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Structural Evidence for the Aromatic–(*i*+1) Amine Hydrogen Bond in Peptides: L-Tyr-L-Tyr-L-Leu Monohydrate

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Abstract

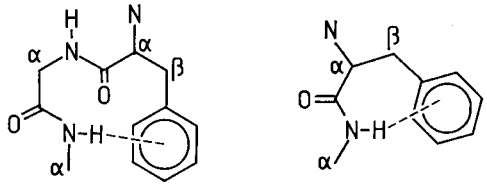
In crystalline Tyr-Tyr-Leu monohydrate, an aromatic–(*i*+1) amine hydrogen bond is observed, that is a weak hydrogen-bond-type interaction between an aromatic side chain and N—H of the next peptide group in the main chain. Unlike the better investigated aromatic–(*i*+2) amine hydrogen bonds, which can adopt almost ideal geometries, the geometry of the discussed interaction is very distorted because of steric constraints. Presumably, this kind of weak hydrogen bond is only formed as a last resort if N—H finds it impossible to engage in the much stronger conventional hydrogen bonding with O-atom acceptors.

1. Introduction

It has long been known that phenyl groups may act as acceptors of hydrogen bonds (often called 'aromatic hydrogen bonds') (Jeffrey & Saenger, 1991). In biological systems, this is of particular importance for protein structures where hydrogen-bond-type N—H···Ph interactions are frequently observed (Burley & Petsko, 1986). In optimal geometry, which is achieved when N—H is positioned exactly above the aromatic center and points linearly at it, the bond energy has been calculated at around 3 kcal mol^{-1} (1 kcal mol^{-1} = 4.184 kJ mol^{-1}) (Levitt & Perutz, 1988; for an experimental gas-phase study on the ammonia–benzene dimer, see Rodham *et al.*, 1993). In competitive situations, however, the stronger conventional hydrogen bonding is normally preferred to N—H···Ph interactions (Mitchell *et al.*, 1994). Several examples have been reported where N—H···Ph bonds are involved in protein–ligand binding (Perutz *et al.*, 1986; Parkinson *et al.*, 1996). Also with O—H donors, 'aromatic hydrogen bonding' has been observed in protein structures (Liu *et al.*, 1993; Dietze *et al.*, 1996), and in a number of peptide crystal structures, water-to-phenyl hydrogen bonds have been found (Steiner *et al.*, 1998).

In this general context, Worth & Wade (1995) analysed the occurrence and properties of what they term the 'aromatic–(*i*+2) amine interaction' in peptides and proteins, that is interactions of aromatic amino-acid side chains with N—H of the second next peptide group in the main chain. In theoretical calculations on tripeptide conformations including possible interactions with solvent molecules, they find a very complex energy hypersurface, in which an N—H···Ph hydrogen bond is not formed if the amine group has the opportunity to engage in conventional N—H···O hydrogen bonding. If the latter is not possible, N—H···Ph hydrogen bonding serves as a last resort, allowing the otherwise unsatisfied N—H donor capacity to be at least partially fulfilled. Consistent with the earlier work of Levitt & Perutz (1988), N—H···Ph bond energies around 3 kcal mol^{-1} are calculated for ideal geometry. Worth & Wade (1995) also briefly mention the occurrence of aromatic–(*i*+1) amine interactions in proteins, but do not investigate it more closely and give no information on the geometry or on the bonding or possibly repulsive character of these interactions, or on their behaviour in competitive situations.

To shed light on the open problem of aromatic–(*i*+1) amine interactions, the observation of such a contact in hydrogen-bond geometry in a tripeptide crystal structure is reported here. The general configuration of aromatic–(*i*+2) and (*i*+1) amine bonds is schematically shown in Fig. 1.



(*a*) (*b*)

Fig. 1. Schemes of (*a*) aromatic–(*i*+2) amine and (*b*) aromatic–(*i*+1) amine hydrogen bonds in peptides.

2. Experimental

A commercial sample (Sigma) of L-Tyr-L-Tyr-L-Leu acetate salt was dissolved in water; slow evaporation yielded rod-shaped colourless crystals of L-Tyr-L-Tyr-L-Leu monohydrate which are stable under ambient conditions (L-tyrosyl-L-tyrosyl-L-leucine monohydrate, $C_{24}H_{31}N_3O_4{\cdot}H_2O$, M_r = 475.5).

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Table 1. *Relevant torsion angles defining the peptide conformation of Tyr-Tyr-Leu*

Residue	1	2	3
ψ	176 (1)	8 (1)	
ω	−167 (1)	168 (1)	
φ		−91.5 (9)	−89 (1)
χ^1	−165.5 (8)	65 (1)	−59 (1)
$\chi^{2,1}$	−126 (1)	−104 (1)	−60 (2)

A crystal of dimensions 0.45 × 0.10 × 0.10 mm was glued on a glass pin and used for all X-ray diffraction experiments (Enraf–Nonius Turbo-CAD4 diffractometer on an FR571 rotating-anode generator, Ni-filtered Cu $K\alpha$ radiation with λ = 1.5418 Å, room temperature). The space group is orthorhombic $P2_12_12_1$ with unit-cell dimensions a = 9.149 (2), b = 12.887 (2), c = 22.124 (11) Å, V = 2608.5 (1.5) Å^3, Z = 4, Z' = 1, D_c = 1.21 g cm^{-3}. 2681 unique reflections were measured to the resolution of $\lambda/2\sin\theta_{max}$ = 0.89 Å, 1308 with $I > 2\sigma(I)$. Structure solution and refinement was performed with standard methods [programs *SIR*92 (Altomare *et al.*, 1994) and *SHELXL*93 (Sheldrick, 1993)]. H atoms were treated in the riding model with exception of the hydroxyl H atoms which were refined isotropically and the N1 ammonium group which was allowed to rotate. The water H atoms could not be unambiguously located in difference Fourier analyses and were, therefore, not included in the model. Refinement converged with R = 0.075 (for observed reflections), $wR(F^2)$ = 0.185 (for all reflections).

3. Results

3.1. *Crystal and molecular structure*

The molecular structure of the tripeptide L-tyrosyl-L-tyrosyl-L-leucine as observed in the monohydrate crystal structure is shown in Fig. 2, some relevant torsion angles are given in Table 1. The molecule, which crystallizes as a zwitterion, adopts a folded conformation with both tyrosyl side chains oriented roughly in the same direction. The O$^\eta$ hydroxy groups of these residues approach to 3.57 (1) Å and donate hydrogen bonds to the carboxy-terminus of a neighbouring peptide molecule. Because of this conformation, N—H of the peptide group linking the two tyrosine residues (N2—H) is shielded from intermolecular contacts. Instead, N2—H is involved in a short contact to the aromatic moiety of Tyr1; the shortest distance is to C$^{\gamma 1}$ with a H···C separation of only 2.42 Å. This contact of the Tyr1 side

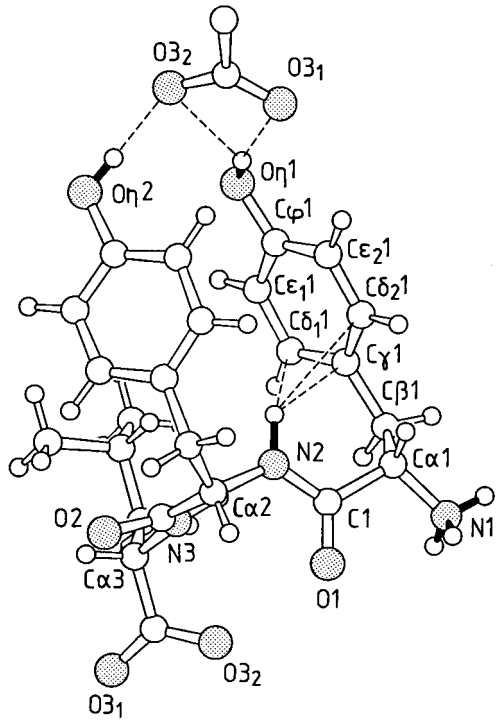


Fig. 2. Molecular structure of Tyr-Tyr-Leu in the monohydrate crystal structure. Only atoms relevant to the discussion are labelled. O and N atoms are drawn shaded. O—H and N—H covalent bonds are drawn filled.

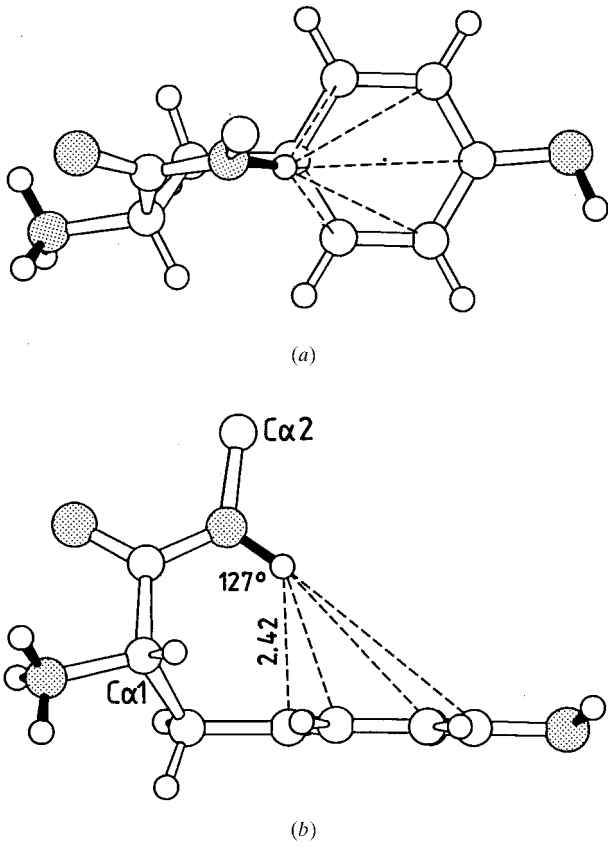


Fig. 3. The aromatic–(i+1) amine contact in Tyr-Tyr-Leu shown in projections (*a*) onto the aromatic plane and (*b*) perpendicular to the aromatic plane. O and N atoms are drawn shaded.

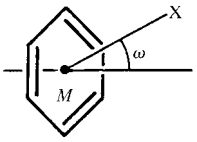
Table 2. *Geometry of the aromatic–(i+1) amine contact in Tyr-Tyr-Leu monohydrate for N—H = 1.03 Å*

Contact	H···C (Å)	N···C (Å)	N—H···C (°)
N2—H···$C^{\gamma 1}$	2.42	3.14 (1)	127
$C^{\delta_1}1$	2.83	3.60 (1)	131
$C^{\delta_2}1$	2.72	3.58 (1)	142
$C^{\varepsilon_1}1$	3.49	4.42 (1)	150
$C^{\varepsilon_2}1$	3.40	4.40 (1)	162
$C^{\varphi 1}$	3.73	4.75 (1)	171
M	2.82	3.78 (1)	156

chain with the next peptide N—H represents an aromatic–amine interaction of the very poorly investigated (*i*+1) type, Fig. 1(*b*), and, therefore, deserves closer inspection. Furthermore, a very similar contact was only recently observed in the crystal structure of Tyr-Tyr-Phe dihydrate (reported without interpretation in the context of solvent–peptide interactions: Steiner *et al.*, 1998), giving additional motivation to this study.

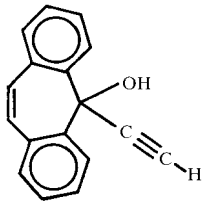
3.2. *The aromatic–(i+1) amine contact*

The geometry of the contact N2—H···Ph(Tyr1) is illustrated in projections onto and perpendicular to the aromatic plane in Fig. 3, and numerical data are given in Tables 2 and 3. The contact is very off-centered with H···C distances ranging from 2.42 to 3.73 Å. When considering the geometry with respect to the aromatic centroid *M*, the H···*M* distance is 2.82 Å, and the angles ω(H) and ω(N) which define the direction of the approach are 32.2 and 38.2°, respectively (Table 3). A look at Figs. 2 and 3 shows that this off-centered geometry is a consequence of steric constraints within the peptide molecule which do not allow a centered contact geometry with ω-values close to 0°. Any rotation around the bonds C1—Cα1 or Cα1—Cβ1 will turn the aromatic moiety away from N2—H rather than bring it into a more perpendicular position. In the related case of Tyr-Tyr-Phe dihydrate, the geometry is even more off-centered (Table 3).



The geometry of the contact makes the interpretation in terms of its bonding or non-bonding nature difficult and possibly controversial ('bonding' here means having a negative interaction energy, 'non-bonding' means having a positive interaction energy). One point of view is to regard the contact as 'forced' by the particular peptide conformation, presumably non-bonding and destabilizing the conformation. The short H···$C^{\gamma 1}$ distance of 2.42 Å (the H···C van der Waals distance is *ca* 2.7 Å) would then be a sign of compression and of steric strain. A different view is to point at the well known geometrical softness of *X*—H···Ph hydrogen bonds, and speculate on possible hydrogen-bonding character of the contact. In the energy maps of Worth & Wade (1995), the contact would be well inside the bonding region, but these maps were calculated for a chemically very simple model, and their application for the present intramolecular contact might be a severe oversimplification. In general, energy calculations on intramolecular hydrogen bonds are questionable, in particular if they are, as in the present case, far from optimal geometry.

At this point, it is helpful to refer to recent studies on a very off-centered intermolecular O—H···Ph contact in the crystal structure of 5-ethynyl-5*H*-dibenzo[a,d]cyclohepten-5-ol, (1), which is shown in Fig. 4.



(1)

The geometry of this contact has been determined very accurately with neutron diffraction (Steiner *et al.*, 1997). O—H points almost linearly at an individual C atom of the acceptor group. The geometry with respect to the aromatic centroid is characterized by ω(H) = 41.7° and ω(O) = 35.3°, *i.e.* it is even more off-centered than the aromatic–(*i*+1) amine contact in Tyr-Tyr-Leu monohydrate. Since the contact is intermolecular, it is certainly not forced by stereochemistry and must be taken as a relevant interaction between neighboring molecules. For this particular contact in a simple organic structure, hydrogen-bond nature could be shown unambiguously by IR-spectroscopy, and *ab initio* molecular orbital quantum chemical calculations estimated a bond energy of *ca* −1.3 kcal mol^{-1} (Steiner *et al.*, 1996). This means that the O—H···Ph interaction in (1) is in fact a hydrogen bond, but with drastically reduced bond energy compared with one in optimal geometry. It is reasonable to assume that the relatively small variation in geometry leading to the aromatic–(*i*+1) amine interaction in Tyr-Tyr-Leu monohydrate does not lead to completely different properties: the contact should represent a hydrogen bond, but the interaction energy is certainly much smaller than for the perpendicular N—H···Ph hydrogen bonds considered *e.g.* by Levitt & Perutz (1988) in their classical study.

For reasons of comparison, it is appropriate to show an aromatic–(*i*+2) amine hydrogen bond in the same way as the contacts discussed above. As an example containing a decent hydrogen bond of this kind, the structure of the trihydrated tetrapeptide L-Phe-Gly-Gly-D-Phe (Fujii *et al.*, 1987) was extracted from the Cambridge Structural Database (CSD, Allen &

Table 3. *Data for the intramolecular N—H···Ph contacts discussed (for normalized H atom positions with N—H = 1.03 Å)*

Peptide	Tyr-Tyr-Leu	Tyr-Tyr-Phe	L-Phe-Gly-Gly-D-Phe
Reference	This work	Steiner *et al.* (1998)	Fujii *et al.* (1987)
Type	*i*+1	*i*+1	*i*+2
Acceptor	Tyr	Tyr	Phe
H···C range (Å)	2.41–3.73	2.47–4.16	2.63–3.29
H···C spread (Å)	1.32	1.75	0.66
N···C range (Å)	3.14–4.75	3.25–5.14	3.59–4.18
N···C spread (Å)	1.61	1.89	0.59
H···*M* (Å)	2.82	3.13	2.65
N···*M* (Å)	3.78	4.07	3.74
N—H···*M* (°)	156	153	142
ω(H) (°)	32.2	44.2	23.3
ω(*M*) (°)	38.2	45.9	13.0
N—H···$C_{shortest}$ (°)†	127	131	155

† N—H···C angle with the C atom having the shortest distance to H.

Kennard, 1993) and is shown in Fig. 5; numerical data are given in Table 3. It is obvious that in this case, unlike for the aromatic–(*i*+1) amine interaction, donor and acceptor are separated by a relatively long flexible chain of atoms, allowing the N—H···Ph bond to adjust close to ideal geometry. In that particular example, the peptide conformation is also stabilized by a short edge-to-face Ph–Ph interaction (Fig. 5).

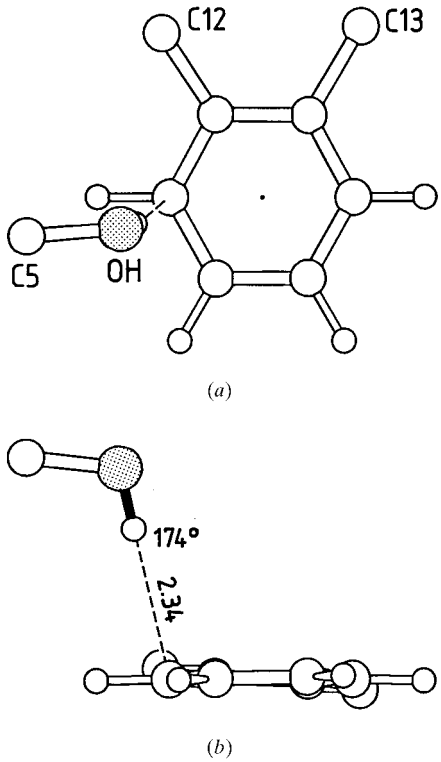


Fig. 4. The O—H···Ph hydrogen bond in the low-temperature neutron diffraction structure of 5-ethynyl-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ol (Steiner *et al.*, 1997) shown in projections (*a*) onto the aromatic plane and (*b*) perpendicular to the aromatic plane.

4. Conclusions

The crystal structure of L-Tyr-L-Try-L-Leu monohydrate contains a clear-cut example of an aromatic–(*i*+1)-amine N···H···Ph contact, which is a novel finding for a peptide structure. Because of stereochemical constraints, the geometry of the N—H group with respect to the aromatic moiety is extremely off-centered, and it is not immediately possible to judge on whether it represents a non-bonding 'forced contact' or, possibly, a weak and distorted hydrogen bond. Geometrical similarity with an O—H···Ph interaction in a simple organic crystal structure, for which hydrogen-bond nature has been clearly shown, suggests that the aromatic–(*i*+1)-amine interaction also represents a weak but noticeable hydrogen bond. The same analogy, however, also suggests that because of the strong geometrical distortion, the energy is reduced to values far below those for aromatic–(*i*+2) amine hydrogen bonds. Estimating this energy would be speculative here.

Worth & Wade (1995) report that aromatic–(*i*+2) amine hydrogen bonds are formed if conventional N—H···O hydrogen bonding is not possible, and then serve as a last resort to satisfy the N—H donor potential at least partially. This means that aromatic Ph(*i*−2) acceptors are a 'second choice' as hydrogen-bond partners for N—H donors, clearly inferior to O (or N, Cl^-, *etc.*) acceptors. Following this line of argumentation, Ph(*i*−1) acceptors would have to be considered as the 'third choice'. Nevertheless, engaging in such weak third-choice hydrogen bonds is still more favourable than engaging in no bonding interaction at all (in the context of the role of weak and weakest hydrogen bonds in protein structure, also see McDonald & Thornton, 1994). This is in line with complementary findings on the role of weak hydrogen bonds in satisfying acceptor potentials: for water molecules, it was shown that if they find no O—H or N—H donors suitably available, they normally satisfy their acceptor potential at least partially with C—H···O_w interactions (Steiner & Saenger, 1993;

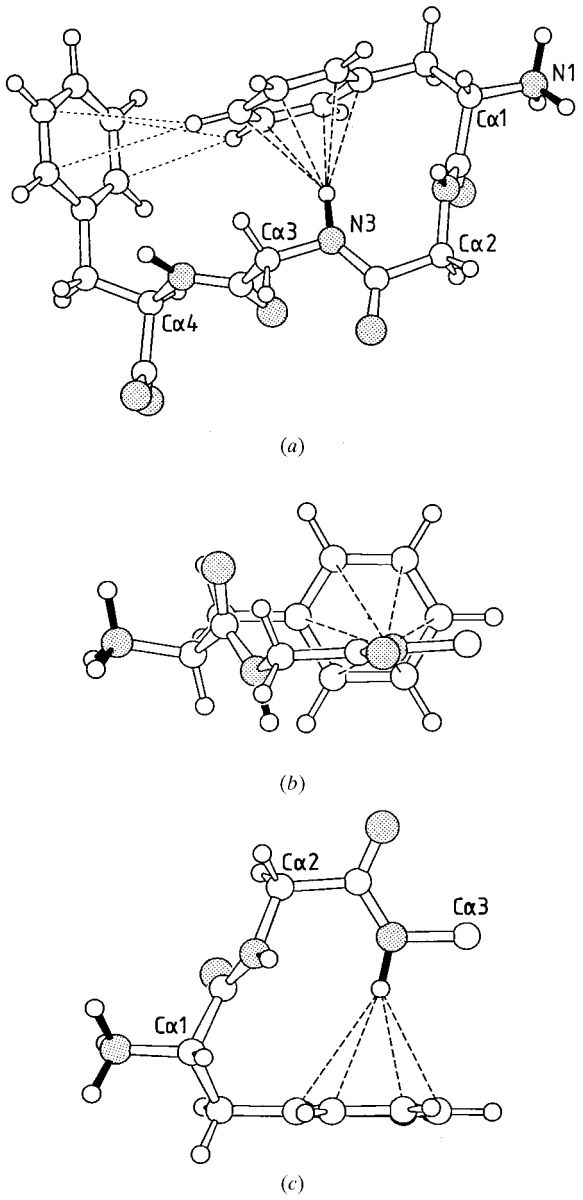


Fig. 5. (*a*) Molecular structure of the tetrapeptide L-Phe-Gly-Gly-D-Phe as observed in the trihydrate crystal structure (Fujii *et al.*, 1987; refcode in the CSD: FEYZEO). O and N atoms are drawn shaded. An edge-to-face Ph–Ph interaction, which was discussed by the original authors, is shown by light dashed lines indicating the shortest (C)H···C separations (minimum: 2.76 Å). The aromatic–(*i*+2) amine interaction is shown in projections (*b*) onto the aromatic plane and (*c*) perpendicular to the aromatic plane.

for background on C—H···O interactions, see Desiraju, 1996; Steiner, 1997).

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