SHORT COMMUNICATIONS

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Structural and computational analysis of published neutron diffraction data shows that crystalline vitamin B_{12} coenzyme contains a strong intramolecular N—H···Ph hydrogen bond

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Abstract

The neutron diffraction crystal structure of vitamin B_{12} coenzyme [Bouquiere *et al.* (1993). *Acta Cryst.* B49, 79–89] contains a yet unnoticed intramolecular aromatic hydrogen bond donated by a propionamide side chain and accepted by the benzimidazole group. The distance from the H-atom to the aromatic midpoint is only 2.58 Å and the bond energy is calculated to be 16.7 kJ mol^{-1}.

1. Introduction

Crystalline hydrated vitamin B_{12} coenzyme is a classical biological model system for structural studies of complex hydrogen-bond effects. The chemical structure of the coenzyme and the standard labeling of side chains is shown in Fig. 1. The core of the molecule is formed by a corrin ring which complexes Co[1]. Apart from eight peripheral methyl groups, the corrin ring has seven longer flexible side chains carrying amide groups (labeled *a*–*g*). A nucleotide with a 5,6-dimethylbenzimidazole base is bonded to side chain *f via* the phosphate group and linked to Co *via* the benzimidazole atom N3. A 5′-deoxyadenosine ligand is bonded to Co *via* C5′. Owing to its intermediate size between a small molecule and a macromolecule, hydrated vitamin B_{12} coenzyme forms highly complex hydrogen-bond systems which can still be studied in atomic detail. High-resolution neutron diffraction crystal structures of excellent quality have been determined at 277 K by Savage *et al.* (1987) and at 15 K by Bouquiere *et al.* (1993). Previous analysis and interpretation of noncovalent interactions in these crystal structures has concentrated on water–biomolecule and water–water interactions, and on disorder phenomena (Savage, 1986; Bouquiere *et al.*, 1994; Steiner & Saenger, 1993), whereas interactions between neighboring coenzyme molecules and possible intramolecular hydrogen bonds were left out of the focus.

When re-inspecting the neutron diffraction studies we unexpectedly find a remarkable hydrogen bond of the type N—H···π(Ph), which has not been noticed previously. Such nonconventional 'aromatic hydrogen bonds' are currently under intense investigation in structural biology (*e.g.* Levitt & Perutz, 1988; Parkinson *et al.*, 1996), but for no system of biological relevance has accurate structural information from neutron diffraction been published as yet. The only available neutron data of an aromatic hydrogen bond is on an O—H···Ph interaction in an alkynol: Steiner *et al.* (1997). Therefore, the structural data from vitamin B_{12} coenzyme is of greater value and deserves a closer look and more detailed discussion.

2. Structural data

The neutron diffraction crystal structure of vitamin B_{12} coenzyme ~$18H_2O$ at 15 K (1), determined by Bouquire *et al.* (1993), is shown in Fig. 1 in a projection perpendicular to the benzimidazole plane. In this projection the corrin ring is shown in the side view. Two conventional intramolecular hydrogen bonds are formed: the primary hydroxyl group of the nucleotide ribose unit forms a hydrogen bond to a phosphate O atom and the amide group of side chain *e* forms a hydrogen bond to the ribose ring O atom. These two interactions are 'normal', as observed in many biomolecules (Jeffrey & Saenger, 1991). Most notably, side chain *d* is oriented such that an amide N—H bond points at the benzimidazole benzyl group. The geometry of this contact (given in Table 1) is clearly

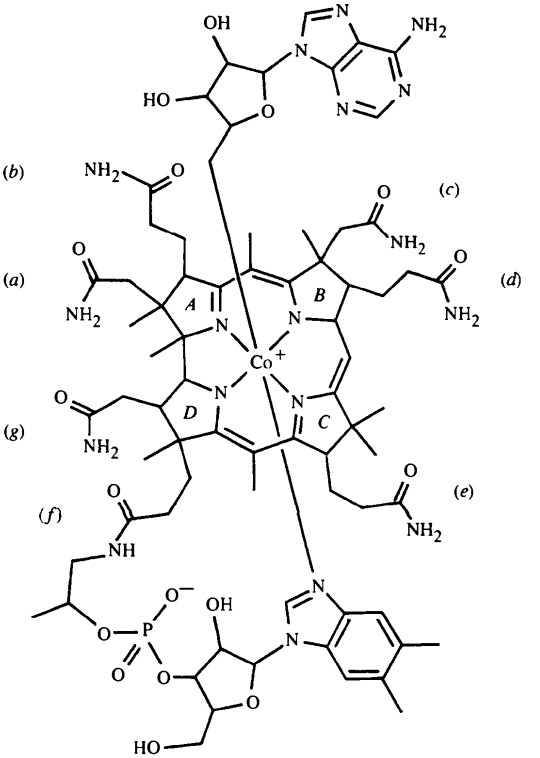
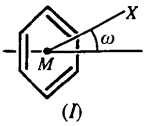


Fig. 1. Chemical structure of vitamin B_{12} coenzyme with standard numbering of the side chains.

indicative of aromatic hydrogen bonding: the distance of the donor H atom to the aromatic midpoint *M* is only 2.58 Å and the distance from the N atom to *M* is only 3.42 Å. As characterized by the angle ω [defined in (I)], the N—H donor is placed more or less 'above' *M*, *i.e.* the geometry is roughly centered. Comparing with literature values based on X-ray diffraction data (Viswamitra *et al.*, 1993; Bakshi *et al.*, 1994; Steiner *et al.*, 1998), this is not only the most reliably determined, but also one of the shortest and best centered N—H···Ph hydrogen bonds reported as yet.

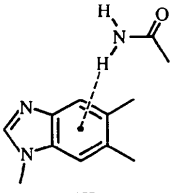


(*I*)

In the original neutron diffraction studies, *two* amide N—H groups are reported as 'free' from hydrogen bonding. One is from side chain *d*, which forms, in fact, an aromatic hydrogen bond. It is of interest to see if the other one (from side chain *b*) might similarly not be 'free', but form a weak hydrogen bond. A close look shows that it is actually engaged in a long N—H···N hydrogen bond with the adenine N1 atom of a neighboring coenzyme B_{12} molecule ($x, y, z - 1$), H···N = 2.48, N···N = 3.36 Å, N—H···N = 143.3°. Apparently, this interaction has been overlooked because it falls out of a restrictive distance cutoff. This means that in fact, *all* amide N—H groups of the molecule form hydrogen bonds (*cf.* the analysis of McDonald & Thornton, 1994, on the relation between distance cutoff criteria and 'unsatisfied' hydrogen-bond functional groups in proteins).

3. Computations

The energies of aromatic hydrogen bonds cover a wide range: for an amine N—H donor in idealized geometry, bond energies around 12 kJ mol^{-1} have been calculated (Levitt & Perutz, 1988), whereas for an O—H donor in strongly off-centered geometry, a bond energy of only 5.4 kJ mol^{-1} has been calculated (Steiner *et al.*, 1996). To estimate the strength of the aromatic hydrogen bond in (1), *ab initio* molecular orbital quantum chemical calculations were performed on the molecular dimer acetamide–1,5,6-trimethyl-benzimidazole (II) in the



(*II*)

geometry of the hydrogen bond in coenzyme B_{12} at 15 K, using the published atomic coordinates of Bouquiere *et al.* (1993) [MO LCAO SCF (HF + MP2) approximation using the *Gaussian*92/*DFT* package (Frisch *et al.*, 1993); atomic partial charges (Mulliken scheme) and intermolecular bond energy calculated using the 6-31G** basis set by taking into account electron correlation and the basis set superposition error (BSSE); CRAY Y-MP4D/464 of the Konrad-Zuse-Zentrum, Berlin]. This yielded an energy of 16.7 kJ mol^{-1}, indicating a surprisingly strong bond, not much weaker than 'normal' N—H···O interactions (Jeffrey & Saenger, 1991). Presumably, this strength is due to the high polarization of the amide donor (caused by the electron-withdrawing C=O group). For the somewhat less accurate structure at 277 K (Savage *et al.*, 1987), an energy of 17.6 kJ mol^{-1} was obtained for the N—H···Ph hydrogen bond.

Table 1. *Geometry of the aromatic hydrogen bond in (1); M = midpoint of the aromatic group; ω is defined in (I)*

N—H (Å)	0.99
H···*M* (Å)	2.58
H···C range (Å)	2.75–3.14†
H···C spread (Å)	0.39
N···*M* (Å)	3.42
N···C range (Å)	3.36–4.02
N···C spread (Å)	0.66
N—H···*M* (°)	143.3
N—H···C range (°)	115.0–170.7
N—H···C spread (°)	55.7
ω(N) (°)	15.1
ω(H) (°)	9.7

† The shortest contact is to C6, the longest to C9 (see Fig. 2*a*).

4. Related molecules

To see if the discussed interaction is unique to (1), or occurs also in related compounds, the molecular structures of the published coenzyme B_{12} analogs were inspected using the Cambridge Structural Database (Allen *et al.*, 1991). It was found that the orientation of side chain *d* with respect to the benzimidazole group is identical in both neutron structures of (1), and also in the earlier X-ray crystal structure of (1) (Lenhert, 1968), but different for the other structures (six cases). As an example, the best refined of the coenzyme B_{12} analog structures is shown in Fig. 2(*b*) (an analog where 5′-deoxyadenosine is replaced by an adenylpropyl moiety, published by Pagano *et al.*, 1991). In this structure, the functional groups which are relevant here are identical as in (1), but the hydrogen-bond interactions are completely different. Most of the flexible side chains are oriented differently as in (1) and no intramolecular hydrogen bonds are formed. In particular, the amide group of side chain *d* is found to be stacked with respect to the benzimidazole moiety and is engaged in conventional hydrogen bonding with other molecules. This shows that intramolecular aromatic hydrogen bonding is not an inherent feature of coenzyme B_{12}, but may or may not occur depending on the side chain orientation which is induced by environmental effects.

5. Conclusions

Revisiting the neutron diffraction crystal structure of vitamin B_{12} coenzyme reveals a remarkable intramolecular aromatic hydrogen bond between an amide group and the benzimidazole moiety (overlooked by the original authors). This observation is of great interest, because it represents the only N—H···Ph hydrogen bond for which highly reliable neutron diffraction data are available, far superior in quality to the X-ray data on which all the previous discussions are based. The energy of this hydrogen bond is calculated to be around 16.7 kJ mol^{-1}, which is not much less than for conventional hydrogen bonds. Thus, the hydrogen-bond pattern in crystalline coenzyme B_{12} turns

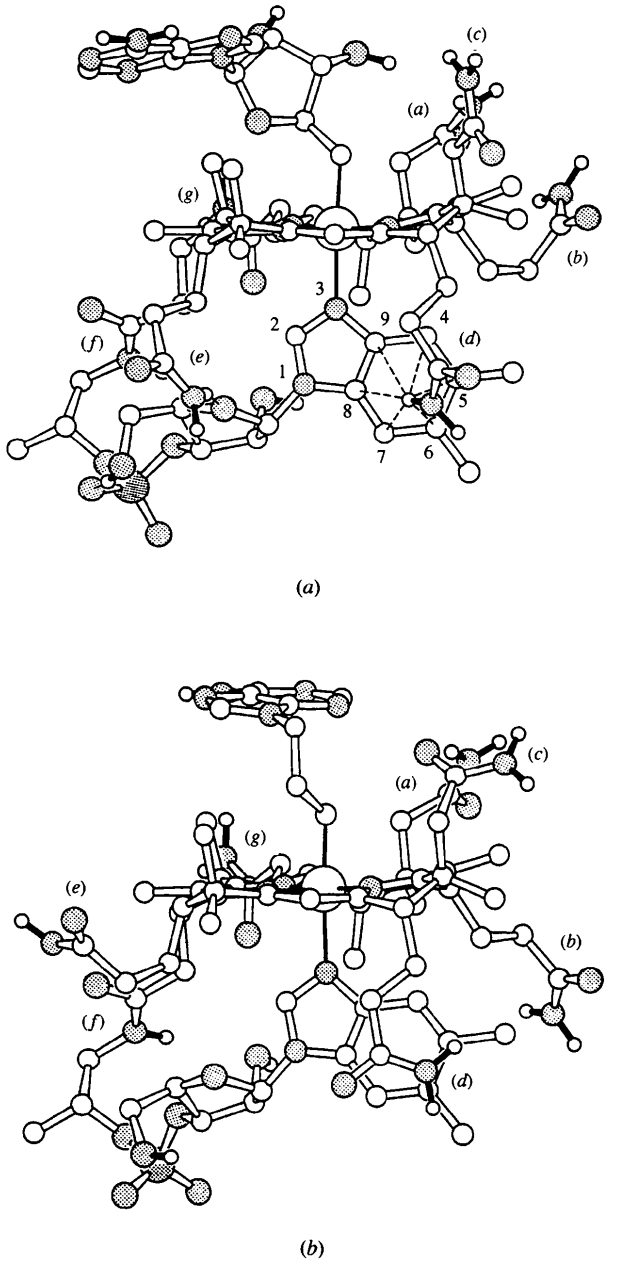


Fig. 2. (*a*) Molecular structure of vitamin B_{12} coenzyme (5′-deoxyadenosyl-cobalamin), as observed in the low-temperature neutron diffraction crystal structure of Bouquiere *et al.* (1993). Atom numbering for the benzimidazole moiety is shown. O, N and P atoms are drawn shaded. N—H and O—H bonds are drawn black. H atoms bonded to C are omitted for clarity. In the original publication, the atomic coordinates of the amide H atoms of side chain *a* have been omitted; for aesthetic reasons, they are drawn here in calculated ideal positions. (*b*) Molecular structure of a coenzyme B_{12} analog where 5′-deoxyadenosine is replaced by 9-$(CH_2)_3$-adenine (adeninylpropyl-cobalamin), as observed in the X-ray crystal structure of Pagano *et al.* (1991). Symbols as for (*a*).

out to be even more complex than reported previously, being composed of subtly interconnected O/N—H···O/N, C—H···O and N—H···Ph interactions.

The discussed aromatic hydrogen bond does not occur in the crystal structures of coenzyme B_{12} analogs, although they contain the same relevant functional groups. In the analog structures, stacked arrangements between the aromatic and amide groups are formed, where the amide N—H donors are engaged in conventional hydrogen bonding. This shows that the aromatic hydrogen bond is not inherent to the molecule itself, but occurs only under specific conditions of the molecular environment. These observations are in line with a statistical analysis of protein X-ray crystal structures, which shows that neighboring amino and aromatic groups (in proteins) can form N—H···Ph bonded as well as stacked arrangements, with the latter being preferred because then the amino N—H donor is free to form stronger conventional hydrogen bonds with other groups (Mitchell *et al.*, 1994).

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