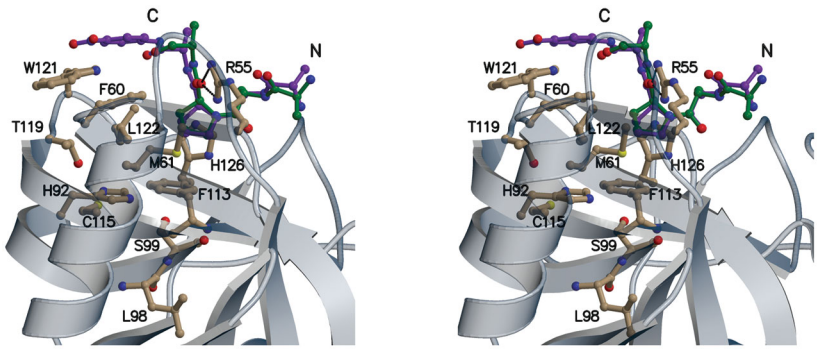

Figure 4 Superposition of CypA complexes with the *cis* conformations of CA^N and Suc-Ala-Ala-Pro-Phe-NA (ref. 23). Residues 88–91 of CA^N AAA-B, green; Suc-Ala-Ala-Pro-Phe-NA, purple. Both ligands are shown truncated to Cβ, except for proline and the tetrapeptide C-terminal nitrophenyl group. Residues of CypA that presumably move to allow the *trans* peptide substrate to contact Leu98 and Ser99 (bottom of figure) are shown explicitly[25]. These CypA residues and the protein substrate residues C-terminal to the isomeric proline are essentially unchanged in the *cis* and *trans* complexes of CA.

apparently with no space for a side chain and with (ϕ, ψ) angles (+123°, +168°) that are favored only for glycine[17]. Consistent with this, the CA^N G89A substitutions weaken the binding considerably; the K_d increases from ~20 μM to ~500 μM (ref. 18), with only slight variation in these values observed in a variety of sequence contexts (data not shown). The G89A substitution also shifts the equilibrium of the bound AAA species toward the *cis* conformation, with the result that both *cis* and *trans* structures are present in the same crystal (**Table 1**) and the *trans* conformation buries less deeply into the active site.

Remarkably, the AMA-A and AMA-A′ complexes show a mixture of 80% *trans* and 20% *cis* conformations, each of which show close similarity to the fully occupied single conformation structures (**Fig. 3a**). Contact of the CA^N *trans*-Ala89 Cβ with CypA Arg55 (absent in the Gly89 structures) seems to hold the CA^N Pro90 side chain ~1.5 Å away from its preferred binding positions for the Gly89 and *cis*-Ala89 structures (**Figs. 2a** and **3b**). Thus, for a non-glycine residue preceding the substrate proline, movement from the *trans* conformation toward the transition state would relieve the steric clash with CypA Arg55 and allow optimal binding of the proline side chain (**Fig. 3b**).

Curiously, substitution of Gly89 to alanine in the AMA construct prompted a partial shift from the *cis* toward the *trans* conformation (**Table 1**). Comparison of the structures suggests that this results from intramolecular interactions within the CA^N protein loop. The ψ angle of Gly89 is unfavorable for non-glycine residues at this position in the *cis*-AMG structure (where ψ = −163° and −176° for AMG-A and AMG-B, respectively). This potentially strained conformation is relieved in AMA-A/A′ by partial switch to *trans* and by smaller adjustments to a ψ angle to +158° in *cis*-AMA-B/B′. The structures are therefore consistent with our earlier finding that CypA is a Gly-Pro-specific binding protein and support our proposal that, conversely, catalytic rates may be greatest for suboptimal binding sequences such as Ala-Pro[17,18].

Comparison to earlier proposals

The catalytic pathway proposed here for the CA protein substrate differs from the pathway for peptide substrates reported recently[25] on the basis of chemical shift and NMR relaxation rate changes of main chain amides in the presence and absence of a model tetrapeptide substrate, Suc-Ala-Phe-Pro-Phe-NA, where Suc is succinyl and NA is *p*-nitroanilide. Catalytic pathways for both substrates seem to involve the same *cis* conformation, because all known CypA–*cis*-peptide structures overlap closely with the CypA–*cis*-Pro90 CA^N structures described here, including a Suc-Ala-Ala-*cis*-Pro-Phe-NA substrate[23] that is closely related to the substrate used by Eisenmesser *et al.*[25] (**Fig. 4**). There are important differences, however, upon moving to the *trans* conformation. As described above, the CA^N protein–substrate complex shows minimal conformational changes that are localized N-terminal to the isomeric proline. In contrast, the observation of catalysis-correlated relaxation rate changes at the main chain nitrogen atoms of CypA residues Leu98 and Ser99 indicated that formation of the *trans* Suc-Ala-Phe-Pro-Phe-NA peptide complex involved substantial conformational changes in substrate and enzyme that position the substrate's C-terminal residues adjacent to CypA Leu98 and Ser99 (ref. 25). Curiously, the different pathway for Suc-Ala-Phe-Pro-Phe-NA does not seem to be simply the result of using a peptide substrate rather than protein, because structures of CypA bound to CA-derived hexapeptides[19,20] overlap closely with the *trans* CypA–CA^N complexes.

A recent molecular dynamics study[31] of four tetrapeptides proposed the same transition-state geometry suggested here for the CA substrate. The crystallographic and dynamics studies are not in complete agreement, however, because the simulation suggested that both *cis* and *trans* ground-state structures bind with the peptide twisted ~20° from planarity, whereas we find that the X-Pro peptides of the protein substrate are not strongly twisted in the CypA crystal complexes with CA^N (**Table 2**), nor even in the peptide used as a starting point in the simulations (see Methods). The average ω-angle for the eight CA^N *trans*-X-Pro90 peptides is just 0.2° away from a planar 180° angle (range 173°–190°), and this value increased to only 1.0° after refinement to convergence (sparse matrix, iterative conjugate gradient) in the absence of peptide planarity restraints. The eight *cis* conformations (excluding the 20% occupied structures) all show some twist (average 9.5°; range 2°–18°) toward the transition state, with an average value of 10.0° after refinement in the absence of peptide planarity restraints. Thus, the *trans*-X-Pro90 peptides are planar in CypA–CA^N

Table 2. ω angles of CA^N Gly/Ala89-Pro90 peptide bond[a]

Trans								
HAG-A	HAG-B	AAG-A	AAG-B	AAA-A	AAA-A′	AMA-A	AMA-A′	Average
190.1	178.4	188.9	185.1	175.8	175.7	174.5	172.9	180.2
197.1	174.9	189.5	185.6	176.4	177.3	174.8	172.6	181.0
Cis								
O-loop-A	O-loop-B	AMG-A	AMG-B	AAA-B	AAA-B′	AMA-B	AMA-B′	Average
2.2	9.7	18.1	9.1	10.9	11.3	6.6	7.9	9.5
2.7	11.1	18.3	9.3	11.4	12.3	6.7	8.0	10.0

[a]Values shown are ω angles in degrees as determined by PROCHECK[42]. Upper values are in the presence of standard stereochemical restraints. Lower values are after 30 cycles of refinement in the absence of planar restraints. In agreement with expected values[43], the r.m.s. deviation from planarity of all peptides in the model ranges from 5.0° to 5.9° for the different refined structures.