A Thermodynamic Model for the Helix-Coil Transition Coupled to Dimerization of Short Coiled-Coil Peptides

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ABSTRACT A simple thermodynamic formalism is presented to model the conformational transition between a random-coil monomeric peptide and a coiled-coil helical dimer. The coiled-coil helical dimer is the structure of a class of proteins also called leucine zipper, which has been studied intensively in recent years. Our model, which is appropriate particularly for short peptides, is an alternative to the theory developed by Skolnick and Holtzer. Using the present formalism, we discuss the multi-equilibriatory nature of this transition and provide an explanation for the apparent two-state behavior of coiled-coil formation when the helix-coil transition is coupled to dimerization. It is found that such coupling between multi-equilibria and a true two-state transition can simplify the data analysis, but care must be taken in using the overall association constant to determine helix propensities (w) of single residues. Successful use of the two-state model does not imply that the helix-coil transition is all-or-none. The all-or-none assumption can provide good numerical estimates when w is around unity ($0.35 \leq w \leq 1.35$), but when w is small ($w < 0.01$), similar estimations can lead to large errors. The theory of the helix-coil transition in denaturation experiments is also discussed.

INTRODUCTION

α-Helix formation by monomeric short peptides in aqueous solution has been well studied (for a recent review, see Scholtz and Baldwin, 1992). It has been demonstrated that the helix-coil transition theory (Poland and Scheraga, 1970), in contrast to the simple two-state model, is required to understand the physical chemistry of these short peptides. Experiments have also shown that some short polypeptides, 28 residues or longer, can form helical homodimers which have distinct biological functions. A class of new proteins called "leucine zippers" has been intensely studied in recent years. These studies include the biological functions, chemical structures, and thermodynamic stabilities of leucine-zipper proteins (O'Shea et al., 1989, 1991; Kim, 1992). The structure of these molecules is based on a quasi-repeating heptad motif, often referred to as the "coiled-coil" structure, which was discovered 35 years ago in tropomyosin (Cohen and Parry, 1990). In the native state, both peptide chains in a dimer are almost completely α-helical. Furthermore, for short peptides, tight coupling between dimerization and the random coil to α-helix transition, i.e., a thermodynamic two-state transition, has been proposed (O'Neil and DeGrado, 1990).

A statistical mechanical model for tropomyosin dimerization has been studied extensively by Holtzer et al. (1990). To model this long polypeptide of 284 residues, several complex but necessary features were built into the model; these include two types of loop entropy and mismatch (Skolnick, 1983, 1984). The first type of loop entropy is due to "bubble" formation in a dimer, while the second type is due to forming a circle, or hairpin, by a single chain. It was shown that the helix-coil transition would be more cooperative when loop entropy is taken into account. On the other hand, the presence of mismatch causes the transition to be less cooperative. In general, however, the effect of both types of loop entropy and mismatch should diminish when peptides become shorter.

Studies on monomeric short peptides have shown that the one-helix approximation is sufficient to understand α-helix formation (Schellman, 1958; Scholtz et al., 1991), and results from experiments on single short peptides suggest that of loop entropy might be important only in a long coiled-coil dimer. The importance of mismatch is not clear in short peptides, and we have neglected this effect in the present work. Including this effect would only strengthen our argument. While we neglect these features here, at the same time we adopt the more realistic helix-coil transition model of Lifson and Roig (1961), which results in a simple and straightforward matrix formalism for the transition from random-coil to coiled-coil helical dimer. Our particular interest is in the relation between coiled-coil multi-equilibria and apparent thermodynamic two-state behavior. The appropriate interpretation of the two states, and possible errors involved in such a two-state picture, are also discussed.

Our matrix formalism is conceptually quite simple and technically accessible to anyone who has encountered conventional helix-coil transition theory. The formalism involves a large (9×9) matrix, but with the assistance of a computer this should not be an obstacle. In addition, since the matrix manipulation is done by a computer, specific sequenced peptides can be modeled. Various matrix methods have been used in modeling the helix-coil transitions of monomeric peptides (Chakrabartty et al., 1991; Qian, 1993; Scholtz et al., 1993). Comparing calculations from the

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present model with recent experimental results from O'Neil and DeGrado (1990), we find that over a wide range of parameters, a two-state transition can be observed for dimerization experiments. However, the nature of the two states is different from those invoked in the simple all-or-none picture; instead, there is an ensemble of molecular conformations in each state. The advantage of the dimerization experiment is that it provides a concrete definition of the two states involved. But one has to be cautious in equating the two-state picture to an all-or-none model.

THE MODEL

The dimer

Our model for a dimer formed by a quasi- heptad repeat (coiled-coil) is an extension of Lifson-Roig's (LR) helix-coil theory for a monomeric polypeptide (Lifson and Roig, 1961; Qian and Schellman, 1992). In the LR model each residue can be in either helical or non-helical (conformational) states, and there is thermodynamic equilibrium between the helical and non-helical states. To accommodate hydrogen bonds that span three consecutive residues, the equilibrium constant of any single residue is determined if, and only if, the conformations of the two neighboring residues are also known. These different equilibrium constants, which are also referred to as statistical (Boltzmann) weights, can be written in a matrix form called a correlation matrix. Since there are four possible conformations for a pair of residues, *cc*, *ch*, *hc*, and *hh*, the correlation matrix has dimension of 4×4, which can be reduced to 3×3 mathematically (Poland and Scheraga, 1970). We adopt the asymmetric form of the LR matrix (Qian and Schellman, 1992), since it is more intuitive for building the "zipper." The physical picture of the asymmetric LR model is as follows: for a residue in a helical conformation that has one of its two preceding neighbors in a non-helical conformation, the statistical weight is v; if both of its two preceding neighbors are in a helical conformation, the statistical weight is w; a non-helical residue always has a statistical weight u.

$$\mathbf{W}_1 = \begin{array}{c} \\ hh \\ ch \\ (h \cup c)c \end{array} \begin{array}{c} \begin{array}{ccc} h\bar{h} & c\bar{h} & (h \cup c)\bar{c} \end{array} \\ \begin{pmatrix} w & 0 & u \\ v & 0 & u \\ 0 & v & u \end{pmatrix} \end{array} \qquad (1)$$

where the bars over the c and h indicate the residues under statistical weight assignment, and $h \cup c$ indicates h or c. The corresponding end vectors $\mathbf{a}_1 = (0, 0, 1)$ and $\mathbf{b}_1^\dagger = (1, 1, 1)^\dagger$.

Because coiled-coil is a dimer with two chains, we have to deal with two residues, one on each chain, so the full matrix for the present problem is 16×16. However, just as LR's matrix can be reduced to 3×3, the 16×16 matrix for the dimer can be reduced to a 9×9 matrix. If there is no interaction between the counterparts in the dimer, the 9×9 matrix is a simple direct product of two LR 3×3 matrices. We assume that all the interhelical interactions are from the a and d residue contacts in the heptad repeat (denoted by the conventional notation a-g, see Fig. 1; see also Cohen and Parry, 1990; Holtzer et al., 1990). If a and its counterpart d are both in a helix, i.e., both have statistical weights of w, an additional statistical weight p (it is the w of Skolnick and Holtzer's notation) will be introduced to denote the interaction between the two helices. $p > 1$ further stabilizes the helices in both monomers. Therefore, for residue a (d') and d (a'), an extra factor p is introduced to the direct product of two 3×3 matrices:

$$\mathbf{W}_2 = \begin{pmatrix} w'\begin{pmatrix} wp & 0 & u \\ v & 0 & u \\ 0 & v & u \end{pmatrix} & 0\begin{pmatrix} w & 0 & u \\ v & 0 & u \\ 0 & v & u \end{pmatrix} & u'\begin{pmatrix} w & 0 & u \\ v & 0 & u \\ 0 & v & u \end{pmatrix} \\ v'\begin{pmatrix} w & 0 & u \\ v & 0 & u \\ 0 & v & u \end{pmatrix} & 0\begin{pmatrix} w & 0 & u \\ v & 0 & u \\ 0 & v & u \end{pmatrix} & u'\begin{pmatrix} w & 0 & u \\ v & 0 & u \\ 0 & v & u \end{pmatrix} \\ 0\begin{pmatrix} w & 0 & u \\ v & 0 & u \\ 0 & v & u \end{pmatrix} & v'\begin{pmatrix} w & 0 & u \\ v & 0 & u \\ 0 & v & u \end{pmatrix} & u'\begin{pmatrix} w & 0 & u \\ v & 0 & u \\ 0 & v & u \end{pmatrix} \end{pmatrix} \qquad (2)$$

where $-kT \ln p$ is the free energy of dimeric interaction, u, v, w are LR weights for one monomer, u', v', w' are for the other. The end vectors are

$$\mathbf{a}_2 = \mathbf{a}_1 \otimes \mathbf{a}_1 = (0, 0, 0, 0, 0, 0, 0, 0, 1)$$

and

$$\mathbf{b}_2^\dagger = \mathbf{b}_1^\dagger \otimes \mathbf{b}_1^\dagger = (1, 1, 1, 1, 1, 1, 1, 1, 1)^\dagger,$$

where $\otimes$ denotes direct product of matrices. Note that a row (column) vector is a non-square matrix. When $p = 1$, the two corresponding residues from each monomer are independent to each other, and mathematically the matrix in Eq. 2 can be written as direct product of two LR matrices given in Eq. 1.

$$\begin{pmatrix} w & 0 & u \\ v & 0 & u \\ 0 & v & u \end{pmatrix} \otimes \begin{pmatrix} w' & 0 & u' \\ v' & 0 & u' \\ 0 & v' & u' \end{pmatrix}$$

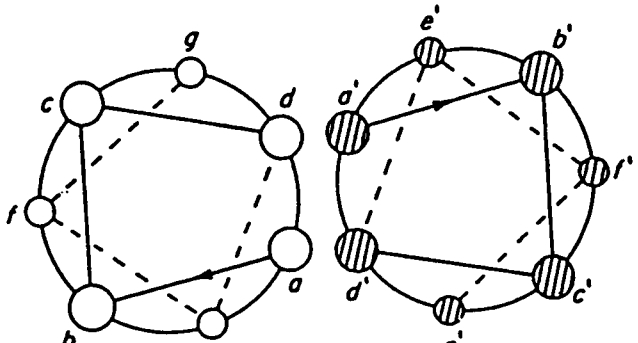

FIGURE 1 Schematic diagram for the quasi-repeating heptet "coiled-coil." The residues a (a') and d (d') are involved in the interhelical interactions. For the reside a to interact with the residue d', it is necessary that e, f, g, a are all in the helix conformation; likewise, for the residue d to interact with the residue a', it is necessary to have b, c, d all in the helical conformation. These requirements are accommodated by the asymmetric LR model, where b and c have to be in the helical conformation to have residue d in a helix. [Adapted from Cohen and Parry (1986) with permission.]

The partition functions for the monomers and dimers are given by straightforward matrix products (Poland and Scheraga, 1970):

$$Z_1 = \mathbf{a}_1\left[\prod_i \mathbf{W}_1(i)\right]\mathbf{b}_1^\dagger, \qquad Z_2 = \mathbf{a}_2\left[\prod_i \mathbf{W}_2(i)\right]\mathbf{b}_2^\dagger \tag{3}$$

where $\mathbf{W}(i)$'s are the matrix for ith residue, $1 \le i \le N$. N is the number of residues per chain, i.e., the number of C_α atoms which are flanked by peptide units on both sides (LR, 1961; Qian and Schellman, 1992). If $p = 1$, then we have two totally independent chains:

$$\begin{aligned} Z_2 &= \mathbf{a}_2\left[\prod_i \mathbf{W}_2(i)\right]\mathbf{b}_2^\dagger \\ &= (\mathbf{a}_1 \otimes \mathbf{a}_1)\left[\prod_i \mathbf{W}_1(i) \otimes \mathbf{W}_1'(i)\right](\mathbf{b}_1^\dagger) \\ &= \left\{\mathbf{a}_1\left[\prod_i \mathbf{W}_1(i)\right]\mathbf{b}_1^\dagger\right\} \otimes \left\{\mathbf{a}_1\left[\prod_i \mathbf{W}_1'(i)\right]\mathbf{b}_1^\dagger\right\} = Z_1 Z_1' \end{aligned}$$

i.e., the partition function for a system with two noninteractive subsystems can be written as the product of the partition functions of the subsystems.

The monomer-dimer equilibrium

The dimerization equilibrium constant can be introduced as (Skolnick and Holtzer, 1985):

$$K = K_0 Z_2 / Z_1^2 \tag{4}$$

where Z_1 and Z_2 are partition functions for monomer and dimer, respectively. When $p = 1$, $K = K_0$, that is to say, thermodynamically, even two non-interactive monomers have a finite association probability, that is of entropic nature. Following Skolnick and Holtzer (in their notation, u), K_0 is a geometric encounter factor which includes volume and orientation considerations. A simple estimation led Skolnick and Holtzer to choose K_0 to be 359 Å^3/molecule, thus 0.216 M^{-1}. It has been shown that calculations based on their model are not sensitive to the choice of K_0 (Skolnick and Holtzer, 1985).

Fraction of helix

Circular dichroism is the most commonly used experimental measurement for the helix-coil transition. Direct measurements of dimer formation would be very valuable in studies of coiled-coil dimerization, but unfortunately, relatively few data are available (O'Neil and DeGrado, 1990). The fraction of helicity in the monomer-dimer equilibrium system is (Skolnick and Holtzer, 1982):

$$\theta_h = g\theta_1 + (1 - g)\theta_2 \tag{5}$$

where g is the fraction of peptide, by weight, in the monomeric state:

$$g = \frac{-1 + \sqrt{1 + 8KC_0}}{4KC_0} \tag{6}$$

where K is the dimer association constant given in Eq. 4, and C_0 is the total peptide concentration. θ_1 and θ_2 are the helical fractions in monomer and dimer, respectively:

$$\theta_1 = \frac{1}{N}\left(\frac{\partial \ln Z_1}{\partial \ln w}\right); \qquad \theta_2 \approx \frac{1}{2N}\left(\frac{\partial \ln Z_2}{\partial \ln w}\right) \tag{7}$$

Combining Eqs. 1–7, for given parameters in correlation matrices (Eqs. 1 and 2), one can calculate the experimentally measurable fraction of helix by Eq. 5.

Correlation matrices for quasi-repeating heptet

We consider a 29-residue peptide which can form a parallel homodimer with four quasi-repeating heptets. To be simple, we will also assume $p = 1$ for residues other than the a's and d's, where interhelical interactions are present. We further assume that all the "host" residues having the same values of u, v, and w, except for a central residue, number 15, where various different "guests" can be introduced (cf. O'Neil and DeGrado, 1990). Similar studies on homopolymers with single hetero-residue substitutions in monomeric peptide can be found elsewhere (Chakrabartty et al., 1991; Qian, 1993).

METHODS

The formalism given in the Model section is coded in a FORTRAN program on a VAX computer. The program has similar structure as the HCONTENT program used in the studies of monomeric helix-coil transition (Chakrabartty et al., 1991; Scholtz et al., 1991). It takes a peptide sequence and a table of parameters (w, v for the 20 different types of amino acids) as input and calculates the fraction of helix for the monomer-dimer equilibrium system according to Eq. 5. The interhelical interaction parameter p is chosen to be >1 for residues at a and d positions (attractive interaction), and p = 1 (no interaction) for others. The partition functions are calculated as matrix products (Eq. 3), hence the program is suitable for heteropolymer calculations. The matrices used are described in the Model section. The calculations in this paper, however, assume a homogeneous sequence of 29 residues (the host) with a single exception at position 15 (the guest).

No fitting to the experimental data are attempted, all the theoretical calculations in this paper are for illustration.

RESULTS

Dimerization

To compare our model with O'Neil and DeGrado's experimental results, we have chosen $(w/u) = 0.85$, $(v/u) = 0.054$, and $p = 100$. Using $(v/u) = 0.054$ is in accord with the values from monomeric peptide studies in which $(v/u)^2$ ranges from 0.0023 to 0.0043, see Rohl et al. (1992) and Scholtz et al. (1991). With fixed (v/u), we find that the choices for (w/u) and p are almost unique, to mimic both the transition midpoint and its slope in dimerization experiments (O'Neil and DeGrado, 1990) and to force the transition to be as complete as possible. Table 1 also gives predictions of the present

TABLE 1 Association constant for dimer, K_a, as function of interhelical interaction parameter p*

p	$kT \ln p$ (kcal)	K_a	θ_1 (%)	θ_2(%)
100	2.76	420306	3.18	87.2
30	2.04	146	3.18	82.2
10	1.38	0.496	3.18	38.6
3.0	0.66	0.216	3.18	3.95

* The peptide has 29 residues, with $w/u = 0.85$ and $(v/u)^2 = 0.0029$. Calculations are based on Eqs. 5–7.

model for other values of p. With different residues at the central position of the chain, the transitions are markedly different (Fig. 2). These results can be represented by a two-state association transition if appropriate baselines are used (see below). The four curves in Fig. 2 are for different residues at the central (guest) position, with (w/u) being 0.01, 0.35, 0.85, and 1.35. The corresponding two-state equilibrium association constants are $10^{2.26}$, $10^{4.88}$, $10^{5.62}$, and $10^{6.0}$, respectively.

The ratios between these association constants for monomers are $(10^{5.6}/10^{4.85})^{1/2} = 2.37$ and $(10^{6.0}/10^{4.85})^{1/2} = 3.76$, while ratios between original values of w are 0.85/0.35 = 2.43 and 1.35/0.35 = 3.86. They are agree very well. However, for a different case, $(10^{2.26}/10^{6.0})^{1/2} = 0.013$ while

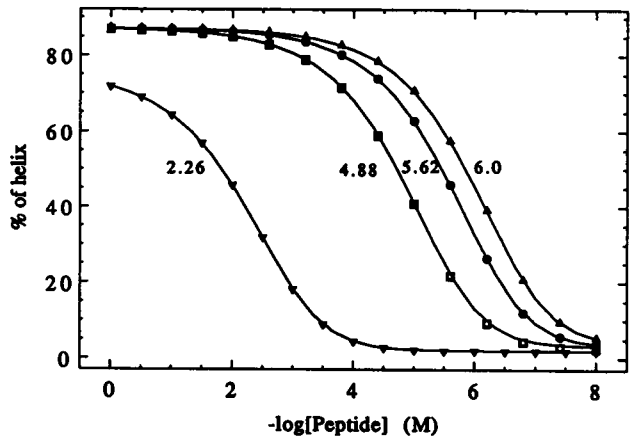

FIGURE 2 The helix content as a function of the peptide concentration. The monomeric peptide has 29 residues ($w = 0.85$, $v^2 = 0.0029$, $u = 1$) with a single guest residue at the central position. The guest residue has $w = 0.01$ (▼), 0.35 (□), 0.85 (○), and 1.35 (▲). The interhelical interaction parameter $p = 100$ for residues at a and d positions, and no interaction ($p = 1$) for others. The lines are from the simple two-state association model:

$$\theta_h = \frac{\sqrt{1 + 8K_aC_0} - 1}{4K_aC_0}\theta_1 + \left(1 - \frac{\sqrt{1 + 8K_aC_0} - 1}{4K_aC_0}\right)\theta_2$$

with association constants $K_a = 10^{2.26}$, $10^{4.88}$, $10^{5.62}$, $10^{6.0}$, and appropriate baselines $(\theta_1, \theta_2) = (2.1, 75.5)$, $(2.5, 87.1)$, $(3.1, 87.2)$, $(3.7, 87.2)$, respectively. So the ratio of w values for the host to the guest are 85.0, 2.43, 1.0, and 0.63, while the corresponding ratios of the equilibrium association constants (for each monomer) are 47.9, 2.34, 1.0, and 0.65. The results from the two-state association analysis are in good agreement with the results from our helix-coil model if the w value is ~1. When comparing this figure with O'Neil and DeGrado's Fig. 3, notice our abscissa for concentration is in M and theirs, μM.

0.01/1.35 = 0.007, and these values are markedly different. Hence, if we were using the two-state association constants to infer the ratio between values of w of guest residues, we would obtain an accurate ratio of values of w for w around 1, but we would see large deviations when w is small, as for glycine. In an extreme case: $K_a = 10^{1.82}$ when $w_g = 0.001$, hence $(10^{1.82}/10^{6.0})^{1/2} = 0.008$ while 0.001/1.35 = 0.0007; there is more than a 10-fold difference.

Two-state model versus all-or-none

The above results can be understood if we look deeper into our calculations. First, we will try to differentiate the meanings of all-or-none and two-state. In the context of coiled-coil, all-or-none is a molecular picture in which only monomers with complete random coil and dimers with 100% helicity are present in the solution at any time. With this picture in mind, the helix-coil transition and dimerization are two aspects of one transition, and partially helical peptides are never populated in the solution. On the other hand, a two-state transition is often experimentally defined. If we can classify every molecular conformation into two, and only two, groups, a two-state system is defined (Table 2). For experimentalists to observe a transition between these two states, the requirement is that the fluctuation within each state should be small compared with the mean structural difference between the two states (cf. Lumry et al., 1966). The baseline problem in data analysis for conformational transitions reflects the fluctuations within each state.

We now return to our dimerization calculation. We see that if we treat θ_1 and θ_2 in Eq. 5 as experimental values for monomer and dimer, the measurable quantity θ_h will represent perfect dimerization with the correct association constant. In other words, fitting the dimerization experiments by a two-state association model will give us the correct value for K in Eq. 4. In fact, $-RT\ln K$ is the free energy difference between two molecular ensembles: the monomers and the dimers. Notice that, within each ensemble, individual molecules have different helicity, hence θ_i ($i = 1, 2$) is the mean value for its ensemble. However, neither of them changes with total peptide concentration. Furthermore, they are relatively insensitive to guest substitutions (Table 2).

If we denote K and K' as dimerization constants for different peptides with different guest residues, from Eq. 4

TABLE 2 Association constant for dimer, K_a, as function of helix propensity (w_g) of the guest residue*

w_g	K_a	$\sqrt{K_a}$	θ_1 (%)	θ_2 (%)
0.001	66.8	8.17	2.05	60.5
0.01	182	13.5	2.06	75.5
0.1	6829	82.6	2.18	86.5
1.0	572663	756.8	3.32	87.2
10.0	28193353	5309.7	11.04	87.3

* The host peptide has 29 residues, with $w/u = 0.85$, $(v/u)^2 = 0.0029$, and $p = 100$. Calculations are based on Eqs. 5–7.

we have:

$$\ln(K'/K) = \ln(Z_2'/Z_2) - 2\ln(Z_1'/Z_1)$$

where Z_1 and Z_1' are partition functions with only a single residue difference. We can write (Qian, 1993):

$$Z_1 = a + bw \quad \text{and} \quad Z_1' = a + bw'$$

where w and w' are helix propensities for different guest residues. Then:

$$\frac{Z_1'}{Z_1} = \frac{a + bw'}{a + bw} = \frac{1 - bw/(a + bw)}{1 - bw'/(a + bw')} = \frac{1 - \theta_g}{1 - \theta_g'}$$

where θ_g and θ_g' are probabilities of guest (single) residues being in a helical conformation. It is clear that for monomers with very little helicity, these probabilities are on the order of few percent, hence $Z_1'/Z_1 \approx 1$. A similar analysis would show $Z_2'/Z_2 \approx (w'/w)^2$ since the dimer is highly helical; hence its central residue should have an even higher probability to be helical.

We therefore have a relationship between the errors in the estimation for helix propensity and the helicity of the monomer and the dimer. For a true all-or-none system, equation $Z_2'/Z_2 \approx (w'/w)^2$ becomes exact.

An interesting corollary from the above discussion is that estimation for large w should be more accurate than for small w. This is because for large w, θ_g should be small in the monomer as it is in denaturing conditions, hence both $Z_1'/Z_1 \approx 1$ and $Z_2'/Z_2 \approx (w'/w)^2$ are good approximations. For small w, however, θ_g may not be large in the dimer, even if its neighbors all have high values of helical probability (Qian, 1993).

Denaturation

The above results simulate situations in solution with 5 M urea. We now consider denaturation experiments according to the present model. We assume that the links between our model and denaturant stem only from p and (w/u), which are intrinsic equilibrium constants. It has been widely observed that free energies are linear functions of denaturant concentration (Schellman, 1987); thus for interhelical interaction p, host residue w_h, and guest residue w_g, we have assumed:

$$p = \alpha_p \exp[-\beta_p([D]\text{-}5)]$$

$$w_h = \alpha_h \exp[-\beta_h([D]\text{-}5)]$$

$$w_g = \alpha_g \exp[-\beta_g([D]\text{-}5)]$$

where $u = 1$ without loss generality, and [D] is denaturant concentration; $\alpha_p = 100$ is the p value when [D] = 5 M. We have chosen [D] = 5 M as the reference point, since it is the midpoint of the transition in O'Neil and DeGrado's experiment. The β values characterize the denaturant dependence of each equilibrium constant. Experimentally, there is an overall β for the denaturant dependence of the entire peptide. An examination of O'Neil and DeGrado's Fig. 2 *B* reveals that the overall β for the whole dimer $\approx$ 1 kcal/M = 1.67 *RT*/M. For a rough estimation of α_g, we take alanine for example: alanine has $w \approx 1.35$ when [D] = 5 M, and $w \approx 2.25$ when [D] = 0 M, so its β is only around 0.1 *RT*/M, which is much smaller than the overall β. The overall β is accumulative from each individual residue, hence the β_g contribution from the guest residue can be negligibly small. Here is a subtle point: if the overall denaturant dependence overwhelms that of w values, the overall denaturant dependence would be a set of parallel lines. However, the difference between the values of β_g for different guests is crucial for extrapolation to zero denaturant concentration (see below and Discussion).

For simplicity, we first assume that w for both host and guest residue have same denaturant dependence, $\beta_h = \beta_g$. The selections of β_p and β_h is made to mimic Fig. 2 of O'Neil and DeGrado, but otherwise these choices are arbitrary. Our Fig. 3 shows that when β_g is relatively small ($\approx$0.03 *RT*/M) for the guest residue, a set of parallel lines is obtained, with overall $\beta \approx 2$ *RT*/M. Even when β_g for the guest residue is as large as -0.3 *RT*/M (inverted triangle), the deviation from the parallel set is still minor. Note that the denaturant dependence is reversed in this case, hence the extrapolation based on overall β will lead to a large error.

DISCUSSION

In our simple coiled-coil model, both monomeric and dimeric loop entropy and mismatch have not been considered. These factors are important for long chains like tropomyosin (Skolnick and Holtzer, 1985), but for short peptides, they may be neglected, just as in the monomeric case, the one-sequence approximation is adequate (Scholtz and Baldwin, 1992). On the other hand, in contrast to Skolnick and Holtzer's model, which used a simplified Zimm-Bragg (2×2) model for the helix, we have used a more realistic model for the helix based on Lifson-Roig's theory. The comparison between these helix-coil transition models has been discussed by Qian and Schellman (1992). The electrostatic interaction between residues e and g has also been omitted from our formula, but it would not be difficult to incorporate it in the future. Using an expanded matrix formalism, electrostatic interactions have been incorporated in interpreting experiments on monomeric helix-coil transitions (Scholtz et al., 1993).

The general conclusion from this model is that coiled-coil dimerization can be a two-state transition, but one should not relate the two states to an all-or-none picture. Only when w is around 1 does the all-or-none interpretation provide a numerically accurate estimation for the helix propensity w (Fig. 4 and Table 2). The estimation for very small w can be off by a factor of 10. Our results show that two-state transition can be invoked in dimerization, but to interpret the two states as all-or-none is problematic. Quantitatively, one can use Eqs. 5 and 6 to analyze dimerization data and obtain the association constant K_a, but to equate K_a with the product of values of w of all the individual residues is an assumption which might not be valid for some peptides. It is also shown that changes in β caused by various values of w can be

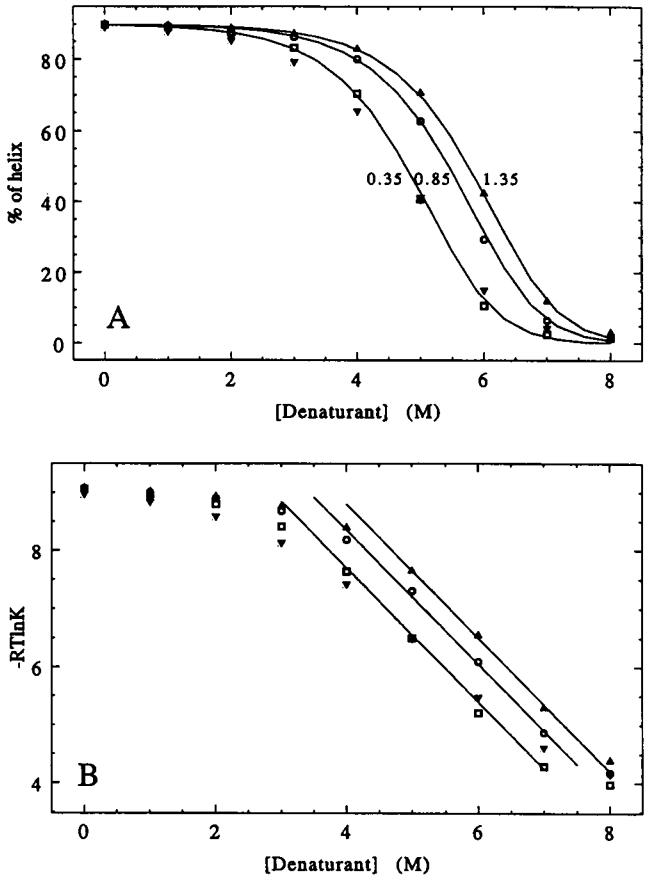

FIGURE 3 (*A*) Helical content as a function of the denaturant concentration, [D]. The same peptides as in Fig. 2 are used. Different symbols are for different guest residues with w = 1.35 (△), 0.85 (○), and 0.35 (□) at 5 M [D]. Peptide concentration is 10^{-5} M. The denaturant dependence is assumed to be linear for ln p and ln w: ln p = 5.36 − 0.15[D]; ln w = ln w_0 − 0.0325[D], where w_0 = 1.59 (△), 1.0 (○), and 0.41 (□) at 0 M [D], respectively. The solid lines are calculated from a simple two-state model using association constant K_a (kcal/mol), with the corresponding ΔG° (= RTln K_a) as a linear function of denaturant concentration: RTln K_a = 14.6 − 1.32[D], 14.2 − 1.32[D], and 13.3 − 1.32[D] from top to bottom, respectively. ▽, for the same host residue, but assuming very different denaturant dependence for the guest residue: ln w = −2.3 + 0.25[D], which gives w = 0.35 at 5 M [D], but 0.1 at 0 M [D], that means a reversed denaturant dependence. (*B*) For each denaturant concentration, ΔG° in kcal/mol was calculated by a two-state model (*symbols*); *lines* are linear fit to the symbols in transition regions (equations for these lines are 24.91 − 1.15[D], 24.46 − 1.15[D], and 23.81 − 1.15[D], respectively).

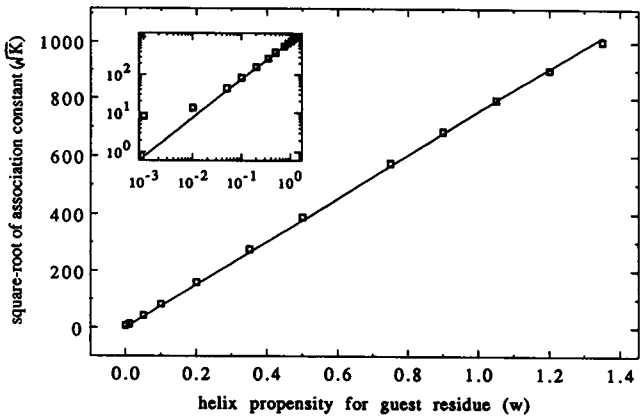

FIGURE 4 The correlation between square-root of the association constant ($\sqrt{K_a}$) and the helix propensity of the guest residue (w_g). The host peptide has 29 residues with w = 0.85, v^2 = 0.0029, u = 1, and p = 100. The line is the best least-squares fit to the symbols: $\sqrt{K_a}$ = 754.17 w_g. If this linear relationship were accurate, then we would have $\sqrt{K_a'}/\sqrt{K_a} = w_g'/w_g$. However, it is shown in the inset with logarithm scales that for small w_g, the linear relationship is no longer valid. In fact, in the most extreme case: $\sqrt{K_a}$ = 1004 when w_g = 1.35, and $\sqrt{K_a}$ = 8.17 when w_g = 0.001. Hence their ratios are 122.9 and 1350, respectively. There is a more than 10-fold underestimation from association constants (Table 2).

overwhelmed by the large contribution to β from p, the interhelical interaction; hence a set of parallel denaturant denaturation curves can be observed.

Another interesting point that comes out of the helix-coil transition model for a dimer helix is that even the dimer should not be 100% helix at a given temperature. This is in sharp contrast to the behavior of a true two-state transition. Therefore, temperature denaturation experiments will improve our understanding of coiled-coil dimerization. The low temperature limit can provide a better estimate of the circular dichroism signal for 100% helix. Correct evaluation of the experimental base line in circular dichroism measurements is essential for analyzing the data of helix-coil transitions of short peptides.

For short monomeric peptides, it has been demonstrated that two-state description is not an appropriate model for α-helix formation in general. The unique property of the coiled-coil system is that the helix-coil transition is coupled to a two-state transition, which is well defined. Because of this coupling, an accurate association constant can be obtained from experimental data. In some experiments with monomeric peptides, two states can also be defined rigorously. For example, if we can monitor the helix probability of a single residue, then a meaningful two-state equilibrium constant can be obtained. Such an equilibrium constant can be directly related to the helix propensity of the single residue (Qian et al., 1994).

A theoretical study based on Skolnick and Holtzer's model for thermal denaturation of coiled-coil helices has also shown two-state behavior. It was suggested that such behavior is caused by some combination of the temperature dependence of w for different amino acid residues (A. Holtzer, R. Fairman, and W. F. DeGrado, personal communication).

The intrinsic helix propensity of alanine to glycine

Based on different experimental systems, O'Neil and DeGrado (1990) and Chakrabartty et al. (1991) have obtained quite different values for the ratio of w_{ala}/w_{gly}. If we accept the common premise that intrinsic helix propensities

do exist for each amino acid, there are several possible explanations for this large difference. 1) It can be shown that using the homopolymer assumption for the host peptide, the ratio of helix propensities of two substituted residues at a single guest position in a monomeric peptide can be overestimated (data not shown). 2) If coiled-coil dimerization can indeed be represented by an all-or-none model in 5 M denaturant, but not in the native (0 M denaturant) condition, the extrapolation should be made for each residue separately, and the value of β_g for different amino acid residue might be different. 3) We see that the disagreement between O'Neil and DeGrado and Chakrabartty et al. is largest for w_{ala}/w_{gly}, where alanine is known to have a large w and glycine is a strong helix breaker. Their experimental results for other amino acids with w values of $\sim$1 are in fact more consistent. Our results show that it is possible that in the extreme case of small w for glycine, the all-or-none model might fail. 4) A recent experiment also shows that trimers are involved in O'Neil and DeGrado's experimental system. However, dimerization and equilibrium sedimentation studies have shown that peptide is primarily dimeric at the condition of O'Neil and DeGrado's experiment (cf. Lovejoy et al., 1993).

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