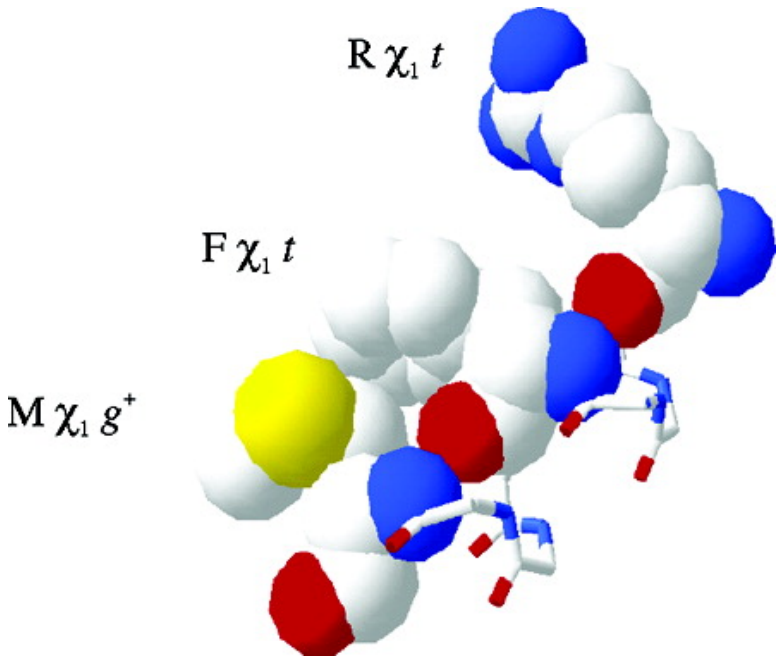
Communication

Pairwise Coupling in an Arg-Phe-Met Triplet Stabilizes -Helical Peptide via Shared Rotamer Preferences

Teuku M. Iqbalsyah, and Andrew J. Doig

J. Am. Chem. Soc., **2005**, 127 (14), 5002-5003• DOI: 10.1021/ja043446e • Publication Date (Web): 18 March 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 03/18/2005

Pairwise Coupling in an Arg-Phe-Met Triplet Stabilizes α-Helical Peptide via Shared Rotamer Preferences

Teuku M. Iqbalsyah and Andrew J. Doig*

Faculty of Life Sciences, Jackson's Mill, The University of Manchester, P.O. Box 88, Sackville Street, Manchester M60 1QD, U.K.

Received October 29, 2004; E-mail: andrew.doig@manchester.ac.uk

α-Helices are stabilized by the helix-forming tendencies of constituent amino acids,[1] capping interactions at the amino and carboxyl termini,[2] solvent environment,[3] and side chain interactions.[4−8] Interaction energies, in particular, have been measured for salt bridge,[4] aromatic−basic,[5] hydrogen bond,[6] and hydrophobic[7,8] interactions.

In proteins, side chains interact within a complex network of multiple noncovalent bonds, which may reinforce or weaken each other. The simplest system to investigate these effects is to study triplets of side chains. This has previously been studied as salt bridge interactions in α-helical peptides. A triplet of charged RER residues spaced *i*, *i* + 4 or *i*, *i* + 3 stabilizes α-helical peptides by more than the additive contribution of two single salt bridges.[9] Other triplets have also been studied, for example, EFR and EFE,[6f,10] although they do not show significant effects on peptide stability.

Here, we investigate the coupling effect of pairwise interactions. The coupling is stabilizing when the free energy of the pairs present simultaneously is greater than the sum of the individual pairs. Conversely, when the free energy of the simultaneous pairs is less than the sum of the individual parts, the interaction is destabilizing.

We have previously measured the ΔG of the interaction between Arg and Phe spaced *i*, *i* + 4 in isolated α-helix peptides. The ΔG of the Arg-Phe interaction is −0.1 kcal/mol.[5b] The ΔG between Phe and Met interaction in an *i*, *i* + 4 spacing has also been measured.[8] In this study, we look at the effects of these interactions on helix stability when present simultaneously via the shared Phe in *i*, *i* + 4, *i* + 8 spacing. The *i*, *i* + 3 interactions in a helix are generally weaker, so a coupling effect in *i*, *i* + 4, *i* + 7 or *i*, *i* + 3, *i* + 7 interactions would not be so clear.

Table 1 shows that in both the RF and FM pairs, Phe tends to be in the *t* conformation. The preference for the trans rotamer for Phe in helices and in all proteins are 67 and 34%, respectively.[11] This suggests that the two pairs incorporated in a RFM triplet could be stabilizing. The Phe residue in the middle of the triplet will be locked into the *t* conformation. RF and FM interactions will, therefore, not both have to pay the entropic cost of restricting the Phe to *t*. The stabilizing free energy resulting from the RFM triplet formation may, therefore, be greater than the sum of the individual RF and FM energies. The preference of Met χ_1 g^+ in the FM pair (0.81) is higher than that in the individual Met χ_1 g^+ in helices (0.69).[11] The high flexibility of the χ_3 rotamer (S−C bond) of Met[12] may also favor binding. This PDB-derived rotamer analysis, however, is based on the assumption that the rotamer distributions determined in the presence of tertiary contacts in proteins are applicable to peptides.

A series of alanine-based peptides was designed to allow the quantitative evaluation of the role of RFM triplets on helix stability (Table 2). Peptides were monomeric throughout the concentration range studied, as shown by the invariance of CD molar ellipticity with concentration.

Table 1. χ_1 Rotamer Populations of RF and FM *i*, *i* + 4 Pairs in α-Helices

residue	χ1 rotamer populations[a] g^+	t	g^-	total found in α-helices
Arg in Arg-Phe	0.46 ± 0.06[b]	0.44 ± 0.06	0.10 ± 0.04	71
Phe in Arg-Phe	0.34 ± 0.06	0.65 ± 0.06	0.01 ± 0.01	71
Phe in Phe-Met	0.16 ± 0.04	0.81 ± 0.05	0.03 ± 0.02	69
Met in Phe-Met	0.81 ± 0.05	0.19 ± 0.05	0	69

[a] Data from domain search of 1135 nonredundant proteins containing 4778 helices in ASTRAL database (*SCOP 1.63 Sequence Resources*). Rotamer definitions: $-120° < \chi_1 < 0° = g^+$; $0° < \chi_1 < 120°$, $= g^-$; $-120° < \chi_1 < 240° = t$. [b] Errors were calculated as $\sigma p_i = [p_i*(1 - p_i)/N_i]^{1/2}$, where p_i is the rotamer populations and N_i is the total number of pairs in helices.

Table 2. Peptides Used to Study Coupling in RFM Triplet

peptide sequence	predicted helicity[a]	predicted helicity[b]	exp helicity[c]
Ac-AAK**R**AAAA**F**AAAA**M**KGY-NH_2	30%	24%	24%
Ac-AAKA**R**AAA**F**AAAA**M**KGY-NH_2	33%	27%	32%
Ac-AAK**R**AAAA**F**AAA**M**AKGY-NH_2	47%	40%	36%
Ac-AAKA**R**AAA**F**AAA**M**AKGY-NH_2	50%	44%	63%

[a] Predicted using the Scint2 program. Values used for side chain *i*, *i* + 4 interactions (*p* values) for RF and FM were 1.2[5b] and 4.0[8a], respectively. Other values of *n*, *v*, *w*, and *c* were taken from ref 1b. [b] Helicity calculated as in 1, but with *w* values multiplied by 0.967. [c] Ellipticities θ were measured with a Jasco J810 CD spectropolarimeter at 222 nm, 273 K in 5 mM Na/phosphate containing 10 mM NaCl, pH 7.4. CD data (in mdeg) were converted to $[\theta]_{222} = \theta$/(molar concentration × 17 residues × 10) (in deg·cm^2·dmol^{-1}). Helix content was calculated as $[\theta]_{222(\text{observed})}/[\theta]_{222(\text{max})}$. $[\theta]_{222(\text{max})}$ is given by $-40\,000 \times (1 - 2.5/N)$, where N is the number of residues. Alternate equations or other proposed parameters give a small (∼1%) uncertainty in helical content.

A helicity prediction of the control R5F5M peptide using the *w* values (the helix interior preferences) of Rohl[1b] gives a predicted helicity about 6% higher than the experimental result. All *w* values of residues in the sequence were then corrected by a factor of 0.967. The discrepancy might be due to the fact that Scint2 does not take into account a possible destabilizing effect of Lys with the helix dipole at the N-terminus.[1j] Helicity disagreements in all peptides could also be due to changes in the ratio of α to 3_{10} conformations that might affect CD-derived helicity.

Helicity predictions of a series of homologous peptides with Ala replacing Phe show only a small difference for all sequences (50−53% helicity). Helix-coil theory takes account of the effect of moving the Arg and the Met when calculating the side chain interaction free energies.

In R5F4M and R4F5M, the theoretical and experimental helicities are somewhat different. The previous *p* value used to calculate the helicity of the FM interaction[8a] was obtained from the peptides: Ac-YG**F**AKA**M**AAKAAAAKAA-NH_2, Ac-YGAAKAA**F**-AKA**M**AAKAA-NH_2, and Ac-YGAAKAAAAKAA**F**AKA**M**-NH_2.

10.1021/ja043446e CCC: $30.25

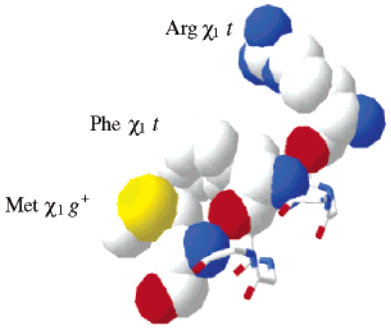


Figure 1. Example of a helical RFM triplet in 1lw3.

There may be additional i, $i + 3$ side chain interactions between Met and Lys residues in these peptides. This would give an overestimate of the p value of the FM interaction.

A sequence of Ac-AAAKA**R**AAA**F**A**R**AAA**F**AKAGY-NH_2 was used to calculate the previous p value for the RF interaction. The peptide contains two RF pairs. It is not clear why the value is lower than that found in this study.

We, therefore, refitted the p value for RF and FM interactions. The p values for each pair were fitted by varying them until the calculated helix contents agreed with experiment. Refitting p values for RF and FM gives values of 1.70 and 2.94, respectively, equivalent to −0.29 and −0.59 kcal/mol.

The predicted helicity for R4F4M is 44% using the refitted p values, which is much lower than the experimental result of 63%. This large difference proves qualitatively that there is a large stabilizing effect as a result of pairwise coupling in the R4F4M triplet. To get the predicted helicity to agree with experiment, the p value of each pair was then increased by a multiplication factor (mf) of 1.99. This increases the statistical weight of all helical residues from Arg to Met simultaneously by $(\mathrm{mf})^2$. The p values for RF and FM in the R4F4M peptide become 3.38 and 5.85, respectively, equivalent to −0.96 and −0.66 kcal/mol.

ΔG for pairwise coupling was calculated as $-RT \ln(1.99^2) = -0.75$ kcal/mol (−0.60 to −0.91 kcal/mol), reflecting a strong stabilizing effect in the R4F4M triplet. The energy is nearly as much as the additive energy of the individual pairs. The error in p was calculated by repeating the fitting procedure using experimental helicities increased or decreased by 3% to account for the experimental error in the measurement of helicity. Alternative parameter sets are available that would give different values, though the qualitative conclusions would not change.

We suggest that stabilizing pairwise coupling is a result of the R4F4M peptide only needing to pay the cost of restricting the Phe residue into a t conformation once in the triplet, rather than twice when the interactions are separate. The conformational entropy cost of restricting a residue into a helix is given by $-R \cdot \Sigma p_i (\ln p_i)$, where p_i values are the populations of the three χ_1 side chain rotamers. From our dataset, we found that these are 0.38, 0.60, and 0.02 for the g^+, t, and g^- rotamers, respectively, for the Phe residue, giving $-T\Delta S$ of 0.4 kcal/mol. Conformational entropy can thus account for over one-half of the free energy of pairwise coupling. The remainder (∼0.35 kcal/mol) may be strain energy. This is the cost of giving Phe nonoptimal bond angles, dihedral angles, and bond lengths when forming its noncovalent interactions. Rotamer and χ strain is of similar magnitude to conformational entropy in opposing protein folding.[13] Loss of conformational entropy in χ_2 of Phe may also contribute to the coupling free energy.

We searched crystal structures from the PDB for RFM triplets in helices, finding seven examples. Only structures obtained with the X-ray crystallography method of 2.5 Å resolution or better with less than 50% homology were considered. The Phe residue is in the χ_1 t conformation in all seven structures. Structural analysis of the RFM triplet indicates that the dominant interaction is hydrophobic for both pairs (Figure 1). Adoption of the Phe t rotamer in helices seems to be essential for forming the Arg and Met simultaneously, most likely through hydrophobic interactions with the face of the planar ring.

The shared rotamer preference effect that rationalizes our results is known to be a general property of proteins, giving cooperativity in folding and increasing protein stability. We have quantified this in a simple system. Any bond which restricts a side chain into a conformation that is favorable for forming additional interactions will show this effect. The effect is substantial, as fixing just a single side chain into its preferred conformation is here worth 0.75 kcal/mol.

Acknowledgment. The Michael Barber Mass Spectrometry Facility at UMIST is thanked for verifying peptide identity. T.M.I. thanks the TPSD Project of the Ministry of National Education of The Republic of Indonesia for the scholarship. We thank Neil Errington for helpful assistance in database search.

References

(1) (a) Wojcik, J.; Altmann, K. H.; Scheraga, H. A. *Biopolymers* **1990**, *30*, 121−134. (b) Rohl, C. A.; Chakrabartty, A.; Baldwin, R. L. *Protein Sci.* **1996**, *5*, 2623−2637. (c) Pace, C. N.; Scholtz, J. M. *Biophys. J.* **1998**, *75*, 422−427. (d) Petukhov, M.; Munoz, V.; Yumoto, N.; Yoshikawa, S.; Serrano, L. *J. Mol. Biol.* **1998**, *278*, 279−289. (e) Petukhov, M.; Uegaki, K.; Yumoto, N.; Yoshikawa, S.; Serrano, L. *Protein Sci.* **1999**, *8*, 2144−2150. (f) Cochran, D. A. E.; Doig, A. J. *Protein Sci.* **2001**, *10*, 1305−1311. (g) Cochran, D. A. E.; Penel, S.; Doig, A. J. *Protein Sci.* **2001**, *10*, 463−470. (h) Petukhov, M.; Uegaki, K.; Yumoto, N.; Serrano, L. *Protein Sci.* **2002**, *11*, 766−777. (i) Ermolenko, D. N.; Richardson, J. M.; Makhatadze, G. I. *Protein Sci.* **2003**, *12*, 1169−1176. (j) Iqbalsyah, T. M.; Doig, A. J. *Protein Sci.* **2004**, *13*, 32−39.

(2) (a) Dasgupta, S.; Bell, J. A. *Int. J. Pept. Protein Res.* **1993**, *41*, 499−511. (b) Forood, B.; Feliciano, E. J.; Nambiar, K. P. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 832−842. (c) Harper, E. T.; Rose, G. D. *Biochemistry* **1993**, *32*, 7605−7609. (d) Zhou, H. X.; Lyu, P. C. C.; Wemmer, D. E.; Kallenbach, N. R. *J. Am. Chem. Soc.* **1994**, *116*, 1139−1140. (e) Aurora, R.; Rose, G. D. *Protein Sci.* **1998**, *7*, 21−38. (f) Viguera, A. R.; Serrano, L. *Protein Sci.* **1999**, *8*, 1733−1742.

(3) (a) Sonnichsen, F. D.; Van Eyk, J. E.; Hodges, R. S.; Sykes, B. D. *Biochemistry* **1992**, *31*, 8790−8798. (b) Waterhous, V. D.; Johnson, W. C. *Biochemistry* **1994**, *33*, 2121−2128. (c) Luo, P.; Baldwin, R. L. *Biochemistry* **1997**, *36*, 8413−8421. (d) Vila, J. A.; Ripoll, D. R.; Scheraga, H. A. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 13075−13079.

(4) (a) Stellwagen, E.; Park, S.-H.; Shalongo, W.; Jain, A. *Biopolymers* **1992**, *32*, 1193−1200. (b) Huyghues-Despointes, B. M. P.; Baldwin, R. L. *Biochemistry* **1997**, *36*, 1965−1970. (c) Smith, J. S.; Scholtz, J. M. *Biochemistry* **1998**, *37*, 33−40. (d) Luo, R.; David, L.; Hung, H.; Devaney, J.; Gilson, M. K. *J. Phys. Chem. B* **1999**, *103*, 366−380. (e) Taylor, J. W. *Biopolymers* **2002**, *66*, 49−75.

(5) (a) Fernandez-Recio, J.; Vazquez, A.; Civera, C.; Sevilla, P.; Sancho, J. *J. Mol. Biol.* **1997**, *267*, 184−197. (b) Andrew, C. D.; Bhattacharjee, S.; Kokkoni, N.; Hirst, J. D.; Jones, G. R.; Doig, A. J. *J. Am. Chem. Soc.* **2002**, *124*, 12706−12714. (c) Shi, Z.; Olson, C. A.; Kallenbach, N. R. *J. Am. Chem. Soc.* **2002**, *124*, 3284−3291. (d) Tsou, L. K.; Tatko, C. D.; Waters, M. L. *J. Am. Chem. Soc.* **2002**, *124*, 14917−14921.

(6) (a) Marqusee, S.; Sauer, R. T. *Protein Sci.* **1994**, *3*, 2217−2225. (b) Huyghues-Despointes, B. M.; Klingler, T. M.; Baldwin, R. L. *Biochemistry* **1995**, *34*, 13267−13271. (c) Stapley, B. J.; Doig, A. J. *J. Mol. Biol.* **1997**, *272*, 465−473. (d) Fernandez-Recio, J.; Romero, A.; Sancho, J. *J. Mol. Biol.* **1999**, *290*, 319−330. (e) Vijayakumar, M.; Qian, H.; Zhou, H. X. *Proteins* **1999**, *34*, 497−507. (f) Shi, Z. S.; Olson, C. A.; Bell, A. J.; Kallenbach, N. R. *Biophys. Chem.* **2002**, *101*−*102*, 267−279.

(7) (a) Padmanabhan, S.; Baldwin, R. L. *J. Mol. Biol.* **1994**, *241*, 706−713. (b) Padmanabhan, S.; Jimenez, M. A.; Laurents, D. V.; Rico, M. *Biochemistry* **1998**, *37*, 17318−17330. (c) Andrew, C. D.; Penel, S.; Jones, G. R.; Doig, A. J. *Proteins* **2001**, *45*, 449−455.

(8) (a) Stapley, B. J.; Rohl, C. A.; Doig, A. J. *Protein Sci.* **1995**, *4*, 2383−2391. (b) Viguera, A. R.; Serrano, L. *Biochemistry* **1995**, *34*, 8771−8779.

(9) Olson, C. A.; Spek, E. J.; Shi, Z.; Vologodskii, A.; Kallenbach, N. R. *Proteins* **2001**, *44*, 123−132.

(10) Shi, Z. S.; Olson, C. A.; Kallenbach, N. R. *J. Am. Chem. Soc.* **2002**, *124*, 3284−3291.

(11) Penel, S.; Hughes, E.; Doig, A. J. *J. Mol. Biol.* **1999**, *287*, 127−143.

(12) Gellman, S. H. *Biochemistry* **1991**, *30*, 6633−6636.

(13) Penel, S.; Doig, A. J. *J. Mol. Biol.* **2001**, *305*, 961−968.

JA043446E