Helix-Forming Tendencies of Amino Acids in Short (Hydroxybutyl)-L-glutamine Peptides: An Evaluation of the Contradictory Results from Host–Guest Studies and Short Alanine-Based Peptides†

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ABSTRACT: The helix propensities ("*s*-values") of amino acids measured using short alanine-based peptides are different, in both magnitude and rank order, from those found using random sequence copolymers of a "guest" amino acid and a (hydroxyalkyl)-L-glutamine "host". The origin of these differences is investigated here. In short alanine-based peptides containing 1–5 (hydroxybutyl)-, (hydroxypropyl)-, or (hydroxyethyl)-L-glutamines (HBQ, HPQ, and HEQ, respectively), we find the rank order of helix propensities to be Ala > HBQ > HPQ > HEQ, which is consistent with earlier results for HBQ, HPQ, and HEQ homopolymers and is attributed to helix-stabilizing hydrophobic interactions [Lotan, N., Yaron, A., & Berger, A. (1966) *Biopolymers 4*, 365–368]. The apparent *s*-values of nonpolar amino acids in a 17-residue, HBQ-based peptide cluster around 1, as they do in the host–guest studies, but in contrast to results with alanine-based peptides. The differences between the host–guest results and results obtained using alanine-based peptides may be rationalized in terms of side-chain interactions involving the hydroxyalkyl moiety.

Differences in helix propensities (*s*-values; Zimm & Bragg, 1959) among the various amino acids are small according to host–guest results (Wojcik et al., 1991), but large according to some short-peptide experiments (Padmanabhan et al., 1990; Lyu et al., 1990; O'Neil & DeGrado, 1990; Chakrabartty & Baldwin, 1993; Park et al., 1993; Chakrabartty et al., 1994). The rank order of the helix-forming tendencies of nonionizing amino acids is essentially the same in different short alanine-based peptides (Padmanabhan et al., 1990; Park et al., 1993) and in some other peptide systems (Lyu et al., 1990; O'Neil & DeGrado, 1990), although quantitative differences remain (Chakrabartty et al., 1994). Whether the differences in *s*-values among the 20 amino acids are large or small is important in regard to the mechanism of protein folding, because this issue can decide whether local sequence or tertiary interactions determine the location of α-helices in proteins. If the differences in *s*-values among the amino acids are sufficiently large, the amino acid composition of a sequence alone should decide whether it is likely to form a helix. If the differences are small, tertiary interactions are likely to determine α-helix locations.

In this study, we ask why contradictory results have been found with alanine-based peptides as compared to host–guest copolymers. One possible explanation (Marqusee et al., 1989) is that substitution of a HBQ[1] residue by a guest residue in the HBQ host results in the loss of the helix-stabilizing hydrophobic side-chain interactions involving HBQ that were postulated by Lotan et al. (1966). Recently, Vila et al. (1992) suggested that the high helix content of alanine-based peptides is actually caused by the three or four lysine residues inserted for water solubility by interfering with the hydration of the peptide backbone in the unfolded chain. This explanation requires that the Lys^+ residues have a much higher helix propensity than Ala residues, but the host–guest value of *s* for Lys^+ at 20 °C (0.94) is lower than that of Ala (1.07) (Wojcik et al., 1991).

Our approach to finding the explanation for the differences between the alanine-based peptide results and the host–guest studies is as follows. We first examine the helix-forming tendencies of (hydroxyalkyl)-L-glutamines in short alanine-based peptides. We then determine the helix-forming tendencies of nonpolar amino acids in an HBQ-based short-peptide system and compare them with those obtained in the host–guest studies and with short, alanine-based peptides. Finally, to test the explanation proposed by Marqusee et al. (1989), we search for interactions involving HBQ residues that affect helix formation.

MATERIALS AND METHODS

Peptide Synthesis and Purification. Peptides were synthesized on a Biosearch 9500 automatic synthesizer with stepwise solid-phase procedures (Stewart & Young, 1984) using a *tert*-butyloxycarbonyl (Boc)/benzyl strategy and HF cleavage. Syntheses were performed on a 0.1–0.6-mmol scale starting with N-α-Boc-N-ϵ-2-chlorocarbobenzoxylysine–MBHA (*p*-methylbenzhydrylamine (polystyrene/1% divinylbenzene) resin. For the peptides with one X, where X is a (hydroxyalkyl)-L-glutamine residue, and with three or five contiguous X's, (hydroxyalkyl)-L-glutamine (X) residues were introduced via Boc-glutamic acid with fluorenylmethyl ester (OFm) side-chain protection. Prior to HF cleavage, the OFm group was removed with piperidine, and the BOP [[(benzotriazolyl)oxy]tris(dimethylamino)phosphonium hexafluorophosphate)]-activated (Fournier et al., 1988) free carboxyl

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[1] Abbreviations: A or Ala, alanine; E or Glu, glutamic acid; HBQ, N^5-(4-hydroxybutyl)-L-glutamine; HPQ, N^5-(3-hydroxypropyl)-L-glutamine; HEQ, N^5-(2-hydroxyethyl)-L-glutamine; I or Ile, isoleucine; K or Lys, lysine; L or Leu, leucine; Q or Gln, glutamine; V or Val, valine; Y or Tyr, tyrosine; CD, circular dichroism; TFE, trifluoroethanol.

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was coupled with the appropiate amino alcohol. For all other peptides, HBQ was introduced with N-α-(fluorenylmethoxycarbonyl)-N-γ-(4-benzyloxybutyl)glutamine [Fmoc-Gln(OBzl)], which was synthesized by coupling 4-(benzyloxy)butylamine hydrochloride to N-α-Fmoc-glutamic acid α-*tert*-butyl ester with BOP or O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU) (Knorr et al., 1989) and diisopropylethylamine (DIEA) in acetonitrile (Castro et al., 1976), and the ester was hydrolyzed with TFA [mp 106–108 °C; TLC: R_f = 0.64 (chloroform:methanol:acetic acid = 85:10:5)]. 4-(Benzyloxy)butylamine hydrochloride was prepared by two methods as follows. In the first method, N-Boc-4-aminobutanol, prepared from 4-aminobutanol and di-*tert*-butyl dicarbonate, was O-benzylated with benzyl bromide and purified by chromatography on silica gel. The Boc group was removed with trifluoroacetic acid (TFA) and treated with HCl to yield 4-(benzyloxy)butylamine hydrochloride (35–40% overall yield). In the second method, 4-(benzyloxy)butyronitrile prepared from 3-(benzyloxy)propyl bromide and NaCN in dimethyl sulfoxide (Bennett & Hock, 1927; Friedman & Shechter, 1960) was reduced with borane–dimethyl sulfide (Brown et al., 1982) to yield 4-(benzyloxy)-butylamine hydrochloride (80% overall yield). The Fmoc group was removed with piperidine. After HF cleavage and desalting on G-15 Sephadex in 50% acetic acid, the peptides were purified by reverse-phase HPLC on a Vydac C-18 column, with gradients of water and acetonitrile containing 0.1% TFA. Amino acid composition was confirmed by analysis on a Beckman 6300 amino acid analyzer after hydrolysis for 22 h at 110 °C. The presence of the amino alcohols was confirmed, but the yields were not quantitated. Peptide molecular weights were confirmed by fast-atom bombardment, plasma desorption, or laser desorption mass spectrometry. The peptide with five HBQ residues was prepared by both methods and gave an identical product.

Circular Dichroism (CD) Measurements. CD measurements were made on an Aviv 60DS spectropolarimeter equipped with a Hewlett-Packard 89100A temperature control unit. The spectropolarimeter was calibrated with (+)-10-camphorsulfonic acid (Chen & Yang, 1977). CD spectra of peptides at 0 °C, 0.1 M KF (pH 7.0; 1 mM potassium phosphate), and 40–60 μM peptide were obtained using a 1-mm path length cuvette, a 0.2-nm step size, and a 1-s average time and averaged over four scans. Mean molar residue ellipticity measurements at 222 nm ($[\theta]_{222}$, deg·cm^2 dmol^{-1}) in 1 M NaCl, pH 7.0 [1 mM each of sodium citrate, sodium phosphate, sodium borate (CD buffer)] were made in 10-mm path length cuvettes (10–30 μM peptide) or in 1-mm path length cuvettes (>50 μM peptide). Trifluoroethanol (TFE) and methanol (MeOH) titrations were carried out at 0 °C, 0.1 M NaCl, and pH 7.0 using the procedure described by Nelson and Kallenbach (1988).

Calculation of Helix–Coil Transition Parameters. The apparent values of s were computed by fitting the experimental fractional helix contents to the Lifson–Roig theory (Lifson & Roig, 1961). This theory uses (ϕ, ψ) angles to define helical residues, and the fractional helicity of a peptide is determined by three parameters: n, the chain length; w, which describes helix growth or propagation; and v^2, the helix nucleation constant. According to the theory, each amino acid is characterized by unique values of w and v^2. w and v^2 are related to the corresponding parameters s and σ of the Zimm–Bragg theory (Zimm & Bragg, 1959) by the relations $s = w/(1 + v)$ and $\sigma = v^2/(1 + v)^4$ (Qian & Schellman, 1992). For the calculations in this paper, a unique value of w describes the nonpolar amino acid, X, and the remaining residues, which are Ala-rich or HBQ-rich depending on the reference peptide, are described by an average value of w. A single value of 0.0023 for v^2 (σ = 0.0019) is assumed for all residues (Rohl et al., 1992). Percent helix contents were determined from $[\theta]_{222}$ measurements using +640 and $-40000\{1 - (2.5/n)\}$ for 0% and 100% helix, respectively, n being the number of amino residues in the peptide (=17 in this study) (Scholtz et al., 1991a). The CD data were fit simultaneously using a least-squares procedure based on a binary search algorithm in conjunction with the Lifson–Roig calculations using a Silicon Graphics Iris computer. s-values obtained in this study were calculated using the basic Lifson–Roig theory without any corrections for the aromatic contribution of the N-terminal Tyr to the CD signal (Chakrabartty et al., 1993b), for N-capping effects (Chakrabartty et al., 1993a), or for charge–dipole interactions (Shoemaker et al., 1987). Thus, they represent apparent s-values. The reported host–guest data had also been analyzed without these modifications to the helix–coil theory.

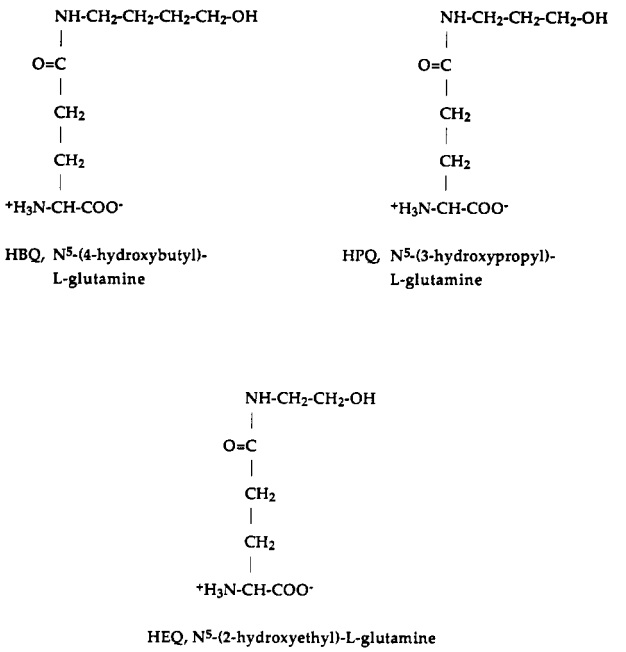

FIGURE 1: Side-chain structures of (hydroxyalkyl)-L-glutamines used in this study.

RESULTS

Peptide Design and CD Properties. The design of the peptides is based on the neutral, water-soluble alanyl-glutamine peptide reported by Scholtz et al. (1991b). The peptides contain mainly Ala and one or more (hydroxybutyl)-, (hydroxypropyl)-, or (hydroxyethyl)-L-glutamine as required (the structures of these side chains are shown in Figure 1) and the neutral, polar amino acid Gln for the solubility of the peptide in water. The single N-terminal Tyr present in all peptides is used for determination of peptide concentration from Tyr absorbance at 275 nm in 6 M guanidine hydrochloride (ϵ_{275} = 1450 M^{-1} cm^{-1}; Brandts & Kaplan, 1973). The N-terminus is acetylated and the C-terminus is amidated. The three N-terminal and the C-terminal residues are identical in all peptides, so that N-capping and C-capping effects are identical in all peptides (Forood et al., 1993; Lyu et al., 1992, 1993; Chakrabartty et al., 1993a). In addition, all peptides contain Glu next to the N-terminal Tyr and Lys at the C-terminus; these residues can stabilize helix formation by

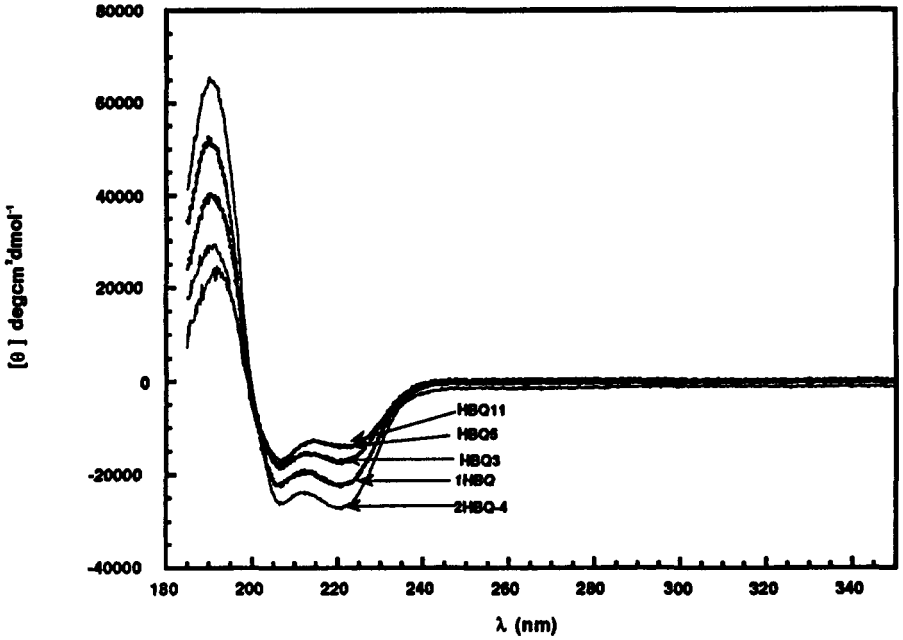

FIGURE 2: CD spectra of the peptides taken at 0 °C, pH 7.0, 0.1 M KF, 1 mM potassium phosphate, and 50–100 μM peptide. The spectra were taken in a 1-mm path length cuvette, a 0.2-nm step size, and a 1-s average time and averaged over four scans. The spectra are for peptides with (i) one HBQ residue (1HBQ), (ii) two HBQ residues spaced four residues apart with an (*i*, *i*+4) Tyr–HBQ spacing (2HBQ-4), (iii) three contiguous HBQ residues (HBQ3), (iv) five contiguous HBQ residues (HBQ5), and (v) 11 contiguous HBQ residues (HBQ11). The sequences of these peptides are shown in Tables 1 and 3.

favorable charge interactions with the helix dipole (Shoemaker et al., 1987) and also enhance water solubility. Most of our results were obtained before it was discovered that the N-terminal Tyr in a helical peptide contributes significantly to the CD signal at 222 nm and can be as much as +4400 to the $[\theta]_{222}$ for a peptide that is about 80% helical (Chakrabartty et al., 1993b). It is not known how the magnitude of this aromatic contribution depends on peptide helicity or the position of Tyr in the sequence, although the latter is accounted for by holding the position of Tyr constant in all of our peptides. For these reasons, the helix contents reported in this paper determined from $[\theta]_{222}$ are approximate.

All peptides used in the present study have CD spectra expected for mixtures of α-helix and random coil, as illustrated by the CD spectra of peptides containing HBQ in 0.1 M KF (pH 7) at 0 °C (Figure 2). The CD spectra have two minima, one at 222 nm and another at around 205 nm, and a maximum at around 190 nm; the existence of an isodichroic point near 203 nm is consistent with the presence of just two conformations for each residue, helix, and random chain (Holzwarth & Doty, 1965). The helix content is directly proportional to the mean molar residue ellipticity at 222 nm ($-[\theta]_{222}$ in units of deg·cm² dmol⁻¹) and is calculated as described in Materials and Methods. The thermal unfolding of all peptides, monitored by CD, is reversible, and the unfolding curves are superimposable at two concentrations that differ 5–10-fold, indicating that the peptides are monomeric.

Helix-Forming Tendencies of HBQ, HPQ, and HEQ in Short Alanine-Based Peptides. Table 1 shows that the helix contents of short alanine-based peptides decrease as the number of Ala to HBQ, HPQ, or HEQ substitutions increases from one to five, indicating that HBQ, HPQ, and HEQ are all helix destabilizing relative to Ala. The observed helix contents for these peptides yield *s*-values of 0.87, 0.80, and 0.67 for HBQ, HPQ, and HEQ, respectively, when the *s*-values given by Chakrabartty et al. (1994) are assigned for the remainder of the peptide (Ala 1.54, Glu^- 0.43, Gln 0.56, and Lys^+ 0.78)

Table 1: Relative Helix-Forming Tendencies of HBQ, HPQ, and HEQ

no. of X's	sequence[a]	% helix[b] X = HBQ	X = HPQ	X = HEQ
1	Ac-Y EAQA$_4$ X A$_4$QAAK-NH$_2$	68	61	57
2	Ac-Y EAQA$_2$ X A X A$_4$QAAK-NH$_2$	58	52	47
3	Ac-Y EAQA$_3$ X$_3$ A$_3$QAAK-NH$_2$	52	49	46
5	Ac-Y EAQA$_2$ X$_5$ A$_2$QAAK-NH$_2$	40	36	22
11	Ac-Y EA X$_{11}$ AAK-NH$_2$	45		

[a] CD spectra of peptides with 1, 3, 5, and 11 HBQ denoted as 1HBQ, HBQ3, HBQ5, and HBQ11 are shown in Figure 2. [b] % helix was calculated as described in Materials and Methods. Conditions: 0 °C, pH 7.0, 1 M NaCl, 1 mM each sodium citrate, sodium borate, and sodium phosphate, and 10–30 μM peptide. Error in % helix is ≤4%. The value of $-[\theta]_{222}$ for 100% helix is taken as −34 100, and the value for 0% helix is +640.

and when a homopolymer approximation is used in which all residues except the guest are described by a single average *s*-value weighted according to the number of residues (Chakrabartty et al., 1994). Thus, the rank order of helix propensities in this system is Ala > HBQ > HPQ > HEQ. This is identical to that observed in homopolymer studies, where poly(HBQ) shows the largest extent of helix formation in water, poly(HPQ) is intermediate, and poly(HEQ) shows practically no helix formation (Lotan et al., 1966). The differences in the helix stability of the HBQ, HPQ, and HEQ homopolymers were explained as resulting from the increase in helix-stabilizing, hydrophobic interactions with longer side chains (Lotan et al., 1966). The correlation between the helix stabilities of the homopolymers and the helix propensities of the isolated residues in an alanine background was not necessarily expected, because the stability of the helical homopolymer may reflect the interaction of a hydroxyalkyl moiety with neighboring side chains. In the more nonpolar trifluoroethanol/water and in methanol/water systems, various peptides containing HBQ all reach a maximum value of $-[\theta]_{222}$ = 32 500 ± 1000 (Figure 3a,b), indicating that the same maximum helix content is reached for each peptide in a

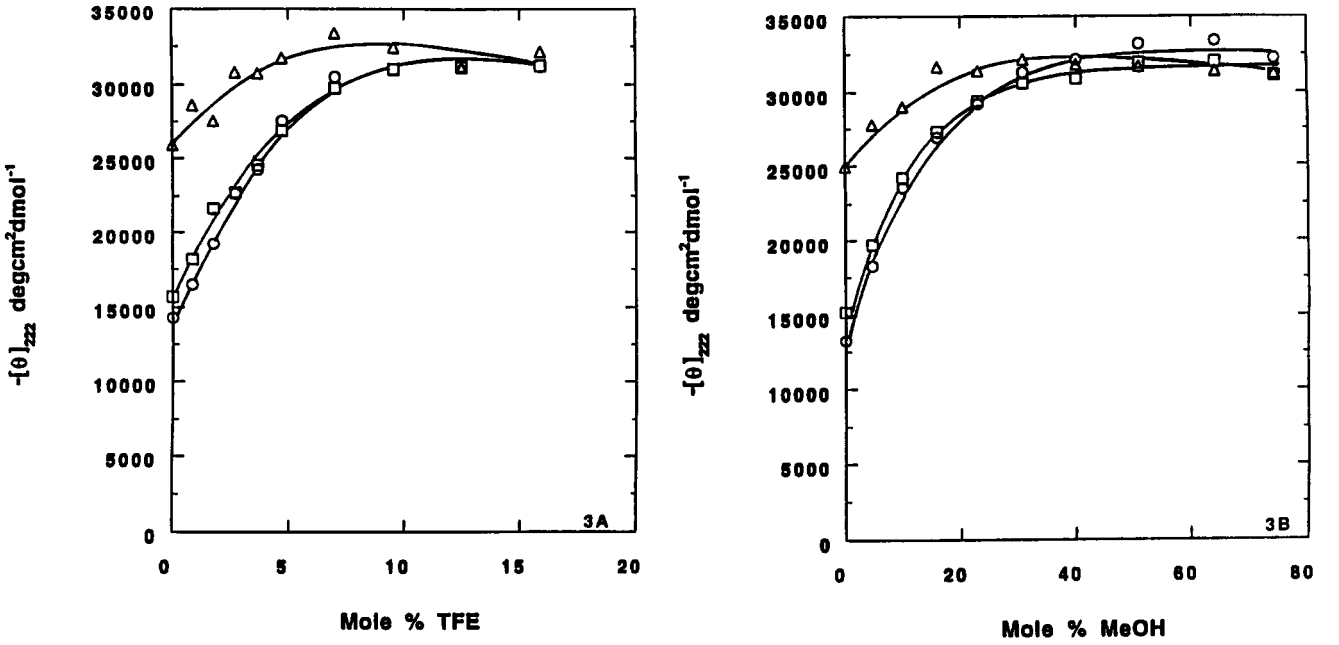

FIGURE 3: TFE and MeOH titrations for peptides with one HBQ residue (Δ), five contiguous HBQ residues (□), and 11 contiguous HBQ residues (○) at 0 °C, pH 7.0, 0.1 M NaCl, and 10–30 μM peptide. The sequences of these peptides are shown in Table 1. The curves are drawn to aid visualization, and they have no theoretical significance.

Table 2: Helix Propensities of Nonpolar Amino Acids in $(HBQ)_{11}$ Peptide[a]

sequence (X = HBQ)	% helix[b]	s_{app}[c]	$s_{H\text{-}G}$[d]	s_{AK}
Ac-Y EAX_5 X X_5AAK-NH_2[a]	45	1.29	1.04	0.87[f]
Ac-Y EAX_5 A X_5AAK-NH_2	40	0.94	1.08	1.54[e]
Ac-Y EAX_5 L X_5AAK-NH_2	44	1.21	1.10	0.92[e]
Ac-Y EAX_5 V X_5AAK-NH_2	35	0.67	0.85	0.22[e]
Ac-Y EAX_5 I X_5AAK-NH_2	42	1.06	1.37	0.42[e]

[a] The sequence of the $(HBQ)_{11}$ reference peptide is given as the first entry of the table. [b] % helix was calculated as described in Materials and Methods. Conditions: 0 °C, pH 7.0, 1 M NaCl, 1 mM each sodium citrate, sodium borate, and sodium phosphate, and 10–30 μM peptide. Error in % helix is ≤3%. The value of $-[\theta]_{222}$ for 100% helix is taken as −34 100, and the value for 0% helix is +640. [c] In HBQ11 reference (see Materials and Methods for calculation). [d] From Wojcik et al. (1991). [e] From Chakrabartty et al. (1994). [f] From this paper.

semipolar solvent, as in homopolymer studies (Lotan et al., 1966).

A striking result in Table 1 is the observation that peptide $(HBQ)_{11}$, containing 11 Ala→HBQ substitutions, is quite helical. This is contrary to the expectation, on the basis of only helix propensities and ignoring possible side-chain interactions, that $(HBQ)_{11}$ should have neglible helix content since, according to peptides with one, two, three, or five HBQ residues, the apparent *s*-value of 0.87 for HBQ is significantly lower than the value of 1.54 found for alanine by Chakrabartty et al. (1994). The significant helix content of $(HBQ)_{11}$ makes it suitable for determining the helix propensities of amino acids in an HBQ context; it also indicates that helix-stabilizing interactions involving HBQ side chains can affect the apparent helix propensities.

Helix Propensities of Nonpolar Amino Acids in an $(HBQ)_{11}$ Reference Peptide System. Table 2 shows the sequences of peptides containing a single HBQ→X substitution, where X = Ala, Leu, Val, or Ile, in $(HBQ)_{11}$; their respective helix contents and apparent *s*-values were determined using the Lifson–Roig theory. Table 2 also lists the *s*-values obtained in host–guest studies (Wojcik et al., 1991) and in short alanine-based peptides (Chakrabartty et al., 1994). The helix contents of all of the peptides differ at most by 10%, with the reference peptide, $(HBQ)_{11}$, and the peptides with a single Leu and Ile having the same helix contents within experimental error; the peptide with a single Val had the lowest helix content, and the peptide with a central Ala was intermediate. The apparent *s*-value of the 11 HBQ residues in the reference peptide is 1.29 (Table 2), when the homopolymer approximation is used together with the *s*-values given by Chakrabartty et al. (1994) for the other residues in this peptide. The *s*-values found for HBQ and for the substituted nonpolar amino acids in the $(HBQ)_{11}$ reference and in the host–guest studies all cluster around 1; $s = 1$ indicates that the residue is indifferent to helix formation, neither helix-breaking nor helix-forming. With the notable exception of Ala, the *s*-values of HBQ, Leu, Val, and Ile determined in the $(HBQ)_{11}$ reference peptide system are larger than the ones determined in Ala-based peptides. These results indicate that substitution experiments are much more sensitive to the nature of the substituted amino acid in Ala-based peptides than in either the $(HBQ)_{11}$ peptide or the host–guest system. These differences may be the result of side-chain interactions among the HBQ residues (see below).

Dependence of Helix Formation on the Spacing between HBQ Residues. Table 3 shows the measured helix contents of peptides with differing spacings between HBQ residues in the sequence. As seen from Table 3, peptides containing two HBQ residues spaced either $(i, i+1)$, $(i, i+2)$, or $(i, i+3)$, with no $(i, i+4)$ Tyr–HBQ spacing, have nearly identical helix contents. They are less helical than peptides that have both an $(i, i+4)$ Tyr–HBQ spacing and two HBQ residues spaced $(i, i+4)$, $(i, i+5)$, $(i, i+6)$, or $(i, i+7)$. Thus, HBQ spaced $(i, i+4)$ from the N-terminal Tyr probably makes a helix-stabilizing interaction similar to the one observed between Tyr and Leu or Val spaced $(i, i+4)$ (Padmanabhan & Baldwin, 1994). Tyr and a nonpolar residue spaced $(i, i+4)$ are one helical turn away from each other in an α-helix, and interactions between their side chains in a helix may result in the burial of nonpolar surface area. The lower helix content of the peptide with two HBQ residues spaced $(i, i+4)$ but without an $(i, i+4)$ Tyr–HBQ spacing, relative to the peptide with both $(i, i+4)$ HBQ–HBQ and Tyr–HBQ spacings, further corroborates the presence of a helix-stabilizing $(i, i+4)$

Table 3: Helix Contents of Peptides with Various HBQ–HBQ and Tyr–HBQ Spacings

no. of X's (X = HBQ)	sequence	% helix[a]
1	Ac-Y EAQA$_4$ X A$_4$QAAK-NH$_2$	68
2	Ac-Y EAQA$_3$ X X A$_4$QAAK-NH$_2$	60
2	Ac-Y EAQA$_2$ X A X A$_4$QAAK-NH$_2$	58
2	Ac-Y EAQA X A$_2$ X A$_4$QAAK-NH$_2$	57
2	Ac-Y EAQA$_2$ X A$_3$ X A$_2$QAAK-NH$_2$	52
2[b] (i, i+4)Y–X	Ac-X EAQ X A$_3$ X A$_4$QAAK-NH$_2$	73
2 (i, i+4)Y–X	Ac-Y EAQ X A$_4$ X A$_3$QAAK-NH$_2$	72
2 (i, i+4)Y–X	Ac-Y EAQ X A$_5$ X A$_2$QAAK-NH$_2$	69
2 (i, i+4)Y–X	Ac-Y EAQ X A$_6$ X AQAAK-NH$_2$	71
3	Ac-Y EAQA$_3$ X$_3$ A$_3$QAAK-NH$_2$	52
3 (i, i+4)Y–X	Ac-Y EAQ X A$_3$ X A$_3$ X QAAK-NH$_2$	72
8	Ac-Y EA X A X$_3$ A X$_3$ A X AAK-NH$_2$	54
8 (i, i+4)Y–X	Ac-Y EA X$_4$ AAA X$_4$ AAK-NH$_2$	52

[a] % helix was calculated as described in Materials and Methods. Conditions: 0 °C, pH 7.0, 1 M NaCl, 1 mM each sodium citrate, sodium borate, and sodium phosphate, and 10–30 μM peptide. Error in % helix is ≤4%. The value of $-[\theta]_{222}$ for 100% helix is taken as −34 100, and the value for 0% helix is +640. [b] CD spectrum of this peptide denoted as 2HBQ-4 is shown in Figure 2.

interaction between Tyr and HBQ. Similar results are obtained for peptides with three HBQ residues (Table 3): the peptide with two (i, i+4) HBQ–HBQ and one (i, i+4) Tyr–HBQ spacings has a significantly greater helix content than the corresponding peptide with no (i, i+4) Tyr–HBQ spacing. Other interactions may be present in HBQ-rich peptides, however, as is shown by the similar helix contents of two peptides, both with eight HBQ residues and identical amino acid compositions, even though one has an (i, i+4) Tyr–HBQ spacing (Table 3). This result illustrates the complexity of the HBQ-based peptide system.

DISCUSSION

Our primary aim in the present study is to reconcile the different numerical values of the helix propensities of nonpolar amino acids in host–guest studies and in short alanine-based peptides. The major differences between these two systems are the following. The short peptide systems have defined sequences and chain lengths (typically around 20 residues long) and primarily consist of alanine residues; alanine has a small, nonpolar side chain that probably is unable to participate in any significant side-chain interactions. The host–guest system consists of copolymers with random sequences and a mixture of chain lengths (typically with degrees of polymerization of 100 or more); these polypeptides chiefly contain (hydroxybutyl)-L-glutamine, whose long side chain, we have demonstrated, can participate in side-chain interactions.

In an alanine-based peptide with few (1–5) HBQ residues, HBQ is less helix stabilizing than alanine and more helix stabilizing than its analogues with shorter chain lengths, HPQ and HEQ. These results are similar to those obtained in homopolymer studies (Lotan et al., 1966), where the increasing helix propensities of HEQ, HPQ, and HBQ with increasing chain length were attributed to helix-stabilizing hydrophobic interactions. The probable interaction of Tyr with HBQ spaced (i, i+4), which is similar to the Tyr–Leu and Tyr–Val (i, i+4) interactions (Padmanabhan & Baldwin, 1994), also indicates that the side chain of HBQ is involved in hydrophobic interactions. Furthermore, the significant helix contents of (HBQ)$_{11}$ and other HBQ-rich peptides, contrary to expectations based on the apparent s-values of isolated HBQ residues in alanine-based peptides, also suggest additional helix-stabilizing interactions involving HBQ in HBQ-rich peptides. Thus, the explanation that we offer here for the differences between the s-values found in host–guest studies versus studies of alanine-based peptides is basically the explanation suggested by Marqusee et al. (1989).

The values of s are, by their intended definition, intrinsic to a given amino acid and independent of context. Their apparent variation with the choice of reference peptide indicates the importance of identifying the side-chain interactions that may occur in a given reference peptide system and affect the apparent values of s. Therefore, peptide systems with the fewest side-chain interactions are best suited to the determination of s-values. In this regard, alanine-based peptides appear to be the simplest.

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