# Effects of charged amino acids at *b* and *c* heptad positions on specificity and stability of four-chain coiled coils

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**Abstract**

An understanding of the balance of chemical forces responsible for protein stability and specificity of structure is essential for the success of efforts in protein design. Specifically, electrostatic interactions between charged amino acids have been explored extensively to understand the contribution of this force to protein stability. Much research on the importance of electrostatic interactions as specificity and stability determinants in two-stranded coiled coils has been done, but there remains significant controversy about the magnitude of the attractive forces using such systems. We have developed a four-stranded coiled-coil system with charged residues incorporated at *b* and *c* heptad positions to explore the role of charge interactions. Here, we test quantitatively the effects of varying sidechain length on the magnitude of such electrostatic interactions. We synthesized peptides containing either aspartate or ornithine at both *b* and *c* heptad positions and tested their ability to self-associate and to hetero-associate with one another and with peptides containing glutamate or lysine at the same positions. We find that interactions between glutamate and either lysine or ornithine are more favorable than the corresponding interactions involving aspartate. In each case, charged interactions provide additional stability to coiled coils, although helix propensity effects may play a significant role in determining the overall stability of these structures.

**Keywords:** Four-chain coiled coil; electrostatic interactions; protein design

Protein design offers the possibility of creating proteins with functions unprecedented in nature that may be applied to such programs as the development of catalysis of important chemical reactions and the creation of biomaterials for nanotechnology. Whereas there has been increased success recently in the design of proteins on the basis of first principles, particularly by use of computer-guided approaches, much has yet to be learned about the balance of chemical forces that dictate protein folding and stability. In particular, the relative importance of the hydrophobic force, packing interactions, and specific electrostatic interactions on protein stability and specificity are yet to be worked out. In all likelihood, these forces will need to be evaluated for each type of structural motif.

One example of a well-characterized structural motif is the coiled coil. As a result of many studies, we are beginning to understand the rules that govern important biophysical parameters that describe several features of coiled coils, including their oligomerization state (i.e., dimers, trimers, and tetramers), heterospecificity, parallel versus antiparallel orientation of helices, stability, and folding pathway. Here we focus on the contributions of interactions between different charged amino acids on the stability of a tetrameric, antiparallel coiled-coil model system based on the tetramerization domain of the Lac repressor (Fairman et al. 1995).

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*Abbreviations:* CD, circular dichroism; FMOC, 9-fluorenylmethyloxycarbonyl; MALDI-TOF, matrix-assisted laser desorption ionization-time of flight; RP-HPLC, reversed-phase-high pressure liquid chromatography; TFA, trifluoroacetic acid; TFE, trifluoroethanol.

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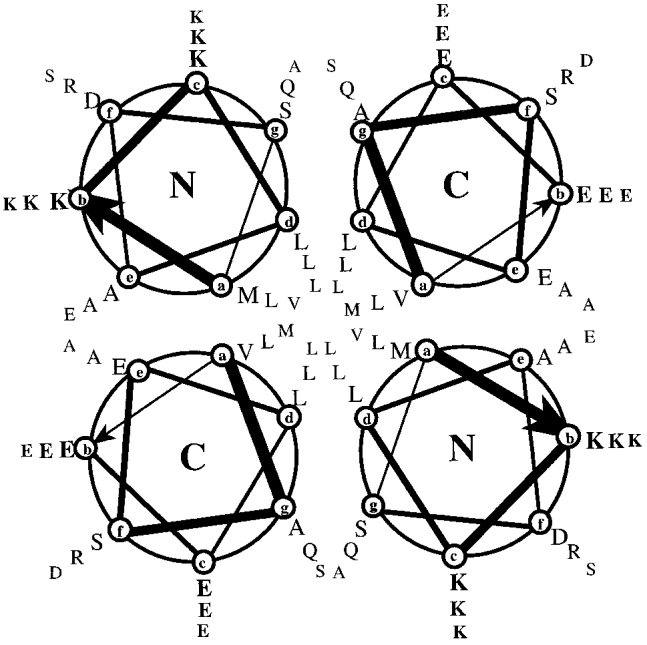
Previously, we have shown that a peptide containing glutamates placed at *b* and *c* positions of the heptad repeating unit (Fig. 1) can interact favorably with a peptide containing lysines placed at these same positions, to form a stable heterotetrameric coiled coil (Fairman et al. 1996). Either peptide alone only weakly self-associates, presumably because of electrostatic repulsion between like charges in the coiled-coil structure.

Here, we probe more deeply the distance dependence of the interhelical charged interaction by varying the sidechain length of the charged residue. We quantify and compare the charged interactions between the acidic residues, glutamic acid and aspartic acid, and the basic residues, lysine and ornithine. The rationale for choosing aspartic acid and ornithine as an expansion of our earlier study is to determine whether there is a correlation between the number of sidechain methylene groups and the strength of the charged interaction.

In response to recent discussions in the literature regarding the importance of added salt to the strength of charged interactions in dimeric coiled coils, we look at the difference of charged interactions in both 0 M and 0.1 M NaCl. This discussion has focused primarily on the stability of dimeric coiled coils, particularly on interactions between the *e* and *g* heptad positions in such coiled coils. Different interpretations of such studies stem from the finding that dimeric coiled coils with charged residues at *e* and *g* positions can show greater stability at low pH, where residues such as glutamic acid or aspartic acid are protonated and uncharged (Lumb and Kim 1995). This controversy has been settled, in part, by Hodges' group, who showed that the pH dependence of stability is governed by the amount of salt present (Yu et al. 1996). They showed that, in the absence of any salt, coiled-coil stability can be greater at neutral pH than under acidic conditions, and that this effect reverses with the addition of as little as 10 mM salt, suggesting that such interactions are effectively screened by even low concentrations of salt. Important work from Vinson's group (Krylov et al. 1998) has quantified the salt effect on the strength of charged interactions in dimeric coiled coils. These conclusions have been confirmed most recently by work from Bosshard's group (Marti et al. 2000) in their nuclear magnetic resonsance (NMR) structural studies on a designed dimeric coiled coil. They concluded that the electrostatic energy that is gained from charged interactions at neutral pH are surpassed by the gain in hydrophobic energy of protonating a charged, glutamate residue near the hydrophobic core, thus explaining the apparently anomalous pH dependence observed earlier. Our previous study of charged interactions at *b* and *c* positions of a tetrameric coiled coil, done in the absence of salt, showed clearly that such coiled coils are less stable at low pH (Fairman et al. 1996), suggesting that, on the basis of Bosshard's interpretation, our charged sidechains may be significantly more solvent exposed.




Peptide sequences:

| | abcdefg abcdefg abcdefg |
|---|---|
| Lac21: | Ac-MKQLADS LMQLARQ VSRLESA-$CONH_2$ |
| Lac21E: | Ac-MEELADS LEELARQ VEELESA-$CONH_2$ |
| Lac21D: | Ac-MDDLADS LDDLARQ VDDLESA-$CONH_2$ |
| Lac21K: | Ac-MKKLADS LKKLARQ VKKLESA-$CONH_2$ |
| Lac21O: | Ac-MOOLADS LOOLARQ VOOLESA-$CONH_2$ |

**Fig. 1.** Helical wheel diagram showing the charged interactions between glutamate and lysine at *b* and *c* heptad positions. Peptide sequences are shown as well. The nomenclature for the peptides (Lac21) suggests their origin from the Lac repressor and the length of individual helices. All peptides are acetylated at the amino-terminal ends and amidated at the carboxy-terminal ends.

## Results and discussion

We studied the distance dependence of charged interactions in our coiled-coil model system by comparing interactions between the negatively charged amino acids glutamate and aspartate with the positively charged amino acids lysine and ornithine. Aspartate and ornithine are one methylene unit shorter than glutamate and lysine, respectively. The sequences of the peptides used here are given in Figure 1 and are based on an earlier study focusing on the glutamate-lysine interaction (Fairman et al. 1996).

We synthesized peptides containing either aspartic acid or ornithine at all *b* and *c* heptad positions using standard FMOC chemistry as described in the Materials and Methods section. These peptides, along with peptides synthesized earlier containing either glutamic acid or lysine at equivalent positions, were used for the studies described below. We characterized the molecular weights of all peptides and

peptide mixtures using equilibrium sedimentation analytical ultracentrifugation either individually, or in combination (Lac21E/K, Lac21D/K, Lac21E/O, and Lac21D/O). Species analyses of data collected at multiple rotor speeds are consistent with each of the samples being either monomeric, tetrameric, or in equilibrium between these two states (data not shown).

We measured the stabilities of the peptides, either individually, or in combination, using circular dichroism (CD) as a probe of the monomer–tetramer equilibrium. Justification of the use of CD to quantify coiled-coil stabilities has been provided by several labs (O'Neil and DeGrado 1990; Thompson et al. 1993), including our own (Fairman et al. 1995; Boice et al. 1996). Spectra collected at constant total peptide concentration show that all peptides, when looked at individually, are largely unfolded with the exception of Lac21E, which shows residual alpha-helix content (Fairman et al. 1996). In contrast, any combination of negatively charged and positively charged peptides results in a significant increase in helix content (Fig. 2).

To quantify the stabilities of these heteromeric interactions, we measured their free energies at 25°C from thermal unfolding experiments. Thermal unfolding of each peptide alone or in the heteromeric mixtures, in 0 M NaCl, is shown in Figure 3A. As shown previously (Fairman et al. 1996), a thermal transition, with a midpoint around 70°C, is seen for the Lac21E/K heterotetramer, whereas Lac21K alone is completely unfolded at all temperatures and Lac21E shows only minimal stability at low temperatures. Similarly, the heterotetramers of Lac21E/O, Lac21D/K, and Lac21D/O show increased stability in comparison with the individually measured peptide stabilities. Like Lac21K, the peptides Lac21D and Lac21O alone are also completely unfolded at all temperatures.

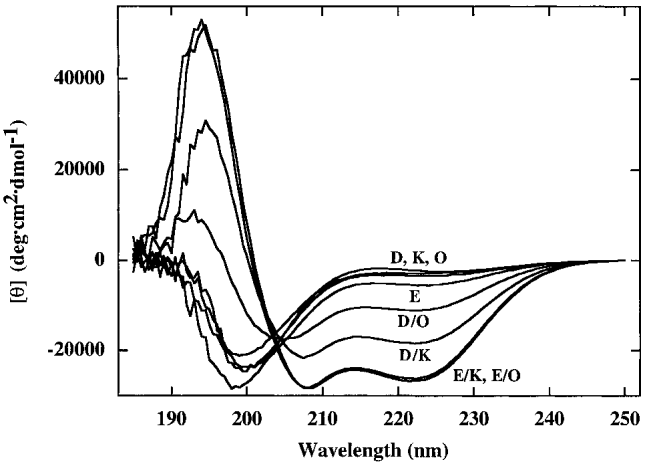



**Fig. 2.** CD spectra of peptides. Spectra include Lac21E, Lac21D, Lac21K, Lac21O, and the mixtures of Lac21E/K, Lac21E/O, Lac21D/K, and Lac21D/O. The total monomeric peptide concentration is kept constant at 100 μM. Samples were prepared in 10 mM MOPS (pH 7.5).

The data were fit by use of the Gibbs-Helmholtz function modified accordingly to describe a monomer–tetramer equilibrium scheme (Boice et al. 1996). Pre- and post-transition baseline corrections were applied to fit the data as described in the Materials and Methods section. Interestingly, we observe evidence for cold denaturation for all of the thermal unfolding curves, except for the Lac21E/K mixture; we had observed this effect previously for related tetrameric and trimeric coiled-coil systems (Fairman et al. 1995; Boice et al. 1996). The presence of cold denaturation is important for rigorous extraction of free energies at temperatures that do not lie close to the unfolding transition. Free energies for the stabilities of these complexes are shown in Table 1.

To a first approximation, the stabilities of the heteromeric mixtures decrease with decreasing sidechain length (Lac21E/K > Lac21E/O > Lac21D/K > Lac21D/O). However, this cannot be directly ascribed to a difference in the strength of interhelical charged interactions as two other factors can affect stability as well; (1) intrahelical interactions, and (2) the change in helix propensities effected by mutating any given position from its wild-type residue to the appropriate charged residue. One approach to separating these parameters is to make corrections for helix propensities to isolate the charged contributions to stability, as described below.

A second, and perhaps more rigorous approach is to screen out the charged interactions and then measure the residual stability of the heteromeric complexes in the absence of these interactions. Figure 4 shows the effect of NaCl concentration on the stability of the Lac21E/K complex as measured by the change in helix content by use of CD. There is a dramatic drop in the helix content of the Lac21E/K heterotetramer on addition of low concentrations of NaCl. This result supports our previous finding that charged interactions drive the stability of the Lac21E/K heterotetramer (Fairman et al. 1996). The charged interactions in the Lac21E/K heterotetramer appear to be largely screened in 0.1 M NaCl, consistent with other studies (Lumb and Kim 1995) and allow us to quantify the stability of the Lac21E/K heterotetramer in the absence of the charged interactions. We can neglect the contributions of self-assocation processes to the overall stability of the system, as neither Lac21E nor Lac21K show any significant stabilization at 0.1 M NaCl. Only on addition of much higher (>1 M) concentrations of NaCl do we see a significant increase in self-association that can be explained by the salting-in effect caused by the hydrophobic interaction (K.M. Werner and R. Fairman, unpubl.).

For purposes of quantitation, we assume that 0.1 M NaCl is sufficient to screen out the charged interactions in the other heteromeric complexes as well. Nevertheless, because of this assumption, the free energies we report below should more aptly be considered lower limits. We measured the stabilities of these complexes, using thermal unfolding, in

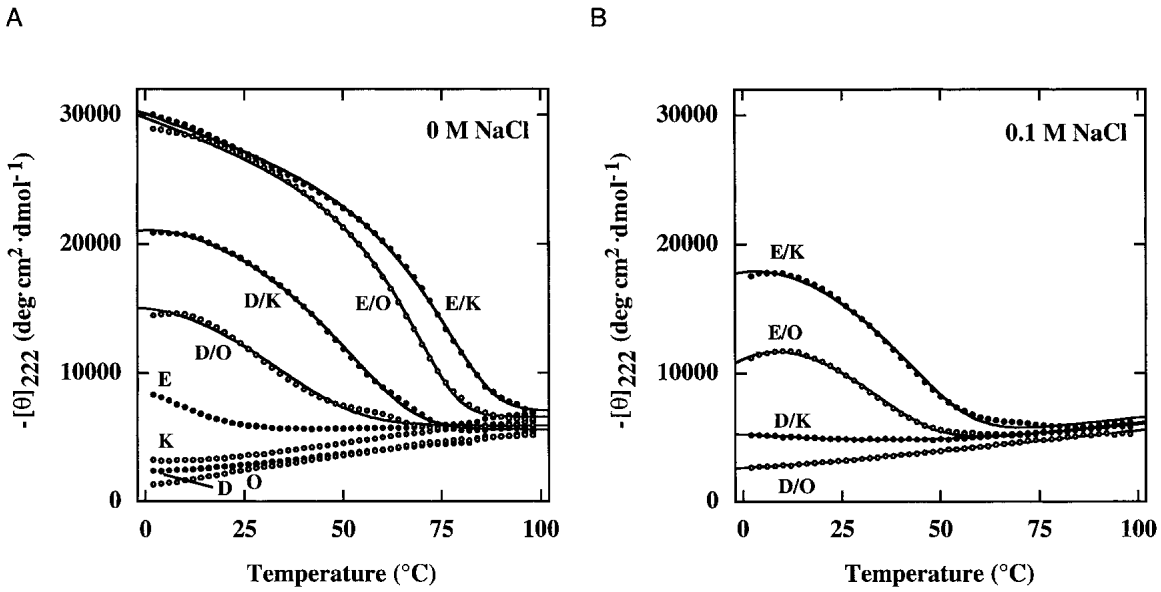



**Fig. 3.** Stability of coiled coils as measured by thermal unfolding experiments in (*A*) 0 M NaCl and (*B*) 0.1 M NaCl. The lines represent fits to the data using the Gibbs-Helmholtz equation to describe the temperature dependence of a monomer-tetramer equilibrium model. In *A*, spectra were taken both before and after the temperature unfolding experiments to demonstrate that >90% of the signal is retained after one heating and cooling cycle.

the presence of 0.1 M NaCl (Fig. 3B) and quantified their stabilities (Table 1). Taking the difference in free energies ($\Delta\Delta G^0$s) between the 0 M and 0.1 M NaCl data for each of the complexes allows us to quantify the strengths of the charged interactions. We find that glutamate-lysine and glutamate-ornithine-charged interactions similarly contribute −7.4 and −7.0 kcal/mole of tetramer to coiled-coil stability. Aspartate-ornithine and aspartate-lysine-charged interactions are significantly weaker, contributing −4.2 and −3.5 kcal/mole of tetramer, respectively. The $\Delta\Delta G^0$s reported here for the charged interactions involving aspartate must be interpreted cautiously, as these values are based on fits to thermal unfolding data that show little or no transition between states. Justification for such an analysis can be warranted, provided that the pretransition and transition slopes are well established by other means (A. Solan and R. Fairman, unpubl.). The magnitude of the free energy for the glutamate-lysine-charged interactions agree closely with our previously published results (Fairman et al. 1996).

To overcome the uncertainty in the measurements of the complexes involving Lac21D in 0.1 M NaCl, we can also extract the magnitude of the charged interactions between this residue and either lysine or ornithine by further analysis of the data collected only in 0 M NaCl. As stated above, this approach requires separating the contributions of intrahelical interactions and helix propensity effects from the contributions of charged interactions. Several model systems have been used to measure the helix propensities of the 20 amino acids and we can apply free energy corrections to our data on the basis of these earlier studies (O'Neil and DeGrado 1990; Chakrabartty et al. 1994; Betz et al. 1995; Padmanabhan et al. 1996). We can compare the free energies for the charged interactions as measured from our salt experiments with that calculated from helix propensity corrections to look for consistency (see Table 1).

Helix propensities of the amino acids are known to play a significant role in coiled-coil stability, and careful studies on a dimer/trimer coiled-coil system have been reported (O'Neil and DeGrado 1990; Betz et al. 1995). The resulting scale of helix propensities, as measured in a coiled-coil system, compare favorably with other model systems, including monomeric helical systems (Chakrabartty et al. 1994; Padmanabhan et al. 1996) and protein systems (Horovitz et al. 1992; Blaber et al. 1993; Myers et al. 1997). Although it is useful to apply such corrections to our data, it is important to point out potential problems (Myers et al. 1997). For example, a recent study has shown that helix propensities have a significant enthalpic component, making them temperature dependent (Luo and Baldwin 1999). Although helix propensities, as determined by a coiled-coil system, are most appropriate for our study, we use two scales for our calculations for the following two reasons: first, for providing some sense of the variance in such a correction procedure; and second, because helix propensity values for ornithine are only available from a monomeric helix model system.

To calculate a helix propensity correction, we apply a free energy correction ( Chakrabartty et al. 1994; Betz et al. 1995) on the basis of mutating the wild-type sequence to the particular residues of interest (i.e., mutating Lac21 to

**Table 1.** *Thermodynamic analysis of heterotetramer stabilities*

| Peptide | $\Delta G^{0,1}$ | $\Delta\Delta G^{0,2}$ | Propensity penalty | | Residual interaction | |
|---|---|---|---|---|---|---|
| | | | cc[3] | m[4] | cc | m |
| Lac21 | | | | | | |
| 0 M NaCl | −17.3 | | | | | |
| 0.1 M NaCl | −18.8 | | | | | |
| Lac21E/K | | | | | | |
| 0 M NaCl | −24.0 | −6.7 | +1.08 | +0.48 | −7.8 | −7.2 |
| 0.1 M NaCl | −16.6 | +2.2 | | | +1.1 | +1.7 |
| Lac21E/O | | | | | | |
| 0 M NaCl | −22.5 | −5.2 | | +3.12[5] | | −7.2 |
| 0.1 M NaCl | −15.5 | +3.3 | | | | +0.2 |
| Lac21D/K | | | | | | |
| 0 M NaCl | −17.4 | −0.1 | +2.40 | +2.88 | −2.5 | −3.0 |
| 0.1 M NaCl | −13.9[6] | +4.9 | | | +2.5 | +2.0 |
| Lac21D/O | | | | | | |
| 0 M NaCl | −15.6 | +1.7 | | +5.52 | | −3.8 |
| 0.1 M NaCl | −11.4[6] | +7.4 | | | | +1.9 |

[1] All free energies are reported in kcal/mol of tetramer using a 1 M standard state at 25°C.
[2] Difference between heterotetramer and Lac21 stabilities.
[3] Helix propensities are calculated using data from Betz et al. (1995) based on dimeric and trimeric coiled coil systems.
[4] Helix propensities are calculated using data from Chakrabartty et al. (1994) based on a monomeric helix system.
[5] Ornithine helix propensity was taken from Padmanabhan et al. (1996), also based on a monomeric helix system.
[6] These values are ill-determined due to incomplete thermal unfolding transitions.

Lac21E results in replacing native residues in *b* and *c* positions with glutamate). Applying a helix propensity correction to Lac21E predicts a loss of free energy, $\Delta\Delta G^0$, of 5.48 kcal/mole of tetramer relative to Lac21. The residual stability observed for this peptide (Fig. 3A) is consistent with this correction. This finding suggests that interhelical repulsive interactions are not significant and that the glutamates are too far apart between the helices to significantly affect one another. In contrast, Lac21K and Lac21O tetramers are predicted to be either slightly more or slightly less stable than the wild-type sequence ($\Delta\Delta G^0 = -3.32$ and +1.92 kcal/mole of tetramer, respectively), whereas experimentally they are shown to be much less stable than the Lac21E peptide. Thus, Lac21K and Lac21O self-association must be strongly opposed by interhelical repulsive interactions. Lac21D is predicted to be much less stable than even Lac21E ($\Delta\Delta G^0 = +8.12$ kcal/mole of tetramer) and thus, we cannot make any conclusions regarding the magnitude of the repulsive interactions in this peptide.

Turning now to the heterotetramer stabilities, we find that the glutamate-lysine charged interactions, after correction for helix propensity effects, contribute in the range of from −7.8 to −7.2 kcal/mole to the stability of Lac21E/K, in excellent agreement with that determined by the salt-screening approach (−7.4 kcal/mole; see Table 1). A helix propensity correction for the glutamate–ornithine system also results in a similar value to the salt-screening measurement (−7.2 kcal/mole vs. −7.0 kcal/mole, respectively). We find good agreement between the values reported for Lac21D/O (−4.2 kcal/mole vs. −3.8 kcal/mole) and Lac21D/K (−3.5 kcal/mole vs. −3.0 kcal/mole), in spite of the poorly defined thermal unfolding transitions observed for these mixtures. Thus, we conclude that each pair of charged residue interactions is capable of increasing the stability of the coiled coil relative to the original wild-type sequence, whether measured by salt-screening experiments or calculated on the basis of helix propensity corrections.

As a final note, we point out that helix propensity corrections for the free energies of the charged interactions measured in 0.1 M NaCl almost always result in anomalous positive values (Table 1). We see values ranging from +0.2 to +2.5 kcal/mole of tetramer, suggesting that the stabilities in the presence of 0.1 M NaCl are lower than expected. This is surprising, as we expected that, if anything, 0.1 M NaCl would not completely screen out a charged interaction, resulting in slightly negative residual interaction-free energies. Several explanations are possible. (1) Screening of the favorable interhelical charged interactions reveals intrahelical charge repulsion, which is certainly possible given the close juxtaposition of the *b* and *c* positions; (2) salt effects on helix propensity values; or (3) the helix propensity tables used are inaccurate for our model system. We believe that local sidechain interactions are the most reasonable source of discrepancy.

The original finding that charged interactions can influence stability of coiled coils at *b* and *c* positions emphasizes

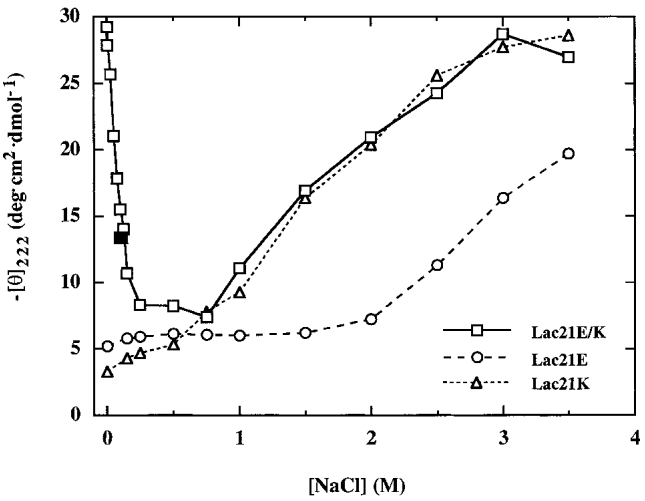



**Fig. 4.** [NaCl] dependence of coiled-coil stability. Experiments were measured at 25°C in 10 mM MOPS (pH 7.5). Data points are based on kinetic scans collected at 222 nm with typical signal averaging of 100–200 sec per data point. (□) Lac21E/K; (○) Lac21E; (△) Lac21K; (■) the 0.1 M NaCl data point; conditions in which the thermal unfolding experiments were carried out.

the importance of all heptad positions in dictating physicochemical parameters of coiled coils, including oligomeric state, stability, and specificity. Modeling studies (Fairman et al. 1996) suggest that glutamate and lysine sidechains are perhaps the minimum length required for bridging these positions between peptides. Our results demonstrate that, whereas ornithine can replace lysine with little or no loss of the strength of the charged interaction, replacing glutamate with aspartate causes a significant reduction in the strength of such an interaction. This result is consistent with previous work comparing aspartate with glutamate in their involvement in charged interactions in a dimeric coiled coil (Krylov et al. 1994). Therefore, there is no simple correlation between coiled-coil stability and distance from the backbone, as afforded by the number of methylene groups added. A molecular explanation for weak charged interactions with aspartate probably involves the strong propensity for aspartate to cyclize and interact with the mainchain, thus swinging the sidechain away from the direction necessary to form an interhelical interaction with either lysine or ornithine. This mainchain interaction has been suggested to make aspartate an effective N-Capping residue and a strong helix breaker (Presta and Rose 1988; Doig et al. 1993).

## Materials and methods

### *Peptide synthesis and purification*

The synthesis and purification of Lac21E and Lac21K have been described previously (Fairman et al. 1996). The two new peptides, Lac21D and Lac21O, were synthesized manually by use of PAL resin (Advanced Chemtech), and standard FMOC-chemistry which, upon TFA cleavage, results in amidation of the carboxyl terminus. The amino terminus was acetylated, by use of acetic anhydride prior to cleavage from the resin. The cleavage cocktail contained 92.5% TFA, 2.5% ethanedithiol, 2.5% thioanisole, and 2.5% anisole. After ether extraction, the peptide was resuspended in 10% acetic acid and lyophilized. After lyophilization, the Lac21O peptide was resuspended in 10 mM acetic acid and passed over a 1.4 × 160-cm column containing a Sephadex medium G50 bed (Pharmacia) and equilibrated in 10 mM acetic acid to remove cleavage byproducts and blocking groups. A constant flow rate was maintained (1.0–1.5 mL/min) using a Pharmacia P-1 pump. Peptides were further purified by RP-HPLC by use of water and acetonitrile, each containing 0.1% TFA, as the mobile phases on a Varian-Rainin HPLC system equipped with a Rainin C18 column. Peptides were purified to >95% homogeneity and were identified by MALDI-TOF mass spectrometry using a PerSeptive Biosystems Voyager-DE Biospectrometer. The molecular weights obtained for Lac21D and Lac21O are 2349.2 and 2343.5 Da, both within 0.5 Da of their respective theoretical molecular weights.

Lyophilized peptides were dissolved in ultrapure water as stock solutions. Concentration of peptide stocks was determined by a modified ninhydrin assay (Rosen 1957); it is well accepted that the combined accuracy and precision of this technique is within the range of ±10%.

### *Analytical ultracentrifugation*

Peptide solutions and reference solutions were prepared by addition of appropriate buffers and used without dialysis. Densities of solvents used were calculated by use of tables from Laue et al. (1992). Partial specific volumes were calculated with tables from Laue et al. (1992) and the partial specific volumes of the heteromeric structures were assumed to be a molar average of the 1:1 mixtures. All experiments were performed at 25°C in 10 mM MOPS (pH 7.0) buffer using 6-sectored charcoal-filled Epon centerpieces. Experiments were performed with an An-60Ti rotor in a Beckman Optima XL-A analytical ultracentrifuge. Ultracentrifuge data were collected by use of 0.001-cm steps with 20 averages and analyzed by the HID program from the University of Connecticut.

### *Circular dichroism spectropolarimetry*

A 62DS Aviv circular dichroism spectropolarimeter, equipped with a piezoelectric device for thermal control, was used to measure spectra and to collect thermal unfolding experiments. Spectra were collected at 25°C by use of a bandwidth of 1.5 nm, an averaging time of 3 sec, and a step size of 0.5 nm. All spectra represent the average of five scans. Thermal unfolding experiments were collected at 222 nm with 2-degree steps using a 30-sec averaging time and a 2-min equilibration time between steps. Stability is quantified by fitting thermal unfolding experiments using the Gibbs-Helmholtz function to define the temperature dependence of a monomer-tetramer equilibrium (Boice et al. 1996). Pre-transition and post-transition baselines were fitted as follows. The slope of the post-transition baseline is well determined for the least stable of the peptides (Lac21K) and is representative of the slopes of all the post-transition baselines determined in this study. Therefore, this slope is kept as a fixed parameter for fitting purposes while allowing y-intercept values to vary to accomodate small differences in these values owing to peptide concentration errors. Likewise, we used the most stable structure (Lac21E/K) to determine accurately the slope and y-intercept of the pre-transition baseline. Slopes and intercepts of the pre-transition baselines were kept fixed to the values determined for Lac21E/K for fitting of all other thermal unfolding curves. Justification for fixed slope and intercept values for the pre-transition baselines for all of the peptides was based on measuring the circular dichroism signal under conditions in which 100% helix formation is expected by use of TFE to induce this effect. Addition of TFE, a helix-inducing solvent, in thermal unfolding experiments using the Lac21K peptide as a test case, was done to demonstrate that the pretransition slope for highly unstable peptides is the same for related coiled-coil peptide systems (data not shown).

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## References

Betz, S., Fairman, R., O'Neil, K., Lear, J., and DeGrado, W. 1990. Design of two-stranded and three-stranded coiled-coil peptides. *Phil. Trans. R. Soc. Lond. B.* **348:** 81–88.

Blaber, M., Zhang, X.J., Lindstrom, J.D., Pepiot, S.D., Baase, W.A., and Matthews, B.W. 1994. Determination of alpha-helix propensity within the context of a folded protein. Sites 44 and 131 in bacteriophage T4-lysozyme. *J. Mol. Biol.* **235:** 600–624.

Boice, J.A., Dieckmann, G.R., DeGrado, W.F., and Fairman, R. 1996. Thermodynamic analysis of a designed three-stranded coiled coil. *Biochemistry* **35:** 14480–14485.

Chakrabartty, A., Kortemme, T., and Baldwin, R.L. 1994. Helix propensities of the amino acids measured in alanine-based peptides without helix-stabilizing side-chain interactions. *Protein Sci.* **3:** 843–852.

Doig, A.J., Chakrabartty, A., Klingler, T.M., and Baldwin, R.L. 1994. Determination of free energies of N-capping in α-helices by modification of the Lifson-Roig helix-coil theory to include N- and C-capping. *Biochemistry* **33:** 3396–3403.

Fairman, R., Chao, H.-G., Mueller, L., Lavoie, T.B., Shen, L., Novotny, J., and Matsueda, G.R. 1995. Characterization of a new four-chain coiled-coil: Influence of chain length on stability. *Protein Sci.* **4:** 1457–1469.

Fairman, R., Chao, H.-G., Lavoie, T.B., Villafranca, J.J., Matsueda, G.R., and Novotny, J. 1996. Design of heterotetrameric coiled coils: Evidence for increased stabilization by Glu(−)-Lys(+) ion pair interactions. *Biochemistry* **35:** 2824–2829.

Horovitz, A., Matthews, J.M., and Fersht, A.R. 1992. Alpha-helix stability in proteins. II. Factors that influence stability at an internal position. *J. Mol. Biol.* **227:** 560–568.

Krylov, D., Mikhailenko, I., and Vinson, C. 1994. A thermodynamic scale for leucine zipper stability and dimerization specificity: e and g interhelical interactions. *EMBO J.* **13:** 2849–2861.

Krylov, D., Barchi, J., and Vinson, C. 1998. Inter-helical interactions in the leucine zipper coiled coil dimer: pH and salt dependence of coupling energy between charged amino acids. *J. Mol. Biol.* **279:** 959–972.

Laue, T.M, Shah, B.D., Ridgeway, T.M., and Pelletier, S.L. 1992. Computer-aided interpretation of analytical sedimentation data for proteins. In *Analytical ultracentrifugation in biochemistry and polymer science*, (ed. S.E. Harding, A.J. Rowe, and J.C. Horton), pp. 90–125. The Royal Society of Chemistry, Cambridge, U.K.

Lumb, K.J. and Kim, P.S. 1995. Measurement of interhelical electrostatic interactions in the GCN4 leucine zipper. *Science* **268:** 436–439.

Luo, P. and Baldwin, R.L. 1999. Interaction between water and polar groups of the helix backbone: An important determinant of helix propensities. *Proc. Natl. Acad. Sci.* **96:** 4930–4935.

Marti, D.N., Jelesarov, I., and Bosshard, H.R. 2000. Interhelical ion pairing in coiled coils: Solution structure of a heterodimeric leucine zipper and determination of pK(a) values of glu side chains. *Biochemistry* **39:** 12804–12818.

Myers, J.K., Pace, C.N., and Scholtz, J.M. 1997. Helix propensities are identical in proteins and peptides. *Biochemistry* **36:** 10923–10929.

O'Neil, K.T. and DeGrado, W.F. 1990. A thermodynamic scale for the helix-forming tendencies of the commonly occurring amino acids. *Science* **250:** 646–651.

Padmanabhan, S., York, E.J., Stewart, J.M., and Baldwin, R.L. 1996. Helix propensities of basic amino acids increase with the length of the side-chain. *J. Mol. Biol.* **257:** 726–734.

Presta, L.G. and Rose, G.D. 1988. Helix signals in proteins. *Science* **240:** 1632–1641.

Rosen, H. 1957. A modified ninhydrin colorimetric analysis for amino acids. *Arch. Biochem. Biophys.* **67:** 10–15.

Thompson, K.S., Vinson, C.R., and Friere, E. 1993. Thermodynamic characterization of the structural stability of the coiled-coil region of the bZIP transcription factor GCN4. *Biochemistry* **32:** 5491–5496.

Yu, Y., Monera, O.D., Hodges, R.L., and Privalov, P.L. 1996. Ion pairs significantly stabilize coiled-coils in the absence of electrolyte. *J. Mol. Biol.* **255:** 367–352.