crystal structures, and the *cis*-peptides are twisted by only ~10° toward the transition state. These results support the idea that CypA catalyzes proline isomerization by stabilizing the transition state of protein substrates rather than selectively binding 'near attack' conformations[31].

CONCLUSIONS

The structures reported here provide mechanistic insight to the isomerization of protein substrates by CypA. Tight binding of the proline side chain and main chain oxygen atom require that conformational changes resulting from isomerization occur at residues N-terminal to the isomeric bond. CypA Arg55 carries out a twin function by anchoring the proline oxygen and activating the proline amide of the isomeric peptide bond. Both *cis* and *trans* structures are accommodated in the same active site with minimal changes in the path of the polypeptide. The peptide bond is planar when bound in the *trans* conformation and only slightly twisted in the *cis* conformation. Finally, steric clash of the side chain in the residue preceding the proline prevents optimal binding of *trans*-proline, and the main chain geometry further appears to destabilize optimal binding of Ala-Pro sequences in the ground state *cis* and *trans* conformations. This explains why CypA preferentially binds Gly-Pro sequences and predicts that suboptimal binding sequences will be better substrates for isomerization.

METHODS

Protein purification and crystallization. Site-directed mutagenesis and protein expression and purification were as described[18,26]. Crystals were grown in sitting drops at 21 °C as described[17], with slight modification. The higher resolution data available in the present study resulted from deletion of five disordered residues from the C terminus of the original CA^N construct, with the result that the proteins used here comprise CA residues 1–146. The protein solution contained 0.4 mM CypA–CA^N, 1 mM βME and 10 mM Tris, pH 8.0.

Table 3. Crystallographic data and refinement[a]

CA^N protein	WT(HAG)	O-loop	AAG	AMG	AAA	AMA
Crystallographic data						
Space group	$P2_1$	$P2_1$	$P2_1$	$P2_1$	$P1$	$P1$
Unit cell dimensions						
a (Å)	38.5	38.9	38.3	38.5	38.4	38.5
b (Å)	113.2	109.3	111.0	110.9	111.2	111.1
c (Å)	67.0	67.7	67.7	67.9	67.8	67.9
α (°)	90	90	90	90	90.0	89.9
β (°)	100.5	99.7	101.0	101.4	101.4	101.6
γ (°)	90	90	90	90	89.7	89.9
Resolution (Å)[a]	38–2.00 (2.03–2.00)	67–1.90 (1.93–1.90)	26–1.72 (1.82–1.72)	20–1.73 (1.78–1.73)	20–1.90 (1.93–1.90)	20–1.70 (1.73–1.70)
Number of observations						
Total	89,358	219,914	188,143	135,448	173,271	261,634
Unique	31,588	37,052	41,047	56,085	84,527	117,639
Complete (%)	83 (60)	94 (82)	78 (31)	96 (93)	98 (96)	97 (95)
R_{sym}[b]	6.3 (19.5)	5.4 (35.2)	5.6 (30.4)	5.7 (36.6)	4.0 (25.1)	5.3 (35.7)
$< I / \sigma (I) >$	14.8 (2.8)	21.1 (3.0)	9.4 (2.3)	15.9 (2.5)	19.0 (2.9)	15.8 (2.3)
Beamline[c]	NSLS	SSRL 9-1	SSRL 1-5	ALS	SSRL 9-1	SSRL 9-1
Detector[d]	Q4 CCD	Mar345	Q4 CCD	Q4 CCD	Mar345	Mar345
Refinement						
R-factor[e]	0.201	0.170	0.178	0.172	0.169	0.172
R_{work}[f]	0.195	0.163	0.173	0.167	0.162	0.165
R_{free}[g]	0.259	0.232	0.226	0.220	0.234	0.230
R.m.s. deviations						
Bond lengths (Å)	0.018	0.018	0.018	0.018	0.018	0.019
Bond angles (°)	2.7	2.9	3.0	2.8	2.6	2.5
φ/ψ angles[h]						
Most favored (%)	86.7	88.1	88.2	88.8	88.1	88.2
Additional allowed (%)	13.1	11.5	11.4	10.6	11.4	11.4
Average *B*-factors (Å^2)						
A: CypA–CA^N	33/32	28/28	21/22	28/26	24/26	21/24
B: CypA–CA^N	26/41	24/30	18/30	21/34	25/43	19/33
A′: CypA–CA^N					43/34	31/28
B′: CypA–CA^N					21/35	16/29
Solvent molecules	32	38	27	38	38	36

[a]Values in parentheses are for the high-resolution shell. [b]$R_{sym} = 100 \times \Sigma_{hkl} | I - < I > | / \Sigma < I >$. [c]NSLS, National Synchrotron Light Source, beamline X12C, Brookhaven, New York; SSRL, Stanford Synchrotron Radiation Laboratory beamlines 9-1 or 1-5; ALS, Advanced Light Source beamline 5.0.2. [d]Q4 CCD, ADSC Quantum4. Mar345 image plate, Mar Research. [e]*R*-factor = $\Sigma_{hkl} | F_o - F_c | / \Sigma | F_o |$. [f]$R_{work}$ is the *R*-factor for 90% of data used during refinement. [g]R_{free} is the *R*-factor for 10% of the data not used in refinement. [h]For non-Gly and non-Pro residues only.

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