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Origin of the different strengths of the (i,i+4) and (i,i+3) leucine pair interactions in helices☆

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Abstract

Pairs of leucine side chains, spaced either (i,i+3) or (i,i+4), are known to stabilize alanine-based peptide helices, Experiments with new peptide sequences confirm that the (i,i+4) pair interaction is markedly stronger than the (i,i+3) pair interaction. This result is not expected from reported Monte Carlo simulations, which predict that the (i,i+3) interaction is slightly stronger. The interaction strength can be predicted from recently reported measurements of buried non-polar surface area, obtained from structures in the Protein Data Bank: the agreement is reasonable for the (i,i+3) LL interaction but underestimates the (i,i+4) LL interaction. Solvation of peptide groups in the helix backbone may contribute to the different strengths of the two LL pair interactions because different χ_1 leucine rotamers are used and the (i,i+3) pair shields two peptide groups whereas the (i,i+4) pair shields only one. A rough estimate of the backbone solvation effect, based on the difference between the helix propensities of leucine and alanine, agrees with the size of the difference between the (i,i+3) and (i,i+4) leucine pair interactions. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

New experiments are presented to resolve the question of whether the (i,i+4) or the (i,i+3) leucine–leucine pair interaction is stronger when measured in alanine-based peptide helices. The question is of interest because older experimental results are in conflict with Monte Carlo simulations of non-polar side chain interactions and, if the older experimental result is confirmed with new peptides, then some factor should be missing from the simulations. The new results confirm that the (i,i+4) LL interaction is substantially stronger than the (i,i+3) LL interaction. We suggest that peptide backbone solvation is a factor which should be considered in estimating the strength of a non-polar pair interaction, because different side chain rotamers are used to make (i,i+4) and (i,i+3) LL pair interactions and the choice of rotamers

☆ This paper is dedicated with respect and affection to Professor Rainer Jaenicke.

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affects the backbone solvation by modulating the access of water to the backbone.

2. Helix stabilization by non-polar sidechain interactions

Leucine–leucine pairs are among the non-polar side chain interactions which contribute to the stability of peptide helices [1] and (i,i+4) LL pairs comprise the most frequently observed class of pairwise side chain interactions in protein helices [2]. Without the aid of helix-stabilizing interactions, only alanine — of the 20 naturally occurring amino acids — forms a stable helix in water [3]. Monte Carlo simulations predict with good success which pairs of non-polar side chains form helix–stabilizing interactions [4]. Formation of the interacting LL pair is assumed to be driven by non-polar contacts [4], probably by burial of non-polar surface area [5], whereas making the interaction is opposed by the loss of side chain conformational entropy [4]. Recently a prediction scheme based on using discrete side chain rotamers, rather than exhaustive search of all possible side chain conformations, has been used with AGADIR [6] and gives similar results for non-polar side chain pair interactions as the Monte Carlo simulations [4].

In the various pairwise (*XY*) non-polar interactions which have been studied experimentally in alanine-based peptide helices, the (i,i+4) *XY* interaction is always stronger than the (i,i+3) *XY* interaction [1]. The same result was found computationally in most, but not all, of the pairwise interactions studied by Monte Carlo simulations [4]. The LL pair interaction is one of the exceptions: Monte Carlo simulations predict that the (i,i+3) LL pair interaction should be somewhat stronger than the (i,i+4) pair [4]. The (i,i+4) LL interaction occurs frequently in helices within protein structures [2] but the (i,i+3) LL interaction does not. Both this observation [2] and the strong (i,i+4) pair interaction found in alanine-based peptide helices [1] suggest that some factor is missing from the Monte Carlo simulations. The recent availability of data for non-polar surface area buried in interacting LL pairs [5] now allows us to predict the strengths of the (i,i+3) and (i,i+4) LL pair interactions, based only on buried non-polar surface area, assuming other factors can be omitted, and the results are in substantial agreement with the Monte Carlo simulations in indicating that the two LL pair interactions are nearly equal in strength. The loss of side chain entropy on LL pair formation is predicted to be a small effect [4]. When electrostatic calculation of solvation free energies is applied to peptide helices, by applying one of the standard approaches used to interpret experimental data for polar small molecules, the results indicate that the hydrogen-bonded peptide groups in an alanine helix still interact substantially with water [7]. We suggest here that modulation of this interaction by leucine side chains should be a significant factor in estimating the strength of a leucine–leucine pair interaction in a helix.

3. Experimental

3.1. Peptides and CD measurements of helix unfolding

Peptide synthesis and purification were made as described [8,9], and measurements of circular dichroism (CD) and thermal unfolding curves of peptide helices, as well as fitting these curves, were also made as described [8,9]. Peptide sequences are given in Table 1.

3.2. Data analyzes

To obtain the standard free energies of the (i,i+4) and (i,i+3) pair interactions, we use a modified form of the Lifson–Roig theory which incorporates pairwise helix-stabilizing interactions [10]. The partial homopolymer approximation is used: all lysine and alanine residues are collectively assigned the same average helix propensity (found by fitting the experimental data: here $\langle w \rangle = 1.44$ at the reference temperature, 0 °C), whereas the known helix propensity for leucine, and also the helix propensities of Gly and Tyr and the *N*-cap propensities are taken from [3]. Because making the (i,i+4) LL interaction requires that the χ_1 rotamers of residues i and i+4 be trans and gauche+, respectively, whereas the (i,i+3) inter-

action is formed with both leucine residues in the gauche+ rotamer, the observed interaction parameters need to be corrected, using the equation given in [10], to the values they would have when the correct rotamer pair is 100% populated. The correction requires knowledge of the fractional amounts of the various leucine rotamers in protein helices, given in [2].

4. Standard free energies of leucine pair interactions in alanine-based peptide helices

Helix formation by the six peptides studied here has been characterized by using CD to measure helix content and by determining thermal unfolding curves to measure stability against unfolding. The relative helix contents of the six peptides are compared in Fig. 1 at temperatures from 0 to 80 °C. The three peptides in Fig. 1a have single LL pairs, identical compositions, and their sequences (Table 1) differ only in the residue positions of the two leucine residues. Simulations indicate that two leucine side chains do not interact when the residues are spaced (i,i+5) [4] and the peptide with the (i,i+5) LL pair is used as a control. It can be seen in Fig. 1a that the (i,i+4) LL pair interaction is roughly twice as strong as the (i,i+3) interaction (before the rotamer distribution is taken into account), and this result is not dependent on temperature between 0 and 80 °C. The three peptides in Fig. 1b, which contain peptides with 0, 4 and 5 leucine residues (and 0, 3 and 4 overlapping LL pairs) were made for another purpose, but they also show by visual inspection that the (i,i+3) and (i,i+4) LL pairs are helix-stabilizing, because the two peptides with LL pairs form more stable helices than the all-alanine peptide, although alanine has a substantially higher helix propensity at 0 °C (1.70) than leucine (0.87) [3].

The helices formed by the three peptides in Fig. 1b are interesting for another reason: the helix contents of the two leucine containing peptides decrease more slowly with increasing temperature than that of the all-Ala peptide. This means that the leucine containing peptides have smaller enthalpies of helix unfolding per residue than the all-Ala peptide. The same phenomenon was

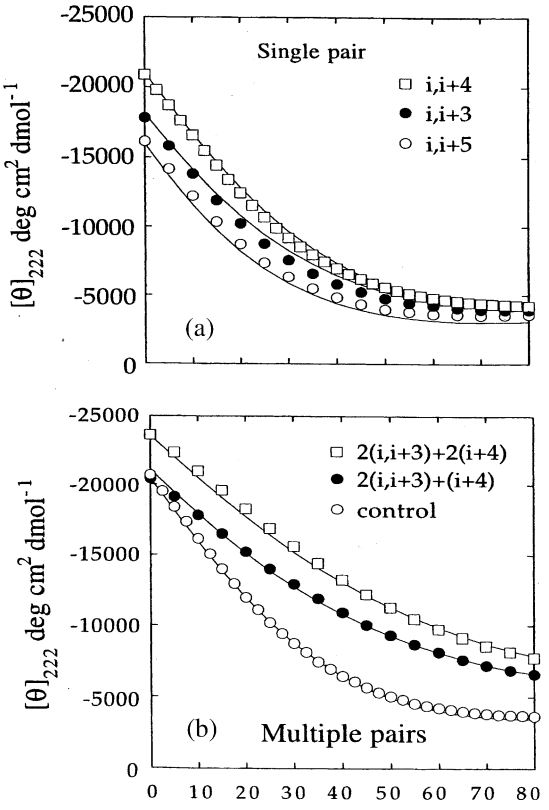

Fig. 1. Thermal unfolding curves of $[\theta]_{222}$ (mean residue ellipticity at 222 nm) vs. temperature (°C) for two sets of three peptides each, containing single (a) and multiple (b) pairs of two interacting leucine residues spaced either (i,i+4) or (i,i+3). Sequences are given in Table 1. The curves are measured in aqueous solution: 0.1 M NaCl, 1 mM each of sodium phosphate, sodium borate and sodium citrate, pH 7.0. The solid lines through the experimental points are fitted to the modified Lifson–Roig theory [3,20] where the helix baseline is $(-44\,000+250T)*(1-3/N_r)$, where T is °C and N_r is the number of residues, and the coil baseline is $2200-53T$ from [9].

observed earlier by us [8] but is more striking here because the effect scales with the ratio of leucine to alanine residues, and in the earlier study [8] short peptides differing only by one non-polar residue were examined. We tried to determine the difference in the enthalpy of helix unfolding between alanine and leucine by fitting the data in Fig. 1b, but the difference cannot be determined accurately because too many parameters must be determined in the fit. The experiment might succeed if new peptides are made in which the ratio

Table 1
Peptide sequences and their side chain interactions

Peptide	Sequence	Side-chain interactions
1	Ac-KAAAAKALAAKALAAKGY-NH_2	(i,i+5) control
2	Ac-KAAAAKAALAKALAAKGY-NH_2	(i,i+4)
3	Ac-KAAAAKAALAKLAAAKGY-NH_2	(i,i+3)
4	Ac-ELAALKAKLAALKAKAGY-NH_2	2(i,i+3)+(i,i+4)
5	Ac-ELAALKAKLAALKAKLGY-NH_2	2(i,i+3)+2(i,i+4)
6	Ac-EAAAAKAKAAAAKAKAGY-NH_2	alanine control

of leucine to alanine is varied as widely as possible and the leucine residues are always spaced either (i,i+5) or (i,i+2), to avoid forming leucine pair interactions. The results for the single LL pair peptides (Fig. 1a) give the standard free energy of forming the (i,i+4) LL pair as -2.26 ± 0.14 kJ/mol at 0 °C, whereas that of forming the (i,i+3) pair is -0.98 ± 0.10 kJ/mol, before correcting these numbers to the values expected when only the correct rotamer pair is populated.

5. Prediction of (i,i+4) and (i,i+3) pair interactions from data on buried surface area

It is necessary first to take account of the fact that pairwise side chain interactions in helices are formed by specific rotamer pairs. A 1994 survey of 167 protein structures containing 802 helices found that (i,i+4) Leu–Leu pair interactions are formed when the χ_1 sidechain rotamers exist as trans, gauche+ rotamer pairs whereas (i,i+3) Leu-Leu pair interactions are formed from gauche+, gauche+ rotamer pairs [2]. The same survey [2] found 0.40 trans and 0.59 gauche+ α_1 leucine rotamers in protein helices, so that the random expectation values of trans, gauche+ and gauche+, gauche+ rotamer pairs are 0.24 and 0.35, respectively. Correction of the measured interaction strengths, by an equation which takes account of the rotamer pair frequencies [10], increases the strength of the (i,i+4) LL pair interaction to -4.8 ± 0.3 kJ/mol and that of the (i,i+3) pair interaction to -2.1 ± 0.2 kJ/mol, so that the corrected difference is -2.7 kJ/mol.

A major energetic factor in the formation of a leucine side chain pair in a helix should be burial of non-polar surface area, caused by the hydrophobic interaction, as proposed originally by Kauzmann [11]. The free energy change is proportional to the non-polar surface area buried [12] and the scale factor commonly used is 25 cal/Å^2 [13], or 10.5 kJ/nm^2. The amount of non-polar buried surface area in (i,i+4) and (i,i+3) LL pair interactions was determined in a 1998 analysis of 328 protein structures [5] to be 39.5 and 32.8×10^{-2} nm^2 for (i,i+4) and (i,i+3) pair interactions, respectively. These values predict pair interaction strengths of -4.1 and -3.4 kJ/mol, respectively, if the calibration factor 10.5 kJ/nm^2 [13] is used and if other possible contributing factors are omitted. The predicted values are reasonably close to the observed values, considering the uncertainty arising from the choice of a reference state for the two leucine side chains when there is no interaction. However the difference in buried non-polar surface area between the (i,i+4) and (i,i+3) pairs is only 6.7×10^{-2} nm^2, corresponding to a free energy difference of only -0.70 kJ/mol, whereas the experimental difference between the strengths of the two pair interactions is -2.7 kJ/mol, somewhat larger than the observed strength of the (i,i+3) interaction.

6. Effect of solvation of the peptide group in the helix backbone

Helix modeling shows that non-polar side chains larger than Ala tend to block the access of water to peptide groups in the helix [8]. Steric blocking of this kind, referred to here as 'side chain shielding', occurs in a rotamer-specific manner. The β-branched side chains of Val and Ile shield two helical peptide groups (those of residues i and i−4), both in the trans and gauche+ χ_1 rotamers,

whereas leucine shields only one peptide group [8]. In the trans rotamer, leucine shields the peptide group of residue i, whereas in the gauche+ rotamer it shields that of residue i−4 [8]. Consequently, the backbone solvation is different in the (i,i+4) and (i,i+3) LL pairs: the (i,i+4) pair shields only one helical peptide group (that of residue i) whereas the (i,i+3) pair shields two peptide groups (those of residues i−4 and i−1).

The size of the shielding effect for a single leucine rotamer can be estimated from the helix propensity difference, measured in alanine-based peptides, between leucine (which shields one peptide group) and alanine (which shields none), if the Leu-Ala difference in helix propensities is attributed solely to backbone solvation. The difference in helix propensities (Ala-Leu) is −1.5 kJ/mol [3] which, if added to the stability difference between the (i,i+4) and (i,i+3) LL pairs expected from buried surface area (6.7×10^{-2} nm^2 equals −0.70 kJ/mol), gives −2.2 kJ/mol, in fair agreement with the experimental value of −2.7 kJ/mol.

Some unknown, or incompletely known, factors have not been taken into account, however. (1) The difference between the contributions to the (i,i+4) and (i,i+3) pair interactions from the loss in side chain entropy on LL pair formation. (2) The contribution to helix propensity from the loss of side chain entropy on helix formation [14]. Its contribution has been estimated at approximately 1/3 of the overall difference in helix propensity measured in alanine-based peptides [3]. (3) Any difference in backbone solvation caused by shielding a helical peptide group with two leucine side chains, in an (i,i+4) pair, vs. shielding with only one leucine side chain. (4) Hydrophobic side chains, such as these LL pairs, may affect the strengths of the peptide H-bonds shielded by them. The average peptide H-bond length in protein helices is different in solvent-exposed and solvent-shielded segments [15].

The strength of the interaction with water of the peptide group in an alanine helix, arising from the peptide dipoles, has been estimated from an electrostatic model to be −10.5 kJ /mol, of which −8.4 kJ/mol is associated with the CO group and −2.1 kJ/mol with the NH group [7]. This estimate of the strength of the polar solvation of a helical peptide CO group is seven times larger than the shielding effect of a leucine side chain, estimated above as 1.5 kJ/mol. Consequently, the shielding effect of a leucine side chain should be only partial; the side chain probably swings slightly to allow access of water to the peptide CO group, at the cost of some strain energy. A steric blocking effect of the leucine side chain, in reducing the access of water to the peptide group, has been inferred from hydrogen exchange rates measured in unstructured peptides [16].

7. Concluding comment

These results for (i,i+4) and (i,i+3) leucine pair interactions suggest that backbone solvation is significant even here, where it is not expected. The underlying reason for the pervasive influence of backbone solvation is the remarkable strength of the interaction between water and the peptide group, first observed by Wolfenden in 1978 [17], who found that the overall solvation free energy of *N*-methylacetamide is −42.3 kJ/mol. This large value of the solvation free energy reflects chiefly the enthalpic interaction of the amide with water [7]. Electrostatic calculations indicate that water still interacts substantially (−10.5 kJ/mol) with the peptide group in an alanine helix after the peptide H-bond has been formed [7], compare [18]. This interaction, especially strong between water and the CO group [7], should be important in stabilizing molten globule folding intermediates [7] and in determining the helix propensities of non-polar amino acids [5,7,8,19].

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