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Peptide–urea interactions as observed in diketopiperazine–urea cocrystal

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Abstract

In order to develop a more complete understanding of urea induced protein denaturation we have investigated the crystal structure of urea with the cyclic dipeptide diketopiperazine. This structure, determined to an *R* factor of 8.1%, shows extensive hydrogen bonding between urea and the peptide groups of diketopiperazine. These studies support a model where hydrogen bonding plays an important contribution in urea-induced protein denaturation. In the companion paper we present thermodynamic data for urea–peptide interactions in aqueous solution that further support this model. (*Biophysical Chemistry* 46 (1993) 171–177).

Keywords: Diketopiperazine; Urea; Cocrystal; Crystal structure

1. Introduction

For some time two models have been used, both convincingly, to describe how highly concentrated urea solutions bring about protein denaturation [2–9]. The first model is based on the ability of a urea molecule to form hydrogen bonds with peptide backbone groups and thereby unfold a protein through competition for the formation of hydrogen bonds within the interior of a protein. However this model has come under question due to the interpretation that hydrogen bonding within the interior of a protein does not play a major role in stabilizing the folded structure [10]. A second paradigm put forth is that urea, at high concentrations, has a strong influence on the hydrophobic effect, thus resulting in protein unfolding by decreasing hydrophobic interactions between water and hydrophobic side chains buried within the interior of a protein.

Both models have strong implications toward our understanding of the forces that are involved in protein stability and structure as well as many other macromolecular interactions. In order to develop a more complete understanding of these models we have begun a systematic study of small solid peptides, that mimic specific interactions within proteins, and their interactions with urea. The cyclic dipeptide cyclo-glycylglycine or diketopiperazine has been used as a model system to explore peptide backbone interactions [11–13].

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Here we report on the crystal structure of a urea-diketopiperazine cocrystal formed by cooling a solution of diketopiperazine in 8 *M* urea. This structure demonstrates the direct involvement of hydrogen bonding between urea and the peptide groups of diketopiperazine. In the companion paper we have used this structural interpretation to determine binding equilibria between urea and diketopiperazine [1].

2. Experimental

Cocrystallization of diketopiperazine with urea results in twinned crystals with the two twins related by a 180° rotation about the $[10\bar{1}]$ axis. Data were collected at 23°C using Cu-K_α(λ = 1.54184 Å) radiation over a 2θ range from 3.0° to 110°. The unit cell is monoclinic with cell parameters: a = 23.199 (4) Å, b = 8.186 (11) Å, c = 23.165 (4) Å, β = 105.519 (13)°. The spacegroup is C 2/c with an asymmetric unit that consists of two diketopiperazine and four urea molecules. Deconvolution of the twinned reflections was accomplished by methods outlined by P. van der Sluis [15]. The structure was refined to a final reliability factor R = 8.10%.

3. Results

Some 30 years ago our lab discovered the formation of a cocrystal of urea and diketopiper-

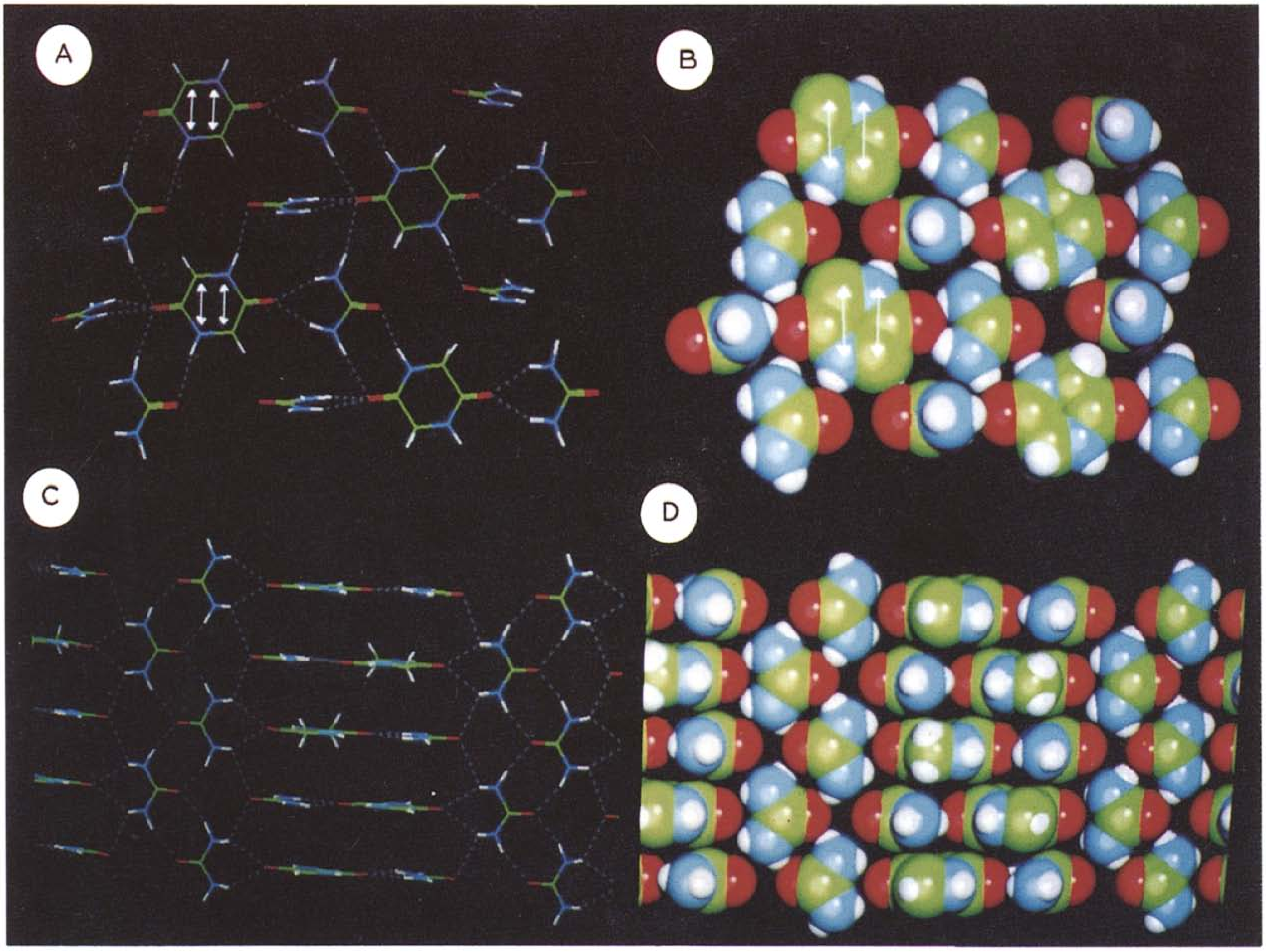

Fig. 1. Crystal structure of diketopiperazine-urea cocrystal. Figures 1A and B show a stick diagram and a ray tracing diagram of the Van der Waals spheres, respectively, for the view taken perpendicular to the plane of the diketopiperazine ring. The arrows show the two possible conformations of the disordered diketopiperazine. Dotted lines represent hydrogen bonds. Figures 1C and D depict similar figures viewed parallel to the plane of the diketopiperazine ring. Figures were generated using the polygen program Quanta.

azine with a stoichiometry of two urea molecules per diketopiperazine [14]. At that time the crystal structure was not solved due to twinning and disorder problems. Reevaluation of these crystals using recently developed computational techniques has allowed for a structure to be determined.

Figures 1A–D shows the structure of the diketopiperazine–urea cocrystal. A complication of this structure is that in one of the two diketopiperazine rings within the asymmetric unit (labeled with arrows in Fig. 1) the alpha carbons are indistinguishable from the amide nitrogens due to crystal disorder. Because of this disorder we have only been able to locate a single hydrogen per alpha carbon as shown in Fig. 1. This disordered structure appears to be an average of two superimposed orientations. The hydrogen bonding pattern and Van der Waals interactions appear quite similar for each orientation, as well as to the molecular interactions observed for the ordered diketopiperazine.

In the diketopiperazine–urea cocrystal, each diketopiperazine is surrounded by six ureas, three in the same plane as the diketopiperazine (horizontal), and three perpendicular to the plane (vertical). The urea molecules are in turn bound to other diketopiperazines, leading to sheets of hydrogen bonded molecules (Figs. 1A and B). The vertical urea molecules are hydrogen bonded to other vertical urea molecules in nearby planes, making columns that hold the planes together (Figs. 1C and D). The net result of these interactions is an extensively hydrogen bonded three dimensional structure.

Examination of the hydrogen bonding pattern between urea and diketopiperazine (Fig. 2) reveals that five of the surrounding urea molecules are capable of forming a net total of eight possible hydrogen bonds with diketopiperazine (using a hydrogen bonding distance of 2.35 Å between proton and acceptor atoms). Interestingly one of the carbonyl oxygens of diketopiperazine is within hydrogen bonding distance of four amide protons

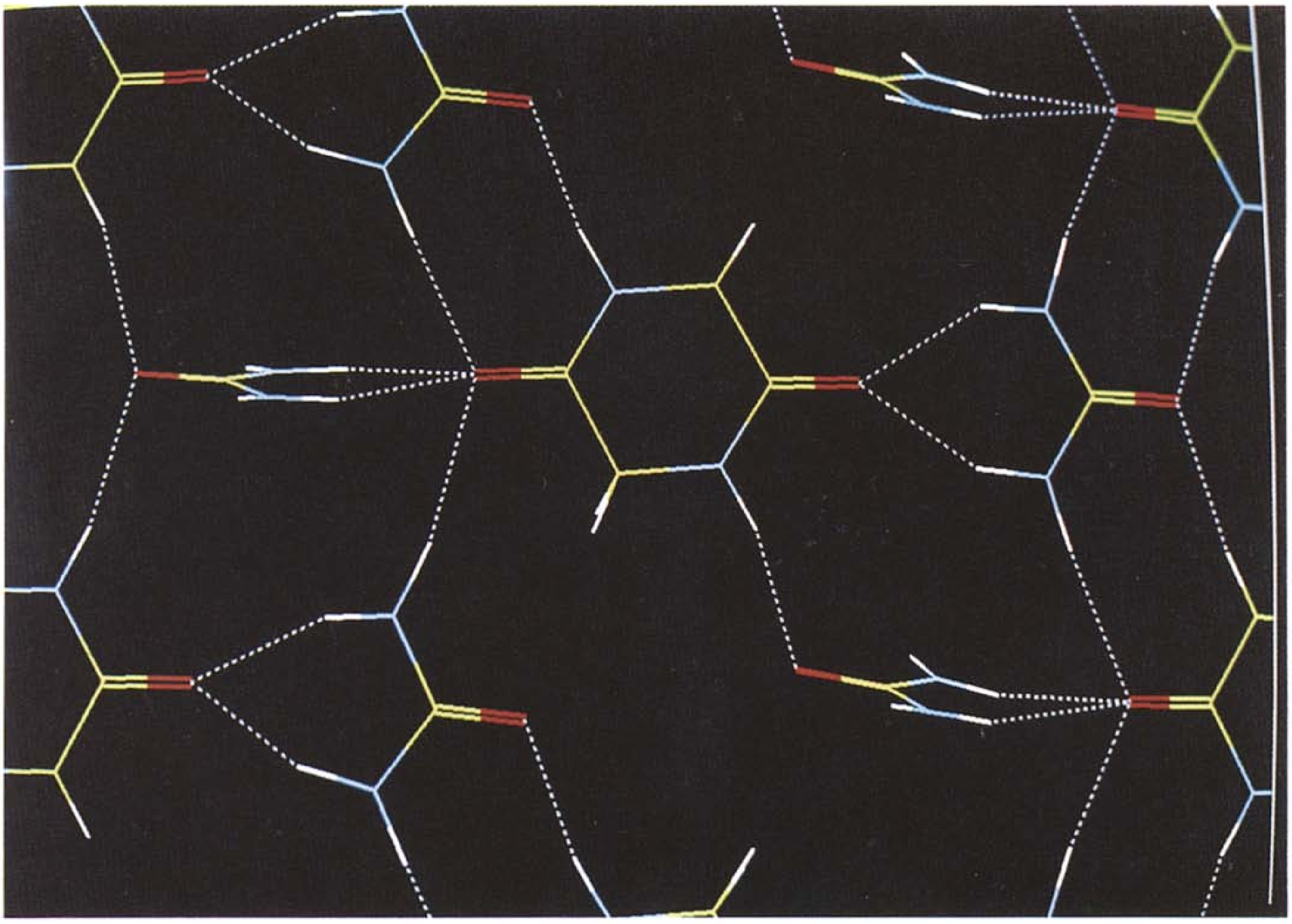

Fig. 2. Hydrogen bonding between diketopiperazine and urea shown as dotted lines.

from adjacent urea molecules. A similar structural motive has been observed in crystals of urea.

Comparison of the cocrystal structure presented here with the crystal structure of diketopiperazine [16] (Figs. 3A and B) shows how extensively the urea molecules solvate diketopiperazine in the cocrystal, precluding all diketopiperazine-diketopiperazine hydrogen bond formation. We also observe a decrease in the stacking interactions between diketopiperazine molecules within the cocrystal due to the inclusion of a urea molecule into the stacking plane (Figs. 3C and D). The distances between these adjacent stacking planes are quite similar in the cocrystal and in the diketopiperazine crystal at 3.33 Å and 3.51 Å, respectively, suggesting similar Van der Waals packing constraints within the two crystals.

4. Discussion

The structure of the diketopiperazine–urea cocrystal presented here provides a structural interpretation of urea induced protein denaturation. The model that these studies support is one where urea effectively competes with internal protein functional groups for the formation of hydrogen bonds, thus contributing to protein unfolding. This interpretation is consistent with the only other known structure of a urea–peptide complex, L-cysteine ethyl ester · HCl · urea [17], where urea has been shown to hydrogen bond

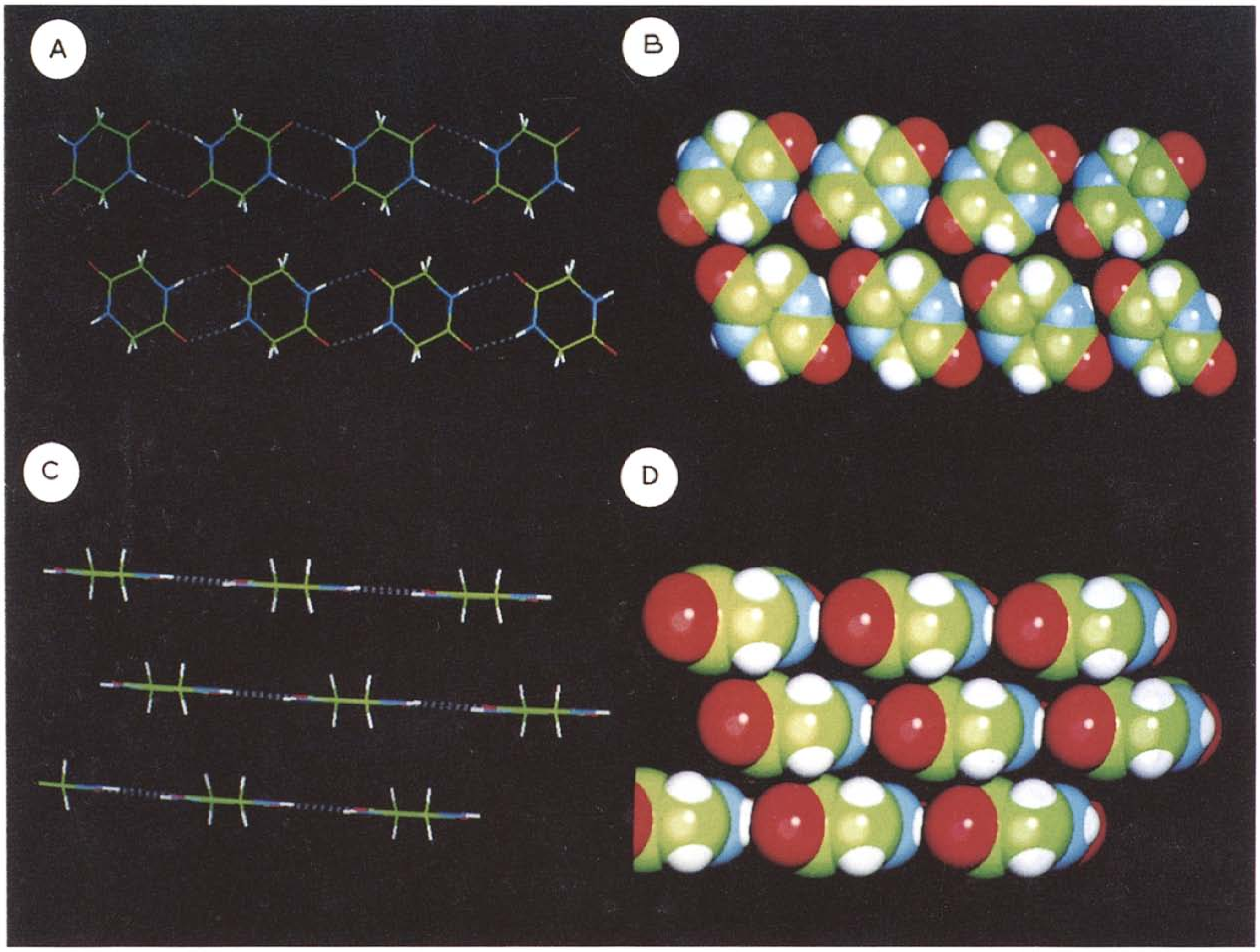

Fig. 3. Crystal structure of diketopiperazine regenerated using the data of Corey [16]. Figures 3A and B show a stick diagram and a ray tracing diagram of the Van der Waals spheres, respectively, for the view taken perpendicular to the plane of the diketopiperazine ring. Figure 3C and D depict similar figures viewed parallel to the plane of the diketopiperazine ring.

with the charged amino group and the carbonyl group of cysteine. It should be emphasized, however, that these data do not discount the importance of the influence of urea on the hydrophobic effect. Instead we obtain a model where both hydrogen bonding between urea and peptide groups, and changes in hydrophobic interactions in the presence of urea, led to urea induced protein denaturation.

In order to obtain an energetic understanding of urea–peptide interactions and the implications of such interactions to protein denaturation we have explored the solubility properties and phase equilibria of diketopiperazine and diketopiperazine–urea as a function of urea activity. These data are presented in the following paper [1].

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