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Anatomy of a *trans–cis* peptide transition during least-squares refinement of rubrerythrin

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A detailed view is presented of the effects of one round of 20 cycles of restrained least-squares refinement of rubreythrin in which a *trans* peptide between Gly78 and Ile79 converts to a *cis* conformation automatically. While the ω angle for the peptide changes by nearly 180°, the maximum shift in any atomic position is 1.32 Å. The peptide converts by passing through a non-ideal structure containing a nearly linear C—N—C$^{\alpha}$ bond angle. The overall motion is not possible for real or virtual molecular models with ideal bond lengths, angles and torsion angles. Strengthening the stereochemical bond length and bond angle restraints halts the structural change.

1. Introduction

When we refined the structure of rubrerythrin (Sieker *et al.*, 1999, 2000; PDB code 1dvb), we were surprised to find that a *cis*-peptide between Gly78 and Ile79 had been generated by the refinement program *SHELXL* (Sheldrick & Schneider, 1997). Non-proline *cis*-peptide conformations are found in a small but significant number of protein structures (Weiss *et al.*, 1998; Jabs *et al.*, 1999) and methods are available to predict their existence on the basis of primary- and secondary-structure information (Pahlke *et al.*, 2005). Thus, the existence of the *cis*-peptide in this structure was not surprising. The fact that human intervention was not needed to generate it was, especially since another refined model for rubrerythrin (deMare *et al.*, 1996; PDB code 1ryt) contains a *trans*-peptide at this position.

To see how the refinement converted the *trans* conformation to *cis*, the peptide between residues 77 and 78 was rebuilt in the *trans* conformation and the structure was re-refined. The

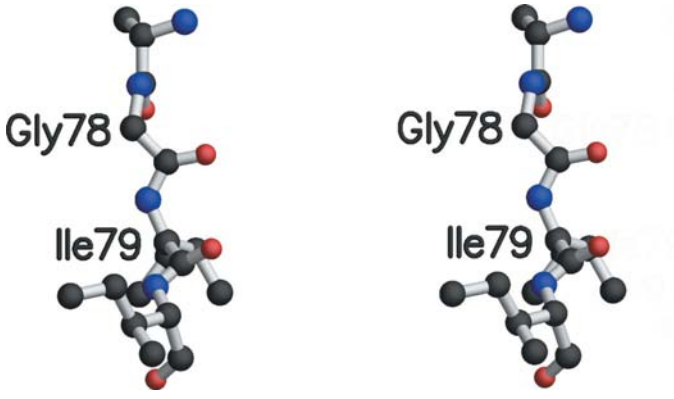

Figure 1
Stereoview of the *trans* peptide connecting Gly78 and Ile79 in the initial model of rubrerythrin.

Table 1
Differences in selected bond lengths, bond angles and torsion angles between the initial *trans* and final *cis* models.

Bond lengths that differ by more than 0.1 Å between the two models are listed, as are bond angles and torsion angles that differ by more than 10°.

	Trans model	*Cis* model
Bond lengths (Å)		
C(Ala77)—N(Gly78)	1.46	1.34
C(Ile79)—N(Ile80)	1.14	1.31
Bond angles (°)		
C^{α}(Ala77)—C(Ala77)—N(Gly78)	96.4	110.4
O(Ala77)—C(Ala77)—N(Gly78)	135.9	119.9
N(Gly78)—C^{α}(Gly78)—C(Gly78)	124.3	104.1
C^{α}(Gly78)—C(Gly78)—N(Ile79)	108.7	118.2
C(Gly78)—N(Ile79)—C^{α}(Ile79)	129.3	144.5
N(Ile79)—C^{α}(Ile79)—C(Ile79)	123.0	106.8
C^{α}(Ile79)—C(Ile79)—N(Ile80)	103.1	120.6
C(Ile79)—N(Ile80)—C^{α}(Ile80)	111.1	123.9
N(Ile80)—C^{α}(Ile80)—C^{β}(Ile80)	112.8	102.5
Torsion angles (°)		
Gly78 φ	73.2	107.6
Gly78 ψ	−158.3	−171.6
Gly78 ω	179.9	4.3
Ile79 φ	84.4	225.0
Ile79 ψ	102.7	156.1
Ile79 χ_1	288.4	226.7

model coordinates were saved after each refinement step and their analysis is described here.

2. Methods

The initial *trans* model for this test (see Fig. 1) was generated by melding a model from our refinement of rubrerythrin with the deposited and isomorphous 1ryt model. Coordinates for the atoms in the *trans* peptide between residues 78 and 79 were taken from 1ryt, while all other atoms came from a model generated near the end of the refinement of our structure. The model fragment from 1ryt matched very closely with our model at the N atom of Gly78, but there was a small gap of 0.31 Å at the N atom of residue 80.

The model with the *trans*-peptide was refined at 1.88 Å resolution using the conjugate-gradient least-squares option in *SHELXL* (Sheldrick & Schneider, 1997) with the standard restraints (Engh & Huber, 1991). Throughout the refinement, both before and after this test, the peptide was restrained to be planar, but no torsion-angle restraints were applied. No restraints were removed or added for refinement of the *trans*-peptide model.

All figures were drawn with *MOLSCRIPT* (Kraulis, 1991) and *RASTER3D* (Merritt & Bacon, 1997).

3. Results and discussion

A single run of 20 restrained least-squares cycles converted the *trans*-peptide into a *cis*-peptide. In the course of doing this, the *R* value for all reflections ($R = \sum \big||F_o| - |F_c|\big| / \sum |F_o|$) fell from 0.201 to 0.198. Fig. 2 shows the structural model for the Gly78-Ile79 peptide at each step in the refinement. There is a continuum of structures between the initial *trans* and final *cis* models with an intermediate structure containing a C—N—C^{α} bond angle of 180° (see Fig. 3).

It is interesting that the seemingly large 180° change in ω is caused by small relatively uniform movements of the atoms. The largest shifts occur in the first least-squares cycle and subsequent shifts are small. The largest overall shifts are observed for the N atom of Ile79 and the carbonyl O atom of Gly78. The first moves 1.32 Å, while the second moves 0.93 Å. The next largest shift is 0.88 Å for CD1 of Ile79. The C^{α} atom of residue 79 does not move much (0.34 Å), but the side-chain atoms move to retain the L-configuration for this residue as the N—C^{α} bond changes its orientation in the peptide.

It is interesting and educational to see which bond lengths, bond angles and/or torsion angles vary as the model goes through the *trans–cis* conformational change. The only bond lengths that vary by more than 0.1 Å during the transition are the C—N peptide bonds between residues 77–78 and 79–80 (see Table 1). These two bonds are located at joints between

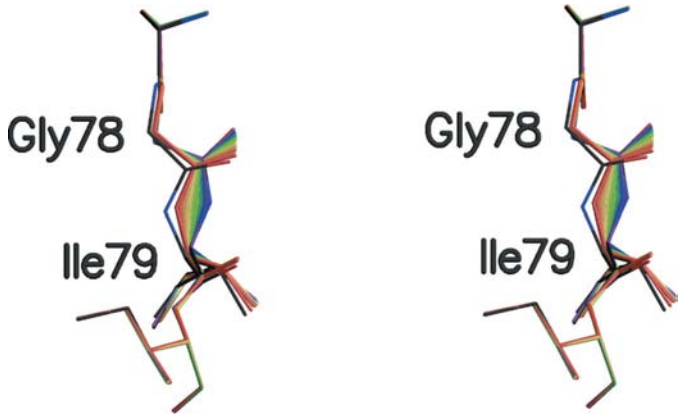

Figure 2
Stereoview of residues Gly78 and Ile79 during 20 cycles of least-squares refinement. The initial *trans* model is shown with standard atom colors (carbon in black, oxygen in red, nitrogen in blue) and the 20 subsequent models are shown in rainbow colors from red to blue.

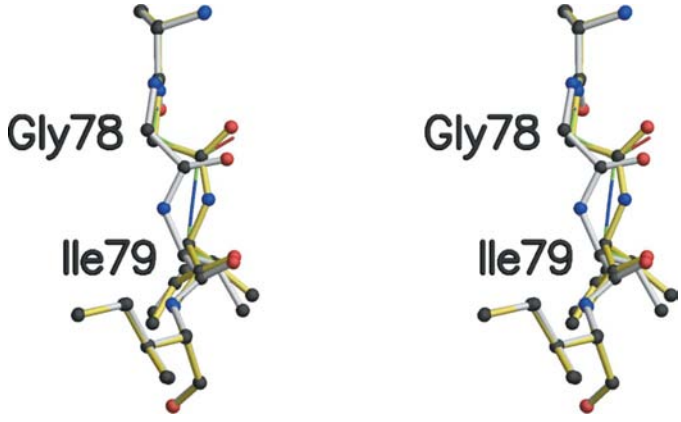

Figure 3
Stereoview of the initial and final models for Gly78 and Ile79 (ball-and-stick representation) as well as the model midway through the refinement cycle (sticks only). The initial *trans* model is shown with gray bonds, the transition structure is shown with bonds colored by atom type (C atoms in green) and the final *cis* model is shown with yellow bonds.

Table 2
Selected quantities as a function of refinement step.

Refinement step	C(Gly78)—N(Ile79)—C$^{\alpha}$(Ile79) bond angle (°)	ω torsion angle Gly78—Ile79 (°)	φ torsion angle Ile79 (°)	C$^{\alpha}$(Gly78)—C$^{\alpha}$(Ile79) distance (Å)
0	129.3	179.9	84.4	3.75
1	141.0	175.5	77.5	3.69
2	144.1	173.8	74.2	3.64
3	150.3	173.8	71.9	3.63
4	154.6	172.5	71.4	3.61
5	158.9	171.2	71.1	3.59
6	162.8	168.8	72.0	3.57
7	166.7	165.5	74.3	3.55
8	170.6	158.9	79.8	3.53
9	174.6	142.8	95.0	3.51
10	176.7	81.1	155.7	3.48
11	173.8	31.1	204.7	3.45
12	169.9	18.6	216.4	3.42
13	166.4	13.7	220.7	3.39
14	163.2	10.7	223.0	3.36
15	159.8	8.7	224.4	3.34
16	156.3	7.4	225.0	3.30
17	152.4	6.1	225.4	3.26
18	148.9	5.2	225.3	3.23
19	146.2	4.6	225.1	3.20
20	144.5	4.3	225.0	3.17

the components making up the initial model, so the large variation here arises from re-imposition of ideal bond-length geometry on an initial non-ideal model. The other bond lengths within the peptide undergoing the *trans–cis* switch all vary by less than 0.1 Å.

Bond angles are more easily distorted than are bond lengths and this is seen in Table 1, in which the bond angles varying by more than 10° are listed. The most interesting bond angle for this study is the C(Gly78)—N(Ile79)—C$^{\alpha}$(Ile79) angle and while the difference between its values in the initial and final models is 15.2°, the more interesting feature is that it passes through a value of 176.7° in going from 129.3 to 144.5°. The values of this bond angle after each refinement cycle are given in Table 2. No large shifts in the bond angle are seen. The values vary smoothly up to the nearly linear model and then back down to the *cis* model.

This bond-angle change is accompanied by an abrupt change in the ω angle. It remains within 20° of its initial value for the first seven refinement cycles and it is within 20° of its final value during the last eight steps. It changes by 130° during the five cycles midway through the round of refinement.

Most investigators would associate a large change in ω like this with a large change in the molecular structure, at least initially. In this case though, the torsion-angle variation is associated with only small changes in the structure and is rooted in the properties of torsion-angle calculations. If the atoms in the peptide were coplanar and their shifts were constrained to the plane, ω would have only two values: 180 and 0°. Also, if the C, N and C$^{\alpha}$ atoms were ever to become exactly collinear, ω would be indeterminate. The fact that the refinement steps yield models with neither planar peptides nor collinear C, N and C$^{\alpha}$ atoms gives rise to the smooth variation in ω from 180 to 4°.

The other torsion angles that undergo large changes during this refinement cycle are φ, ψ and χ_1 of Ile79. The large shift in the position of the N atom of that residue, coupled with the need to retain the L-configuration at its C$^{\alpha}$ atom, causes small shifts in the C$^{\alpha}$, C$^{\beta}$ and C atoms. These shifts result in large changes in the torsion angles for the residue. As seen in Table 2, the φ angle goes through an abrupt change at the midpoint in the refinement cycle much as did the ω angle. These large changes are all associated with the large shifts in the N atom of Ile79. Smaller changes are observed for the main-chain torsion angles at residue 78 because its carbonyl C atom does not shift as much during the refinement.

As would be expected, the C$^{\alpha}$—C$^{\alpha}$ distance for the peptide varies smoothly from the 3.8 Å value for a *trans*-peptide to 3.2 Å for a *cis*-peptide (see Table 2). No restraints were applied to this distance in the refinement. The change in the peptide conformation naturally leads to the shorter value. The atomic shifts provide strong evidence that the peptide is in fact a *cis*-peptide and the criteria developed by Weiss & Hilgenfeld (1999) for identifying potentially mis-restrained peptides, when applied to the 1ryt model, agree with this assignment. The most recent rubrerythrin models deposited in the PDB (*e.g.* 1lkm, 1lko, 1lkp, Shi *et al.*, 2002; 1qyb, Shi *et al.*, 2004*b*; 1s2z, 1s30, Shi *et al.*, 2004*a*) contain a *cis*-peptide between residues 78 and 79.

Thermal parameters (*B* values) often serve as markers for problem areas in models undergoing crystallographic refinement. In this case, no obvious aberrant *B* values or shifts were noticed for the atoms in these residues during the *trans–cis* transition. In general, the *B* values for atoms in residues 78 and 79 fell during the refinement from near to 40 to 25 Å^2 and they fall smoothly to their final values. The larger initial values came with the atoms from the 1ryt model. The 1ryt structure is isomorphous with 1dvb and differs largely in the composition and arrangement of the metal centers (Sieker *et al.*, 1999, 2000). The overall *B* values for the two structures are about the same, but in 1ryt residues 78 and 79 have slightly higher *B* values, probably owing to the modeling of them with a *trans*-peptide instead of a *cis*-peptide. Conversion to a *cis*-peptide improves the quality of the model and results in lower *B* values for the atoms in these residues. The *cis* model also fits the electron-density map better than does the *trans* model.

Finally, the influence of different weighting schemes on the conformational transition was investigated. Torsion-angle restraints are not part of the default restraints produced by the setup program for *SHELXL* (*SHELXPRO*). However, the peptide linkages are restrained to be planar. This allows for either *trans* or *cis* peptides and probably accounts for the ability of the refinement process to make the conformational change described here. Reducing the strength of the planarity restraint did not significantly alter the pattern of atomic shifts observed for the transition.

On the other hand, strengthening the overall bond-distance and bond-angle restraints stopped the *trans–cis* transformation. Reducing the standard deviation of those restraints from 0.032 to 0.01 effectively kept the model in the *trans* conformation. It appears that this weight change was sufficient to

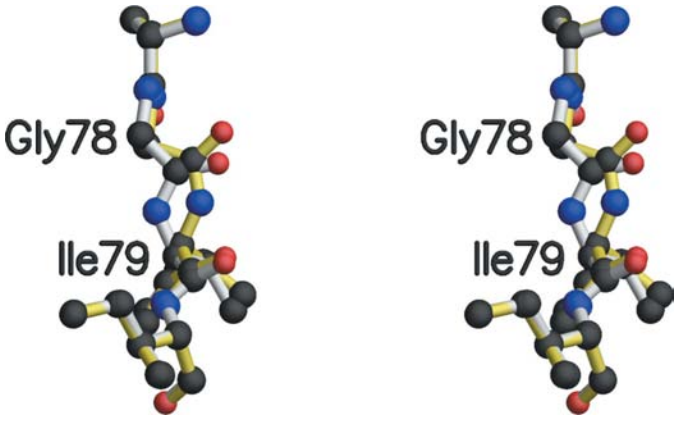

Figure 4
Stereoview of residues Gly78 and Ile79 in the initial model (gray bonds, *trans* conformation) and the final model (yellow bonds, *cis* conformation).

keep the C—N—C^{α} angle from approaching 180°, the transitional structure for the conformational change.

4. Conclusions

Much of our thinking about molecular models and how they respond to crystallographic refinement is shaded by our experiences with physical models and their manifestations in crystallographic graphics programs. Kendrew and Labquip models (and others) have influenced the types of manipulations engineered into graphics systems for adjusting and refining molecular models. Twisting of polypeptides and side chains about their bonds, *i.e.* adjusting the torsion angles, restrains the ways we might think about and manipulate molecular models. It is instructive to realise that refinement programs may or may not be similarly restrained.

The conformational change described here clearly involves a different type of structural change, one that makes great sense, but one that is still surprising at first glance. The observed shifts (see Fig. 4 for a comparison of the initial and final structures) are an efficient way of carrying out the conformational change and it is interesting to see how the refinement program balances the cost of distorting the C—N—C^{α} bond against the reduction in the crystallographic residual in improving the structure.

An unanswered question is how other refinement protocols, *i.e.* torsion-angle refinement, energy minimization with crystallographic restraints and maximum-likelihood methods, would deal with a situation like this one. Torsion angles are not normally restrained in *SHELXL* refinements, but one could imagine that inclusion of such restraints would alter the ease with which the initial *trans* model converted to a *cis* conformation.

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