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# Chiral discrimination in cyclodextrin complexes of amino acid derivatives: β-cyclodextrin/*N*-acetyl-L-phenylalanine and *N*-acetyl-D-phenylalanine complexes

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**Abstract:** In a systematic study of molecular recognition of amino acid derivatives in solid-state β-cyclodextrin (β-CD) complexes, we have determined crystal structures for complexes of β-cyclodextrin/*N*-acetyl-L-phenylalanine at 298 and 20 K and for *N*-acetyl-D-phenylalanine at 298 K. The crystal structures for the *N*-acetyl-L-phenylalanine complex present disordered inclusion complexes for which the distribution of guest molecules at room temperature is not resolvable; however, they can be located with considerable confidence at low temperature. In contrast, the complex with *N*-acetyl-D-phenylalanine is well ordered at room temperature. The latter complex presents an example of a complex in this series in which a water molecule is included deeply in the hydrophobic torus of the extended dimer host. In an effort to understand the mechanisms of molecular recognition giving rise to the dramatic differences in crystallographic order in these crystal structures, we have examined the intermolecular interactions in detail and have examined insertion of the enantiomer of the D-complex into the chiral β-CD complex crystal lattice.

**Abbreviations**: CD, cyclodextrin; Im, intermediate; N-Ac-L-F, *N*-acetyl-L-phenylalanine; N-Ac-D-F, *N*-acetyl-D-phenylalanine.

Chiral cyclodextrin (CD) hosts have been used extensively as models for investigating chiral and molecular recognition. Solution studies of CD inclusion complexes (1-3) and determination of binding constants (2), have provided thermodynamic data useful for chromatographic applications (4-8). In the solid phase, x-ray diffraction studies of inclusion complexes with chiral guest molecules can provide direct information regarding the mechanism of chiral recognition. In the relatively few studies with native cyclodextrins, mixed results have been reported (9-15). The most complete studies are those in which structures are determined for complexes formed with both enantiomers separately and for the racemate (9-14). For example, significant discrimination was observed for the β-CD inclusion complex with (*R*,*S*)-fenoprofen (11) in the solid state, whereas there was no apparent discrimination for (*R*,S)-flurbiprofen (9-14). These results and others suggest that features such as guest fit to the cavity, solvent interactions, hydrogen bonding potential of the guest molecules, and host crystal packing arrangement combine to play a significant role in chiral discrimination.

We have initiated a study in which the crystal lattice of β-cyclodextrin complexes that crystallize in the intermediate (Im) packing motif (16-18) has been characterized as a binding pocket useful for the crystallographic study of structural aspects of molecular recognition at high resolution (19). Briefly, the crystal lattice consists of close packed hydrogen bonded dimers of the host β-cyclodextrin molecules stacked, with an intervening layer of water molecules, along the *a*-axis. Typically two amino acid derivative guest molecules are included in the extended torus of the host dimer. To differing extents, the more hydrophilic backbones of the guest molecules interact with water molecules between the sheets and, in most cases, via bridging water molecules with hydroxyl moieties of cyclodextrins or other guests in the next layer. Examples of *N*-acetyl-L-phenylalanine complexes with different backbone structures (19), with a modified benzyl side chain (20) and for a single complex at different levels of kinetic energy, have been determined (J.L.C. and J.J.S., unpublished results). This report examines the application of the model for the study of chiral recognition.

We report the crystal structures of β-CD/*N*-acetyl-L-phenylalanine (N-Ac-L-F) at 298 K and 20 K and the crystal structure of β-CD/*N*-acetyl-D-phenylalanine (N-Ac-D-F) at 298 K. We examine distinct differences in the arrangement of the guest molecules, ordered and disordered, in the crystals. We also examine the incorporation of numerous water of hydration molecules in the lattice and their hydrogen bonding interactions with both the CD host and the pseudo peptide guests. Analyzing these interactions is expected to improve our understanding of chiral discrimination by β-CD in the solid state as well as to further our understanding of intermolecular interactions giving rise to chiral recognition in general. (This is paper no. 3 in the series "Molecular Recognition in Cyclodextrin Complexes of Amino Acid Derivatives." Paper no. 2 is ref. 20.)

## Experimental Procedures

Single crystals were grown by slow evaporation from an aqueous solution. For data collection, crystals with approximate dimensions of $0.2 \times 0.5 \times 0.5$ mm$^3$ were mounted with a small amount of mother liquor in thin-walled glass capillaries and sealed. Data were collected for the N-Ac-L-F complex at 298 K (room temperature) and at 20 K employing a specially designed Oxford Crysosystems He cryostat (Braintree, MA); data were collected for a crystal of the N-Ac-D-F complex at room temperature. All diffraction measurements were conducted with graphite monochromated Ag $K_\alpha$ X-radiation ($\lambda = 0.5608$ Å) by using a Bruker AXS (Madison, WI) rotating anode x-ray source; data were measured in oscillation scan mode with a MARResearch (Hamburg, Germany) (18 cm) imaging plate area detector and processed by using the MARXDS program (21). Data collection and structure refinement are characterized in Table 1.

Initial phases were derived from isomorphous replacement of the β-CD coordinates from the thermodynamically stable *n*-propanol complex (22, 23). Waters of hydration and guest sites were located in difference electron density. Structural models were refined on $F^2$ (Table 1) by using the SHELXL97 program library (24). All nonhydrogen atoms of the cyclodextrin molecules and numerous water molecules were refined anisotropically. Where defined by the chemical structure, hydrogen atoms were generated geometrically and fixed as a riding model. When disorder was found for water molecules or guest molecules, isotropic atomic displacement parameters

**Table 1. Characterization of the crystal structure refinement**

| | β-CD/N-Ac-L-F (298 K) | β-CD/N-Ac-L-F (20 K) | β-CD/N-Ac-D-F (298 K) |
|---|---|---|---|
| Space group | P1 | P1 | P1 |
| **a** | 18.06 (6) | 17.734 | 18.15 (6) |
| **b** | 15.44 (6) | 15.397 | 15.47 (6) |
| **c** | 15.53 (6) | 15.358 | 15.41 (6) |
| $\alpha$ | 103.44 (6) | 103.26 | 103.53 (6) |
| $\beta$ | 113.02 (6) | 113.34 | 113.72 (6) |
| $\gamma$ | 98.99 (6) | 98.64 | 98.74 (6) |
| V, Å$^3$ | 3,746 | 3,645 | 3,703 |
| Reflections measured | 30,772 | 34,625 | 22,086 |
| Unique reflections | 15,572 | 17,535 | 11,108 |
| $R_{int}$ | 0.025 | 0.024 | 0.027 |
| Resolution | 0.78 Å | 0.74 Å | 0.87 Å |
| % Completeness | 84% | 91% | 94% |
| $R_1/wR_2$/GOF | 0.0823/0.2404/1.135 | 0.0723/0.2053/1.109 | 0.0614/0.1712/0.953 |
| No. parameters/no. data ($F_o > 4\sigma(F_o)$) | 1807/12,323 | 1,804/14,806 | 1,804/9,853 |
| Final $\rho_{max}/\rho_{min}$ (e$^-$·Å$^{-3}$) | 0.526/−0.343 | 1.202/−0.613 | 0.440/−0.390 |

were used. Where necessary, disordered guest molecules were treated as rigid groups. Final ($F_o - F_c$) maps showed no distinct features. Crystallographic data in .cif format are available from the Cambridge Crystallographic Data Center entries CCDC 181781-181783.

To examine the stereochemistry of the enantiomeric substitution of the well ordered D-complex into the crystal lattice, the atomic coordinates of the two N-Ac-D-F molecules and the associated water molecule in the torus were inverted (mathematically converted to N-Ac-L-F) and, using the unit cell boundaries, translated into the "original" unit cell. A second operation on this moiety involved rotating it 180° about an axis passing through the midpoint of the torus; the guests were shifted slightly to achieve acceptable contact distances within the torus. The atom numbering scheme* used here is closely correlated with those reported earlier for analogous complexes (19, 20).

## Results and Discussion

Although the low temperature crystal structure determination demonstrates that two guest molecules must be present in the crystals of CD/ N-Ac-L-F at 298 K, the electron density maps reveal only one. At 20 K, four disordered pairs of guest molecules, for which the total occupancy factors add to 1 in each host monomer, are indicated by the electron density; in contrast, the crystal structure for the β-CD/ N-Ac-D-F inclusion complex at 298 K reveals two unusually well ordered guest molecules and a water molecule included deeply in the torus of the face-to-face β-CD dimeric host (Fig. 1). Numerous water of hydration molecules (≈ 20) are found in each structure. In all cases, guests pack with the phenyl rings positioned near the center of the dimer torus; the backbones for all of the N-Ac-L-F molecules extend across the β-CD primary face. It is noteworthy that the chemical structure of N-Ac-L-F is similar to *N*-acetyl-L-phenylalanine amide, for which a room temperature crystal structure was reported earlier (19). The guest molecules in the latter crystal structure at room temperature are considerably better ordered than those in the present crystal structures either at room temperature or at 20 K.

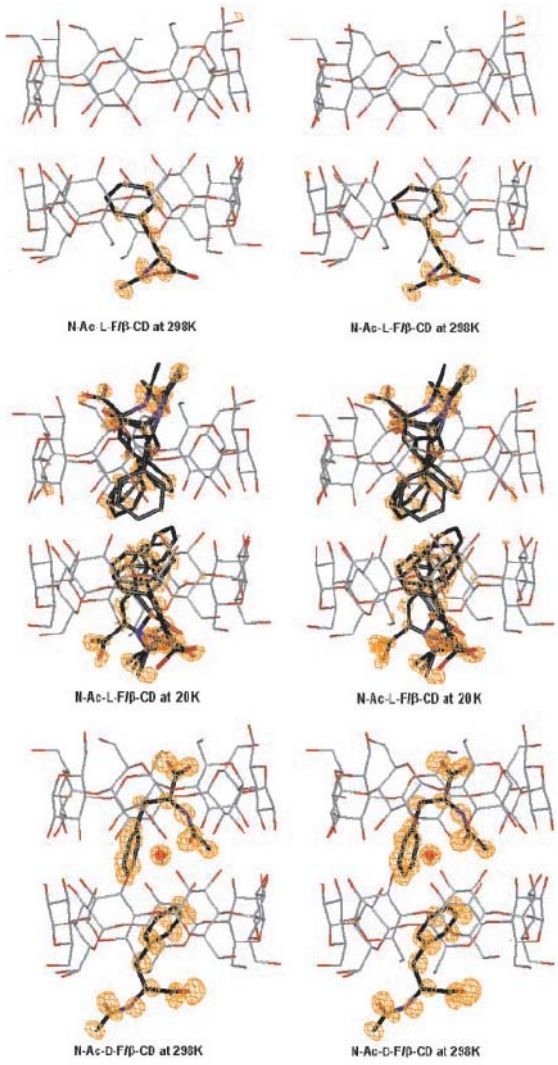


**Figure 1.** A stereoscopic projection of difference electron density plots contoured at 0.45 e$^-$ • Å$^{-3}$. The water of hydration molecules have been removed. In each host dimer, the upper cyclodextrin monomer has been designated **A**, the lower one **B**. The subsequent fit of guest molecules to the electron density is illustrated.

* The naming scheme for β-CD/ N-Ac-D-F is as follows: residues (1)–(14) are glucose residues for the β-CD dimer; residues O(15)–(44), O(62), and O(63) are waters of hydration; residues N-Ac-D-F (47) and N-Ac-D-F (51) are guests (OAc for acetyl oxygen, N for nitrogen, O and OH for acid oxygen atoms). Residues (1)–(44) are the same in the β-CD/ N-Ac-L-F structure; N-Ac-L-F (47) and N-Ac-L-F (51) are guests (with the same atom naming scheme).

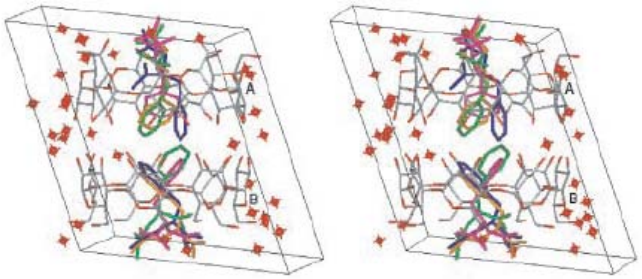


**Figure 2.** A stereoscopic projection of the unit cell contents for the crystal structure of the 2:2 *N*-acetyl-L-phenylalanine/β-cyclodextrin complex at 20 K. Guest molecules believed to be present simultaneously are presented in like colors. The oxygen atoms of water of hydration molecules are illustrated as red spheres. The **A** and **B** monomers are labeled.

The structural features that we believe are relevant to molecular recognition in these complexes are examined first with respect to the positions of the guest molecules in the crystalline complex. The positions of the midpoints of the respective backbone atoms* from the closest C6 atom β-cyclodextrin plane provide an indication of the positions of the guest molecules in the host dimer (20). The respective values are as follows: (*i*) for N-Ac-D-F: **A** 3.34 Å and **B** 2.08 Å; (*ii*) for "probable" pairs in N-Ac-L-F: **A1** –0.22 Å, **B3** 0.46 Å; **A2** –1.01, Å **B2** 0.88 Å; **A3** 0.48 Å, **B1** 1.05 Å; and **A4** –.33 Å, **B4** 1.33 Å. These values demonstrate that the position of the guest molecules in N-Ac-D-F complex are shifted downward, i.e., toward the **B** monomer significantly farther than in any of the structures for phenylalanine derivatives. The backbone for one of the N-Ac-D-F molecules in the **A** pocket has its acetyl group "tucked" into the host cavity and hydrogen bonded to a water molecule that is also included in the torus.

Turning to the distribution of guest molecules in the two complexes, the difference in guest order is one of the most striking features in the two crystal structures; a second is the hydrogen bonded water molecule included in the torus for the D-enantiomer mentioned above. For a modified amino acid complex with β-CD, the N-Ac-D-F guest molecules in this complex and the water molecule included in the torus are unusually well ordered; the included water molecule is more strongly hydrogen bonded to the carbonyl oxygen atom of the guest ($d_{O\cdots O} = 2.79$ Å) than to the secondary hydroxyl groups ($3.12 \leq d_{O\cdots O} \leq 3.40$ Å for the four closest neighbors). A difference electron density map ($F_o - F_c$) calculated with the guest molecules removed shows no evidence for additional guest conformations or water molecules. In contrast, in the β-CD/ N-Ac-L-F complex crystal at 298 K, only one guest molecule, located in the **B** monomer, is well defined in an ($F_o - F_c$) map contoured at 0.45 e·Å$^{-3}$ (see Fig. 1); there is no clear evidence of a preferred conformation or location of included guest molecule(s) in the monomer **A** region of the complex. Cooling a crystal of the complex to 20 K reveals electron densities indicating four different disordered guest molecule sites per monomer—resulting in four different probable pairs of guest molecules (Fig. 2) that are present with varying populations. Compared with the available low temperature structure, the guest molecule in the **B** pocket at room temperature appears to represent an average of two of the low temperature guest sites (**B1** and **B4**).

* A positive displacement for a guest in the **A** monomer indicates that the molecule is displaced toward the center of the β-CD dimer; for the **B** monomer, a positive value indicates that the guest molecule is displaced toward the hydrophilic interlayer region of the crystal lattice.

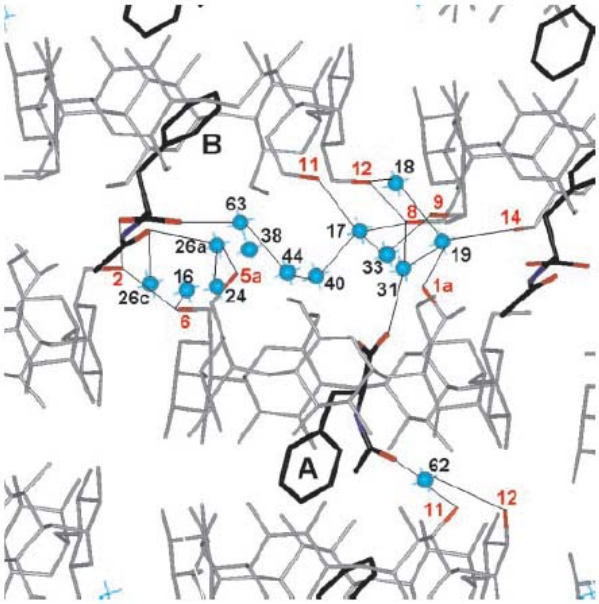


**Figure 3.** The probable hydrogen bonding scheme for the "binding pockets" in the Im crystal lattice for the *N*-acetyl-D-phenylalanine complex at 298 K. The **A** and **B** labels correspond to the β-cyclodextrin monomers and indicate the "pocket" label. Water molecules that are relevant to the guest binding are represented as cyan colored spheres with black labels. The labeling scheme is that used in the reports for the crystal structures of the *N*-acetyl-L-phenylalanine methyl ester complex (19) the *N*-acetyl-L-phenylalanine amide complex (19), and the *N*-acetyl-L-*p*-methoxyphenylalanine methyl ester complex (20). Red numbers refer to either β-cyclodextrin primary hydroxyl groups (upper portion of the figure) or secondary hydroxyl groups (lower portion of the figure).

Whereas the crystals display the nearly isomorphous Im packing type found for *N*-acetyl-L-phenylalanine derivative complexes reported earlier (refs. 19 and 20; J.L.C. and J.J.S., unpublished results), there are dramatic differences in the distribution of guest molecules in these two diasteromeric crystal structures. In the well-ordered β-CD/ N-Ac-D-F complex, the guests pack with the phenyl rings in an edge-to-face arrangement, although not one particularly suitable for a C-H-π interaction. In the low temperature structure for the N-Ac-L-F complex, a number of the guest pairs display geometry for varying degrees of π-π interactions.

The hydrophilic interactions in the binding pocket model involve interactions with peptide derivative backbones, water molecules, and β-cyclodextrin hydroxyl groups. The backbone of guest molecule N-Ac-D-F (**B**) extends completely outside of the torus, interacting with waters and neighboring CD primary hydroxyls. As discussed above, the guest molecule N-Ac-D-F (**A**) has its acetyl amide backbone folded in toward the center of the torus, displaying the rare interaction with a well ordered included water molecule (w62). The observation that its contact distances with secondary hydroxyl groups are long [$O_w\cdots O_{OH}$ distances range from 3.13 and 3.21 Å, respectively for residues 11(O3) and 12(O2) (Fig. 3)] is taken as an indication that this water forms a packing entity with the amino acid derivative to effectively fill a portion of the nonconstraining extended torus of the host dimer. In the β-CD/ N-Ac-L-F complex at 20 K, all of the guest molecule backbones extend across the CD primary face; phenyl rings of probable pairs (color coded in Fig. 2) pack either in edge-to-face, edge-to-edge, or anti-parallel stacked arrangements.

Because the hydrogen bond interactions between it and the secondary hydroxyl groups of the host are weak, the presence of water

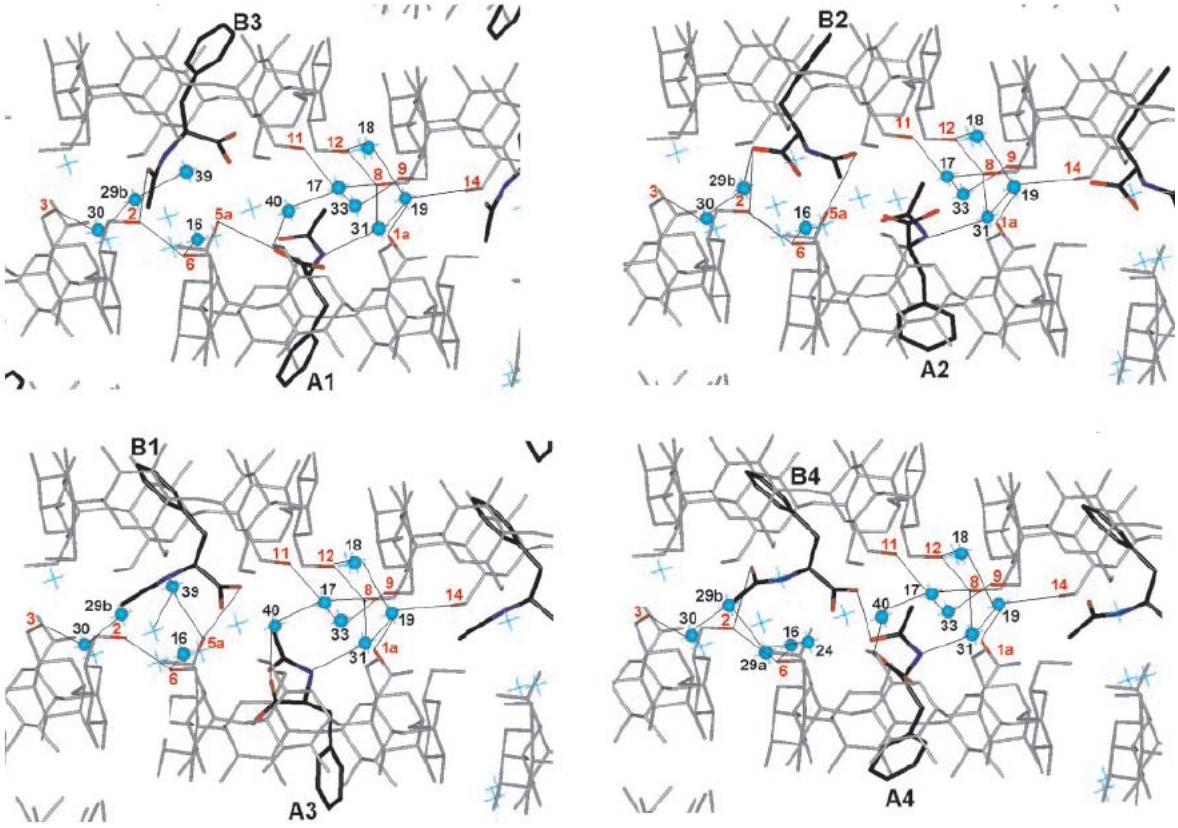


**Figure 4.** The probable hydrogen bonding scheme for the "binding pockets" in the Im crystal lattice for the *N*-acetyl-L-phenylalanine complex at 20 K. Water molecules that are relevant to the guest binding are represented as cyan colored spheres with black labels; those that do not interact with the indicated guest molecules are shown as cyan plus signs. Guest molecules are labeled **A*x*** and **B*y***, where **A** and **B** correspond to the β-cyclodextrin monomers and indicate the "pocket" label and ***x*** and ***y*** indicate guest labels in the .cif file. The numerical labeling scheme is that used in the reports for the crystal structures of the *N*-acetyl-L-phenylalanine methyl ester complex (19), the *N*-acetyl-L-phenylalanine amide complex (19), and the *N*-acetyl-L-*p*-methoxyphenylalanine methyl ester complex (20). Red numbers refer to either β-cyclodextrin primary hydroxyl groups (*Upper* portion of the figure) or secondary hydroxyl groups (*Lower* portion of the figure).

molecule w62 in the torus of the crystal structure of the D-enantiomer is not likely to fully explain the difference in guest order in the two crystal structures. Therefore, an examination of the potential hydrogen bonding interactions with water molecules and neighboring cyclodextrin molecules (Figs. 3 and 4) is appropriate. The same four CD primary hydroxyls are involved in intermolecular H-bonding in each of these structures as in those reported earlier [O6(8)···O6(12) and O6(2)···O6(6)] (19, 20). These hydrogen bonds appear to be an integral component of the Im packing motif. Comparison of the H-bonding interactions of the D-enantiomer at room temperature and of the L-enantiomer at low temperature with those reported for the structures of the L-enantiomers of the *N*-acetylphenylalanine methyl ester, the *N*-acetylphenylalanine amide, and the *N*-acetyl-*p*-methoxyphenylalanine methyl ester (19, 20) reveal a generally conserved network, especially in the **A** pocket. The guest located in monomer **A** of the β-CD/ N-Ac-D-F complex interacts via oxygen OH(47) with water w31, a water commonly observed to interact with amino acid derivative guest molecules that display intermediate packing of the CD dimers. Waters w29 and w30 in the β-CD/ N-Ac-D-F complex **B** pocket are not conserved; however, a new water w63 is involved in a bridging H-bond from the carboxyl moiety of the guest in monomer **B** and waters w38 and w44 at the interface to the **A** pocket. Primary hydroxyl 2 of an adjacent CD molecule is hydrogen bonded to the second oxygen of the carboxyl moiety of the guest in monomer **B** in the D-enantiomer. This group forms a H-bond to the acetyl oxygen atom in three of the four disordered guest molecules in the crystal structure with the L-enantiomer and to the analogous carboxyl moiety in the fourth example; in the other derivatives reported, it H-bonds to the acetyl oxygen atom. Interactions of the guest molecules in the **A** monomer of the 20 K β-CD/ N-Ac-L-F complex with the binding pocket are between the acetyl nitrogen atom and water w31. In three of the four disordered **A** monomer guest molecules, there is a H-bond interaction between water w40 and an oxygen of the carboxyl moiety; similar interactions are observed for the analogous carbonyl moiety in the amide complex (19). As mentioned earlier, the crystal structure of the N-Ac-L-F $NH_2$ complex is much better ordered than the structurally similar N-Ac-L-F complex. The disorder precludes definitive analysis of the reasons for this difference; however, it seems appropriate to suggest that the resonance properties of the carboxylic acid moiety may be at least partially responsible.

In the **B** monomer of the β-CD/ N-Ac-D-F complex, the acetyl oxygen OAc(47) interacts water w26a with primary hydroxyl O6(5), which is in a (+)-gauche conformation. This CD primary hydroxyl is commonly disordered in the amino acid derivative complexes previously reported, but, in this complex, only the one conformer is observed. With these observations in mind, it appears that the unusually well ordered structure for the D-enantiomer complex results from a combination of effects: the presence of a water molecule in the torus (W62), the presence of a bridging water molecule between the **A** and **B** binding pockets (W63), and the interaction via W26a with the

ordered primary hydroxyl O6(5) of a second neighboring CD molecule. It is also noteworthy that, as illustrated in Fig. 4, the guests in the **B** monomer in the low temperature β-CD/ N-Ac-L-F complex are involved in interactions with primary hydroxyls O6(2) and O6(5), along with several waters of hydration, but that, in all cases, the full hydrogen bonding capability of the carboxyl moiety is not used.

This study indicates a dramatic difference in the recognition of the D- and L-enantiomers by the β-cyclodextrin host lattice. The observation that they crystallize in essentially isomorphous crystals but with greatly differing degrees of crystallographic order raises the question of whether or not the L-enantiomer can achieve packing of the complex with the water included in the torus. To probe the possibilities, two model structures were constructed both from an inversion operation applied to a D-enantiomer triad consisting of the two guest molecules and the included water molecule. For model **I1**, the inverted complex was simply placed in the unit cell by a simple (1*x*,1*y*,1*z*) translation, and, for model **I2**, the inverted complex from **I1** was rotated by 180° by an axis perpendicular to the dimer long axis. These models were examined for prohibitively close contacts (Fig. 5). The contact distances between the included water molecule and the cyclodextrin host dimer are all acceptable. However, in model **I1** (colored blue in the figure), the backbone of the guest in the **A** monomer protrudes deeply into the interstitial hydrophobic regions between cyclodextrin molecules in the crystal lattice. There are numerous short intermolecular contacts involving water of hydration molecules and neighboring β-CD molecules as indicated in the figure. Model **I2** places the backbones of the two guest molecules in positions similar to those found in the D-enantiomer complex; again, there are unacceptably short intermolecular contacts involving the guest molecules and other components of the chiral host lattice (magenta-colored molecules in the figure). Although not an exhaustive test of the possible orientations and positions of the inverted guest molecules in the native β-CD lattice, there is clear indication that the chirality of the host lattice and the inverted guest structure are incompatible for steric reasons.

Our expressed goal with this systematic study of intermolecular interactions in crystal structures of supramolecular complexes of amino acid derivatives with β-cyclodextrin is to improve our understanding of molecular and chiral recognition. To accomplish this goal, we are in the process of building a database of structural information based on analysis of a binding pocket model presented by the crystal lattice of supramolecular βcyclodextrin complexes, especially those crystallizing with the Im packing motif. Although still relatively small, that database is beginning to reveal many subtle changes in intermolecular interactions that occur in a series of complexes in which the host molecule provides a nonconstraining hydrophobic environment for guest inclusion and the lattice presents a variety of opportunities for hydrogen bonding interactions, many of which are mediated by water of hydration molecules. The long term goal is to use that database of interactions at the molecular level to calculate macroscopic properties, such as differences in enthalpies and binding constants when selected complexes are compared.

This report has focused on an interesting example of chiral recognition in which one diastereomer, the complex with the N-Ac-D-F enantiomer, is very well ordered in crystals at room temperature and the other, the complex with N-Ac-L-F, is so highly disordered under the same conditions that it is impossible to locate the guest molecule in half the host dimer. Cooling a crystal of the latter complex to 20 K, that is, reducing the kinetic energy of the system, has permitted the location of four "pairs" of guest molecules with different orientations and positions in the complex. Comparison of the crystal structures in these two systems provides indications that the differences in

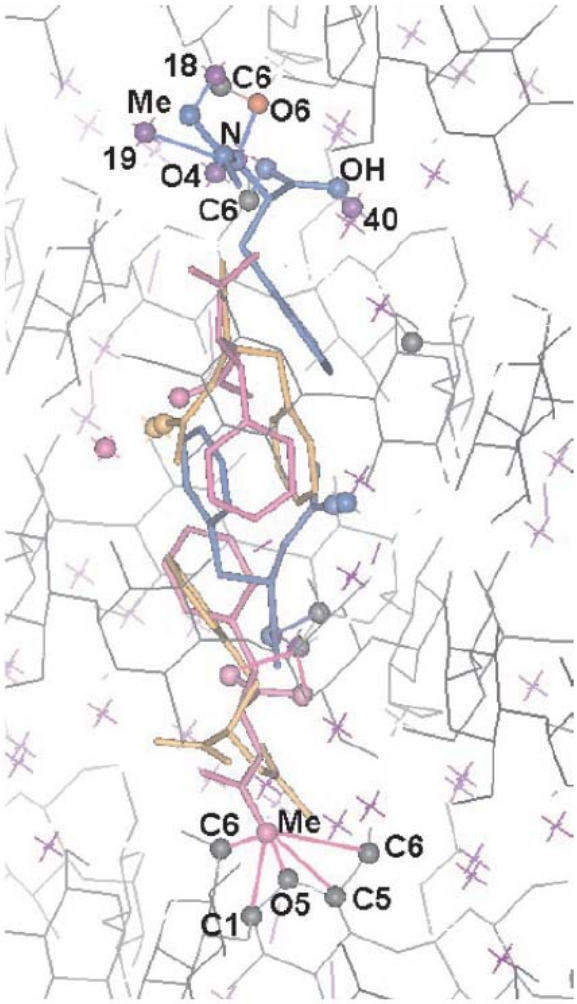


**Figure 5.** A superposition diagram of the crystal structure of the *N*-acetyl-D-phenylalanine complex (guest molecules and included water molecule colored gold), the **I1** inversion model (colored blue), and the **I2** inversion-rotation model (colored magenta). Unacceptably short interatomic distances for the two models are indicated, with the atoms involved drawn as colored spheres and labels. Water atoms are given only numerical labels, β-Cyclodextrin atoms have appropriate C or O prefixes, and guest molecule atoms are given only letter labels.

molecular recognition of the two amino acid enantiomers is not determined by any single change in hydrophobic interactions or in hydrogen bonding interactions. Rather, it is an accumulation of effects that include the presence of a small number of different water of hydration molecules in the crystal structure of the D-enantiomer in the supramolecular complex accompanied by subtle changes in hydrogen bonding interactions. One of the new water molecules is located deeply within the hydrophobic pocket of the inclusion complex. It is in a region of the extended nonconstraining torus where the water molecule has the potential to anchor one of the guest molecules to the extended β-CD torus via hydrogen bonding interactions between secondary hydroxyl groups and the associated guest molecule. The contact distances to this water molecule indicate that it does not fulfill this role. Rather, the contact distances indicate that it has become an integral part of the guest molecule to which it is hydrogen bonded and interacts only weekly with the host.

Primitive molecular modeling (that places the enantiomer of the tri-molecular unit consisting of two N-Ac-D-F molecules and the wa-

ter molecule in the torus that is hydrogen bonded to one of them in hydrated β-cyclodextrin lattice) indicates that this construct interferes with the hydrogen bonding in the hydrophilic interface between sheets of complexes in the crystal. That is, a hydrogen bonding network analogous to that observed for the diastereomer with the D-enantiomer of the phenylalanine derivative is not sterically possible.

To further develop the database, a temperature dependent study of the crystal structure of the *N*-acetyl-L-phenylalanine methyl ester complex would be useful, as would be crystal structures for two complexes with *N*-acetyl-L-tryptophan derivatives. The database is also being expanded to include selected non-amino acid complexes. A crystal structure for a *p*-cresol complex with 3.5:2 guest:host stoichiometry that also crystallizes with the Im packing motif provides an interesting snapshot of the hydrophobic effect (J.J.S. and T. I. Doukov, unpublished results).

## Acknowledgements

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