptide is dissolved at high peptide or TFE concentration and that combinations of high peptide and TFE concentrations only marginally increase the total α-helicity of the peptide. Our findings suggest that these two experimental variables stabilize the α-helicity of this peptide in a similar manner. Since previous 2D NMR studies of this peptide, in 30% TFE, show that this peptide forms an α-helix extending from residues 106 to 128 (Gooley & Mackenzie, 1988), it would be of interest to determine if the same residues of this peptide become α-helical at high peptide concentration.

The TFE-enhanced α-helicity of bGH fragments and the known secondary structure of corresponding protein regions were also compared. We observed that the fragments that develop the greatest amount of α-helicity in aqueous TFE (peptides 2, 7, and 10) correspond to α-helical regions of the protein. These results suggest that lowering of the dielectric constant may be sufficient for the development of α-helical conformations in regions of high α-helical propensity.

Peptide 6, a peptide that also corresponds to an α-helical region of bGH but that is not predicted to contain a significant level of α-helicity, forms much less of this conformation in aqueous TFE than the peptides discussed above. The ability of this protein region to form an α-helix within the protein may therefore be due to long-range intraprotein interactions. Another interesting finding is that peptide 5, which is predicted to contain about 60% α-helix according to the Chou–Fasman algorithm, contains about that amount of α-helix in 10 mol % TFE, but only 24% α-helix in the intact protein. Perhaps this protein region is α-helical during early stages of bGH folding but is constrained from forming this conformation in the native protein.

These results have important implications regarding the use of predictive algorithms, such as the Chou–Fasman procedure, for the estimation of secondary structure in proteins (Yada et al., 1988). Since the correlation between the TFE-enhanced α-helicity and predicted α-helicity is more significant than the correlation between TFE-enhanced α-helicity and corresponding protein structures, it appears that the Chou–Fasman procedure is more indicative of α-helical propensities of specific amino acid sequences than their protein-linked conformations. This is reasonable since the method averages the conformational preferences of individual amino acid residues within proteins of known tertiary structure and will tend to average out unusual conformational preferences which arise from unique protein environments. Similar approaches using short peptide sequences (containing 2–9 amino acid residues) are also limited in their ability to predict protein conformation (Rooman & Wodak, 1988). This is because different protein environments can significantly influence the conformational preferences of short peptide sequences. Similar reasoning explains the ability of pentapeptide sequences to form α-helices and β-sheets within different protein environments (Kabsch & Sander, 1984).

We conclude that the α-helicity of bGH fragments in aqueous TFE is often indicative of their potential for α-helix formation and that the conformation of the corresponding protein regions is significantly influenced by other factors. These findings have focused on α-helical secondary structure and should not be applied to other types of secondary structure. Future efforts will help determine the generality of these findings with other α-helical proteins, and possibly for other types of secondary structure.

Acknowledgments

We thank Wayne Duholke for FAB-MS analyses, Lynn Snyder for amino acid analyses, Drs. Ferenc Kezdy, Eldon Ulrich, and Henry Havel for their helpful comments, and Pat Barr for secretarial assistance.

References

Abdel-Meguid, S. S., Shieh, H.-S., Smith, W. W., Dayringer, H. E., Violand, B. N., & Bentle, L. A. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 6434–6437.

Bierzynski, A., Kim, P. S., & Baldwin, R. L. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 2470–2474.

Brems, D. N., & Havel, H. A. (1989) *Proteins 5*, 93–95.

Brems, D. N., Plaisted, S. M., Dougherty, J. J., & Holzman, T. F. (1987a) *J. Biol. Chem. 262*, 2590–2596.

Brems, D. N., Plaisted, S. M., Kauffman, E. W., & Lehrman, S. R. (1987b) in *Protein Structure, Folding, and Design 2* (Oxender, D. L., Ed.) pp 151–165, Alan R. Liss, New York.

Brems, D. N., Plaisted, S. M., Kauffman, E. W., Lund, M., & Lehrman, S. R. (1987c) *Biochemistry 26*, 7774-7778.
Chen, C.-J. H., & Sonnenberg, M. (1977) *Biochemistry 16*, 2110-2118.
Chen, Y. H., Yang, J. T., & Martinez, H. M. (1972) *Biochemistry 11*, 4120-4131.
Chou, P. Y., & Fasman, G. (1978) *Annu. Rev. Biochem. 47*, 251-276.
Dyson, H. J., Rance, M., Houghten, R. A., Wright, P. E., & Lerner, R. A. (1988) *J. Mol. Biol. 201*, 201-217.
Gooley, P. R., & MacKenzie, N. E. (1988) *Biochemistry 27*, 4032-4040.
Hopp, T. P., & Woods, K. R. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 3824-3828.
Kabsch, W., & Sander, C. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 1075-1078.
Kim, P. S., & Baldwin, R. L. (1982) *Annu. Rev. Biochem. 51*, 459-489.
Kim, P. S., Bierzynski, A., & Baldwin, R. L. (1982) *J. Mol. Biol. 162*, 187-199.
Kyte, J., & Doolittle, R. F. (1982) *J. Mol. Biol. 157*, 105-132.
Leist, T., & Thomas, R. M. (1984) *Biochemistry 23*, 2541-2547.
Lu, Z.-X., Fok, K.-F., Erickson, B. W., & Hugli, T. E. (1984) *J. Biol. Chem. 259*, 7367-7370.
Marqusee, S., & Baldwin, R. L. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 8898-8902.
Merutka, G., & Stellwagen, E. (1988) *Biochemistry 28*, 352-357.
Moroder, L., Filippi, B., Borin, G., & Marchiori, F. (1975) *Biopolymers 14*, 2075-2093.
Nelson, J. W., & Kallenbach, N. R. (1986) *Proteins: Struct. Funct. Genet. 1*, 211-217.
Oas, T. G., & Kim, P. S. (1988) *Nature 336*, 42-48.
Ptitsyn, O. B. (1987) *J. Protein Chem. 6*, 273-293.
Robson, B., & Pain, R. H. (1971) *J. Mol. Biol. 58*, 237-259.
Rooman, M. J., & Wodak, S. J. (1988) *Nature 335*, 45-49.
Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., & Klenk, D. C. (1985) *Anal. Biochem. 150*, 76-85.
Yada, R. Y., Jackman, R. L., & Nakai, S. (1988) *Int. J. Peptide Protein Res. 31*, 98-108.
Zimm, B. H., & Bragg, J. K. (1959) *J. Chem. Phys. 31*, 526-535.

Rotational Diffusion Studies of the Lipoyl Domain of 2-Oxoacid Dehydrogenase Multienzyme Complexes

John P. Harrison,[‡] Ian E. G. Morrison, and Richard J. Cherry*
Department of Chemistry and Biological Chemistry, University of Essex, Wivenhoe Park, Colchester CO4 3SQ, U.K.
Received September 22, 1989; Revised Manuscript Received February 7, 1990

ABSTRACT: Rotational mobility of the lipoyl domain of a number of 2-oxoacid dehydrogenase complexes was investigated by transient dichroism after the domain had been specifically labeled with the triplet probe eosin-5-maleimide. Complexes investigated included pyruvate dehydrogenase complexes from *Bacillus stearothermophilus*, ox heart, and *Escherichia coli* (in which the E2 component had been genetically engineered to contain one lipoyl domain) and 2-oxoglutarate dehydrogenase complexes from ox heart and *E. coli*. Measurements were also performed with ox heart pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase complexes specifically labeled on E1. Anisotropy decays were recorded in glycerol–buffer solutions of varying viscosity and at different temperatures. For E2-labeled complexes, the decays were found to be multiexponential, and the fastest correlation time was considerably shorter than expected for tumbling of the whole complex. This fast correlation time was absent from E1-labeled complexes and was assigned to independent motion of the lipoyl domain. Plots of the fast correlation time against η/T showed a surprisingly weak dependence on viscosity and extrapolated to a time of 30–40 μs at zero viscosity. To explain this result, a model is proposed in which the lipoyl domain is in equilibrium between "free" and bound states. The time of 30–40 μs is shown to correspond to $1/k_{off}$, where k_{off} is the rate constant for dissociation of the domain from binding sites on the complex. This dissociation phenomenon only contributes to the anisotropy decay when the viscosity of the solution is sufficiently high to slow the tumbling of the whole complex to times that are long in comparison to $1/k_{off}$.

The 2-oxoacid dehydrogenase family of multienzyme complexes consists of pyruvate, 2-oxoglutarate, and branched-chain 2-oxoacid dehydrogenase complexes, which catalyze the oxidative decarboxylation of their respective substrates [for reviews, see Reed (1974) and Yeaman (1986, 1989)]. The constituent enzymes of the PDH[1] complex are pyruvate decarboxylase (E1), lipoate acetyltransferase (E2), and lipoamide dehydrogenase (E3). The corresponding enzymes of the 2-OGDH complex are 2-oxoglutarate decarboxylase (E1), lipoate succinyltransferase (E2), and lipoamide dehydrogenase (E3).

[‡]Present address: Department of Clinical Neurology, Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, U.K.

[1] Abbreviations: PDH, pyruvate dehydrogenase; 2-OGDH, 2-oxoglutarate dehydrogenase; TPP, thiamin pyrophosphate; NEM, *N*-ethylmaleimide; DTT, dithiothreitol; MOPS, 3-(*N*-morpholino)propanesulfonic acid; BSA, bovine serum albumin; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

0006-2960/90/0429-5596$02.50/0