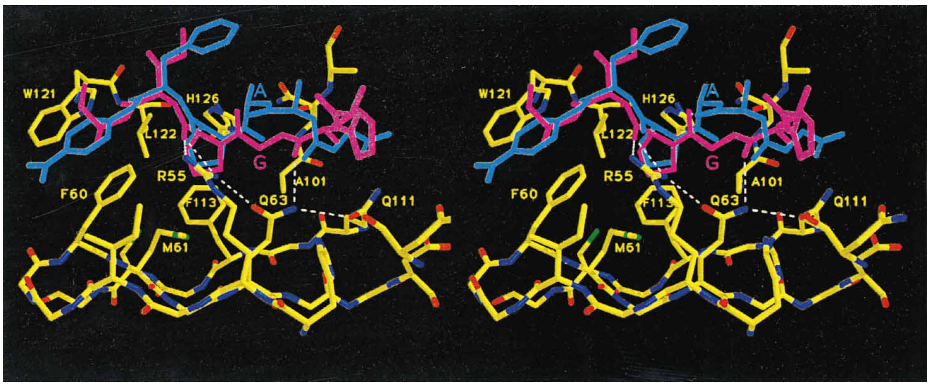

Figure 4. Comparison of CA_{151}/CypA and *succinyl*-Ala-Ala-Pro-Phe-*pNA*/CypA Active Site Conformations

The two structures were overlapped on the CypA molecules. The tetrapeptide (cyan) and equivalent residues from CA_{151} (magenta) have their C-termini on the left and N-termini on the right of this stereo view. CA_{151} Gly-89 is labeled G, and the corresponding tetrapeptide Ala is labeled A. CypA conformations are very similar in both complexes and are only shown for CA_{151} complex.

Our structure of the CA_{151}/CypA complex calls into question the prevailing paradigms that CypA binds preferentially to prolines in the *cis* conformation (Kallen and Walkinshaw, 1992; Ke et al., 1993; Kakalis and Armitage, 1994; Schutkowski et al., 1995; Zhao and Ke, 1996a; Zhao and Ke, 1996b) and that the protein exhibits little sequence specificity (Harrison and Stein, 1990b; Zhao and Ke, 1996a). Instead, the CA_{151}/CypA structure exhibits many of the features seen in the tight, specific complex formed between CypA and cyclosporine, including hydrogen bonding interactions with the following five CypA atoms: Asn-102 O, Gln-63 $N^{\epsilon 2}$, Arg-55 $N^{\eta 1,2}$, and Trp-121 N^{ϵ}; although cyclosporine lies in the opposite orientation in the CypA active site (Pflügl et al., 1993; Thériault et al., 1993).

Schreiber and Crabtree have proposed that CypA does not normally function as an enzyme in vivo (Schreiber and Crabtree, 1992). They suggest, instead, that the protein's active site may normally function to mediate specific protein–protein interactions (e.g., in analogy to SH3 domains) and that the rotamase activity observed in vitro is simply a consequence of the protein's hydrophobic proline binding site (Wolfenden and Radzicka, 1991). The CypA/CA_{151} structure appears to explain how CypA can function as a sequence-specific binding protein and a nonspecific rotamase. We propose that specific binding to HIV-1 CA is facilitated by the absence of a side chain at the Gly-89 position immediately preceding the proline residue. This allows tight binding N- and C-terminal to Pro-90 without distortion of the backbone conformation. In contrast, we propose that when nonspecific peptide sequences bind to CypA, a major driving force for proline isomerization is the backbone distortion induced by the imperfect fit of the substrate in the enzyme's active site. Specifically, when a low affinity sequence, such as *succinyl*-Ala-Ala-*trans*-Pro-Phe-*pNA*, begins to bind in the enzyme's active site, the central proline residue inserts into the hydrophobic binding pocket, and the C^{β} of the residue preceding proline clashes with the enzyme's Arg-55 and Gln-63 side chains. This forces the X–Pro bond toward the transition state and thereby uses the energy of peptide binding to catalyze proline isomerization. The CA_{151}/CypA structure does not rule out the possibility that the CypA Arg-55 guanidinium group contributes to catalysis by hydrogen bonding to the amide nitrogen of CA Pro-90 (Zhao and Ke, 1996a), although the Arg-55 $N^{\eta 2}$ to Pro-90 N distance for both of the CA_{151}/CypA complexes in the asymmetric unit is rather long (3.6 Å) in these ground state structures. Mechanisms involving catalysis by distortion have been suggested previously for CypA (Harrison and Stein, 1990a; Liu et al., 1990; Zhao and Ke, 1996a; Zhao and Ke, 1996b), but this is the first time that this detailed mechanism has been proposed.

Our model predicts that -Gly-Pro- will bind more tightly to CypA than other X-Pro sequences. Others have reported that the tetrapeptide *succinyl*-Ala-Gly-Pro-Phe-*pNA* appears to be a normal CypA substrate as judged by the kcat/Km ratio (Harrison and Stein, 1990b). However, it is possible that kcat/Km is similar to that of other substrates because kcat and Km have been reduced by similar amounts (i.e., that the enzyme-bound *trans*-Pro ground state has been stabilized relative to the transition state). It is also possible that kinetic parameters for the model substrates are influenced by the succinate moiety, which makes a series of nonnative contacts in the *succinyl*-Ala-Ala-Pro-Phe-*pNA* crystal structure (Zhao and Ke, 1996a). An attractive feature of our model is that it explains how the same binding site can be used for tight sequence-specific recognition, as required for HIV-1 packaging of CypA, and for catalyzing the isomerization of a broad range of X–Pro sequences, as is necessary if cyclophilins also play a general role in protein folding.

CA–CA Interactions in the Crystal and Possible Implications for Virion Structure

Mutations that affect Gag assembly generally map to the C-terminal third of CA (Jowett et al., 1992; Von Poblotzki et al., 1993; Dorfman et al., 1994; Franke et al., 1994a; Reicin et al., 1995). Following proteolytic maturation, the liberated CA molecules further self-associate to form the cone-shaped core structure that characterizes infectious viral particles. Mutations in the N-terminal domain of CA frequently give rise to cores with aberrant morphologies, indicating that this domain