SHORT COMMUNICATIONS

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The ordered water cluster in vitamin B_{12} coenzyme at 15 K is stabilized by C—H···O hydrogen bonds.

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Abstract

The hydrogen-bonding interactions of the ordered water cluster in the pocket region of vitamin B_{12} coenzyme at 15 K are analyzed from published atomic coordinates. Several of these water molecules accept C—H···O hydrogen bonds from the pocket wall, most of which have the function to complete tetrahedral hydrogen-bond coordination. This is the largest water assembly for which the stabilizing function of C—H···O interactions has been described.

Introduction

Recently, we have analyzed the role of C—H···O hydrogen bonds in the coordination of water molecules (101 water molecules in 46 neutron crystal structures) (Steiner & Saenger, 1993). The essential finding was that water molecules which cannot arrange in a tetrahedral O—H···O and N—H···O hydrogen-bond coordination, tend to satisfy 'free' hydrogen-bond acceptor potentials by C—H···O_W interactions. In fact, O—H, N—H, C—H and metal ions may coordinate to water molecules in many different configurations, with differences only in the hydrogen-bond distances. These observations, however, were primarily based on small-molecule neutron crystal structures, which contain only very few water molecules per asymmetric crystal unit; the only structures with larger assemblies of water were two cyclodextrin inclusion complexes. We assumed that the general trends should be similar in macromolecular systems, for which hydration phenomena play a most important role, but this must remain an assumption unless it is corroborated by accurate experimental data on large water assemblies.

Very recently, a high-resolution neutron diffraction study of vitamin B_{12} coenzyme at 15 K was published by Bouquiere, Finney, Lehmann, Lindley & Savage (1993). This structure contains the largest neutron-determined cluster of ordered water molecules that is presently available. As shown in Fig. 8 of this publication, several of the water molecules accept only one O—H···O_W or N—H···O_W hydrogen bond (ions are not present) and apparently have a very unfavourable hydrogen-bond coordination (this is particularly obvious for *W*223). As the water assembly is still ordered and stable, it is appropriate to assume the presence of additional stabilizing forces. In the following analysis, it will be shown that in fact these additional stabilizing forces are provided by C—H···O_W interactions.

The analyzed water cluster

The crystal structure of vitamin B_{12} coenzyme contains about 17–18 water molecules per asymmetric crystal unit. They are distributed over a solvent channel, in which they are disordered even at 15 K, and a pocket region, in which

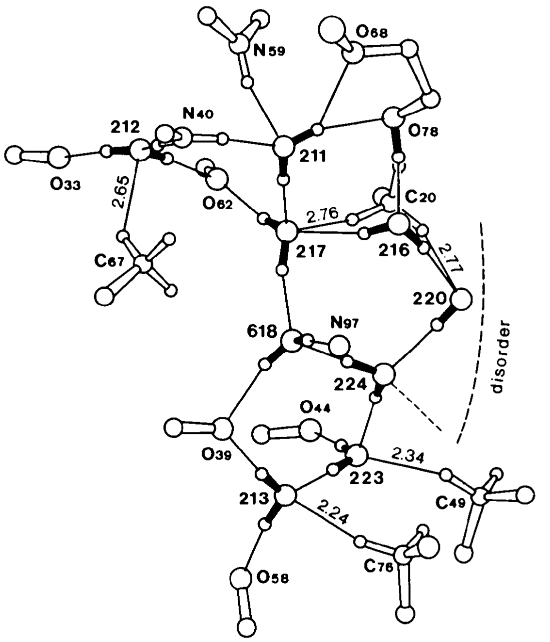

Fig. 1. Hydrogen-bond network in the ordered pocket region of vitamin B_{12} coenzyme at 15 K, drawn using coordinates from Bouquiere, Finney, Lehmann, Lindley & Savage (1993). The projection is very similar to that in Fig. 8 of the original publication of the structure. H···O separations are given in Å for C—H···O_W interactions only. For *W*213 and *W*223, the hydrogen-bond geometries are given in Fig. 2. For the C—H···O interaction accepted by *W*212, H···O = 2.65, C···O = 3.57 Å and the angle = 152°. For the interaction accepted by *W*217, H···O = 2.76, C···O = 3.74 Å and the angle = 147°. *W*213 accepts a further short contact from C66—H139, which is associated with a short H···H—O_W contact of 2.33 Å, and is, therefore, classified as 'uncertain' (H···O = 2.54, C···O = 3.56 Å, angle = 152°). Further short C—H···H—O_W contacts: C8—H111···H306—O213 (2.12 Å) and C36—H170···H516—O618 (1.88 Å). Refinement e.s.d.'s suggest that H···O distances are accurate to ~0.02, *X*···O to ~0.01 Å and angles at H to ~2°.

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they are ordered. At 279 K, the pocket region also shows disorder (Savage, 1986; Savage, Lindley, Finney & Timmins, 1987). The pocket offers to the enclosed water cluster several O—H, N—H, NH_2, C=O and C=N—C groups as classical hydrogen-bond partners, but it is also lined by numerous 'hydrophobic' C—H groups. In this sterically restricted and partly apolar surrounding, only few of the ~10 water molecules can arrange in a more or less ideal tetrahedral O—H···O and N—H···O hydrogen-bond configuration. Of the ordered water molecules, full atomic coordinates and coordinates of the surrounding atomic groups were published for only seven, 211, 212, 213, 216, 217, 223 and 618, to which the following discussion must, therefore, be restricted.

Results and discussion

Hydrogen bonds are defined by cutoff criteria as in the previous study; for C—H···O_W these are that H···O_W < 2.8 Å and the angle at H > 90°. The justification for and the consequences of this proceeding have been discussed in some detail (Steiner & Saenger, 1993). The hydrogen-bond configuration in the pocket region is shown in Fig. 1, for C—H···O_W interactions, the H···O_W distances are given in Å [for other hydrogen bonds, the distances are in the usually observed ranges; see, *e.g.* Jeffrey & Saenger (1991)].

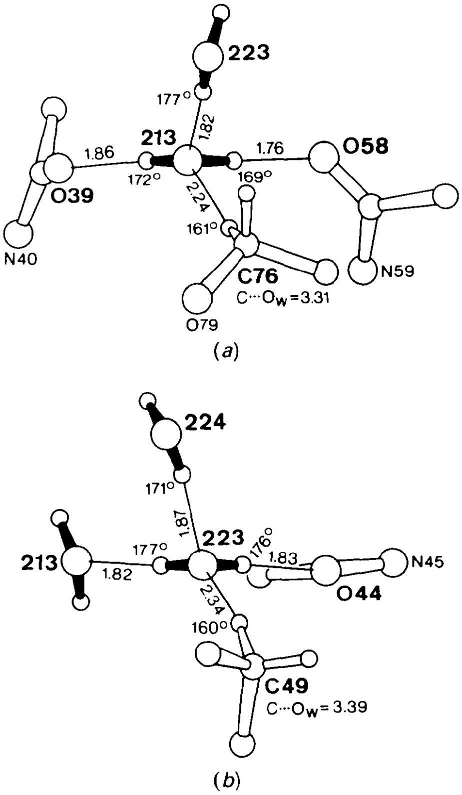

Fig. 2. The water molecules accepting the shortest C—H···O_W hydrogen bonds drawn along the water bisectors. (*a*) Water 213, (*b*) water 223. Distances are given in Å.

As expected, all water hydrogen-bond donor potentials are satisfied. If C—H···O interactions are neglected (as was done in the original structure publication), four of the seven water molecules discussed accept only one hydrogen bond (212, 213, 216 and 223), hence have unsatisfied acceptor potentials and should be prone to disorder. In particular, for *W*213 and *W*223 the resulting coordination is pronouncedly pyramidal and obviously highly unfavourable. If C—H···O hydrogen bonds *are* considered, it becomes clear that three of these four water molecules satisfy the apparently free acceptor potentials by C—H···O_W interactions with the pocket lining (212, 213 and 223) and are actually tetrahedrally coordinated (Fig. 1). One water molecule (217) even accepts three hydrogen bonds (two donated by water molecules and a very long one by a methyl group), and only one water molecule with a single acceptor function remains (216). For the two water molecules accepting the shortest C—H···O_W interactions, 213 and 223, the hydrogen-bond configuration is shown more quantitatively in Fig. 2. Note how neatly the C—H···O_W contacts complete tetrahedral coordinations. Also note that the shortest H···O_W separation is only 2.24 Å (C···O_W = 3.31 Å).

From these observations we conclude that C—H···O hydrogen bonds are an integral part of the hydration pattern of vitamin B_{12} coenzyme, and that they play an important role in the stabilization of the ordered water cluster in the partly apolar pocket region. This is the largest water assembly that has been discussed in this context so far and, therefore, is a most valuable support to the idea that the role of C—H···O_W interactions is principally the same in the hydration of small, medium sized and macromolecular structures (Steiner & Saenger, 1993).

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