Solution structure of cyanocobalamin (vitamin B_{12}) by NMR-restrained molecular dynamics and simulated annealing calculations

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The solution structure and solution dynamics of cobalamins can be determined and studied by NMR-restrained molecular-modelling techniques.

Determination of the crystal structure of vitamin B_{12} (cyanocobalamin, CNCbl) derivatives (Fig. 1), including the coenzyme form, 5′-deoxyadenosylcobalamin (AdoCbl), by X-ray[1] (and neutron[2]) diffraction has provided a major impetus for hypothesis and experimentation in the areas of cobalt corrinoid chemistry and biochemistry. There has been a heavy reliance in the B_{12} community on the details of these crystal structures for hypotheses regarding the enzymatic mechanism of AdoCbl function. However, structural studies of various cobalt corrinoids have shown that the corrin ring is highly flexible.[3] This flexibility, together with the exposed nature of the axial ligands and corrin ring side chains, would seem to make the corrinoids susceptible to substantial conformational modifications in the solid state. Thus, questions remain regarding the correspondence of crystal structures and solution conformations of corrinoids. In addition, important derivatives such as the base-off cobalamins and the axial nucleotide-free cobinamides have resisted crystallization, and so the conformational consequences of de-ligation of the axial nucleotide remain unknown.

The recent development of a molecular-mechanics force field for the cobalt corrins,[4] along with the ability to unambiguously assign the complex ^{1}H NMR spectra of these,[5] has now provided the opportunity to use NMR-restrained molecular modelling[6] to directly determine solution structures for such compounds. We demonstrate the success of such methodology by its application to the solution structure of vitamin B_{12}.

ROESY spectra were obtained on solutions of CNCbl in 90% H_2O–10% D_2O at various mixing times at 600 MHz. A total of 121 cross-peaks, not including those due to geminal hydrogens, could be resolved and unambiguously assigned at the longest mixing time (200 ms). NOE cross-peaks were classified as strong, medium, weak, or very weak[7] depending on whether they first appeared in a ROESY spectrum at mixing times of 50, 100, 150 or 200 ms, respectively. Molecular-dynamics (MD), simulated annealing (SA) and molecular-mechanics (MM) calculations were performed using HYPERCHEM V. 4.5,[8] the potential functions of the MM2 force field[9] and the force-field parameters developed for the cobalt corrinoids.[4a] The NMR restraints were modelled as functions of the form $k(r - r_0)^2$ where k = 1.2, 0.52, 0.3 and 0.075 kcal mol^{-1} Å^{-2} (cal = 4.184 J) and r_0 = 2.5, 3.0, 4.0 and 4.5 Å for the strong, medium, weak and very weak NOE cross-peaks, respectively.[6a] Assignments of prochiral protons were made on a trial-and-error basis until the minimum number of violations of distance criteria were obtained during 25–100 ps MD simulations at 300 K. Details are given in the supplementary material as are tables of the NMR and NOE assignments. Twenty five solution structures were generated by MD/SA procedures (heated from 300 to 1000 K over 5 ps, run at 1000 K for between 5 ps and 50 ps, cooled from 1000 to 300 K over 10 ps, all in steps of 1 fs; followed by full energy minimization).

The superposition of the 25 solution structures obtained in this manner is shown in Fig. 2. A consensus structure was

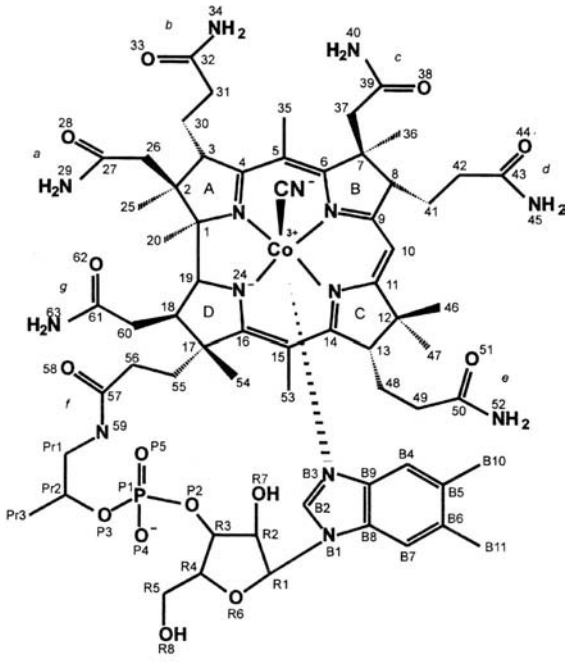

Fig. 1 Standard numbering scheme for cyanocobalamin (CNCbl, vitamin B12)

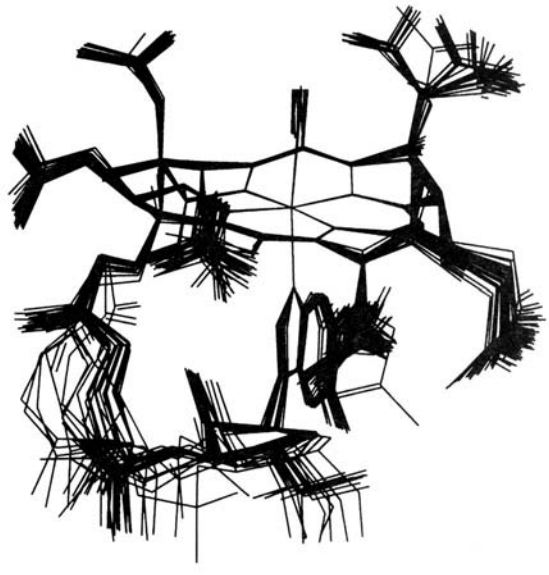

Fig. 2 Superposition of 25 solution structures of CNCbl determined by NMR-restrained molecular-modelling calculations

obtained by averaging the coordinates for these 25 structures and then energy minimizing the resultant structure. The r.m.s.d. for all heavy-atom positions in the 25 structures when superimposed was 0.66 ± 0.27 Å, while the r.m.s.d. for the corrin ring atoms alone (when the structures were superimposed only at the corrin atoms) was 0.045 ± 0.024 Å. Direct evidence that the NMR restraints significantly affect the resultant structures was obtained by modelling calculations after release of the NMR restraints on the side-chain atoms, and release of all NMR restraints.

The consensus structure is quite similar to the solid-state structure, with the principal differences occurring at the nucleotide loop and at several side chains. The corrin ring-fold angle[3] for the consensus structure was 18.5°, while for the 25 contributing structures the average value was 18.7 ± 1.5° (min., 15.7°; max., 21.2°). For comparison, the corrin ring-fold angle in the X-ray structure is 18.0°.[1a] The solution structure is, however, somewhat more folded in the western and northern quadrants of the molecule.

Despite differences in the orientation of the *b* and *d* side-chains in the two structures, the general disposition of the unsubstituted side-chains is quite similar. However, there is a major difference in the orientation of the *f* side chain, which has a profound effect on the orientation of the entire nucleotide loop. The C(16)–C(17)–C(55)–C(56) torsion angle is 133.1° in the solution structure, but −49.1° in the crystal structure. The *f* side chain is thus more horizontal in the former than in the latter (Fig. 2). This affects the entire disposition of the nucleotide loop and the orientation of the axial base. Thus, when viewed from above, the base in the solution structure has undergone an 11° clockwise rotation. The ribose ring also has a different conformation in the two structures; it is an envelope in the crystal structure, but a half-chair in the solution structure. The A, B and C pyrrole rings all have half-chair conformations in both the solution and the solid-state structures, although they are somewhat more twisted in the former. The D ring, in contrast, is in an envelope conformation in both structures, but is significantly flatter in the solution structure than in the solid state.

The variability in the side chains, nucleotide loop, and corrin ring-fold angle seen in the 25 solution structures suggests that there is considerable conformational flexibility in these segments of the structure. To see if this is the case, a 100 ps MD simulation at 300 K was run and selected structural parameters were monitored every 10 fs. The side chains have significant mobility about their mean positions and normally distributed torsion angles with the exception of the *c* side-chain which visits two distinct conformations, one in which the side chain points out from the corrin ring and the amide is vertically above C(36), and one in which the amide is directly above the region bounded by C(36) and C(35).

The axial base flips about its equilibrium position such that its maximum deviations on either side of the mean place the six-membered ring directly under C(6) at one extreme and to the left of C(5) (when viewed from above) at the other. The relative orientations of the base and the ribose, however, remain fairly constant. The conformation about the phosphate is quite flexible, and considerable flexibililty is also evident in the aminopropanol.

Flexibility about the C(1)–C(19) bridge in the corrin ring was also detected, with the N(21)–C(1)–C(19)–N(24) torsion angle (mean −46.5°) oscillating between −61.3 and −25.3°, producing relative up and down motions of the A and D pyrrole rings. In addition, monitoring of a number of corrin ring torsion angles showed that the corrin ring undergoes a breathing motion in which C(5), C(10) and C(15) move perpendicularly up and down above and below the plane of the corrin during the simulation. These results suggest some significant flexibility in the corrin ring which has long been thought to be important for the enzymatic acceleration of carbon–cobalt bond homolysis in AdoCbl.[10–12] Such flexibility has been observed in comparisons of the X-ray structures of various cobalt corrinoids, but has not been previously shown to occur in a given complex.

The apparent success of this methodology suggests that for corrinoids in which there are demonstrated conformational equilibria in solution,[4c,5b] but not in the solid state, and for those corrinoids which have resisted all efforts at crystallization, solution conformations may now be studied by NMR-restrained molecular modeling.

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Supplementary material (^{1}H NMR assignments for CNCbl, observed NOE cross peaks and intensities of CNCbl and atom assignments used in molecular modelling, and configuration of prochiral protons, 9 pp.) is available from the authors.

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