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The oxidized form of nicotinamide adenine dinucleotide

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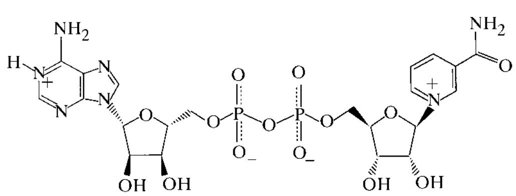
The crystal structure of the free acid form of NAD$^+$ tetrahydrate (nicotinamide adenine dinucleotide tetrahydrate or 3-carbamoyl-1-β-D-ribofuranosylpyridinium hydroxide 5′-ester with adenosine 5′-pyrophosphate inner salt tetrahydrate, $C_{21}H_{27}N_7O_{14}P_2 \cdot 4H_2O$) has been determined at 100 K. NAD$^+$ is the coenzyme of several protein families and plays a dominant role in biological redox processes. In this study, the molecule shows a different conformation from the one usually found in holoenzyme complexes.

Comment

The NAD$^+$ molecule (I) is the oxidized form of the coenzyme NADH. This redox pair holds great biological importance notably in energy-producing processes (Stryer, 1988). It is involved in redox processes catalyzed by various protein families, the dehydrogenases being the largest group.



(I)

As shown in Fig. 1, the molecule contains adenylic acid and nicotinamide-5′-ribonucleotide groups joined by a pyrophosphate linkage. Due to the acidity of the crystallization solution, the N3 atom of the adenine moiety is protonated. This positive charge and that carried by the nicotinamide group compensate the two negative charges on the pyrophosphate link. In the crystallization conditions used here, the molecule is thus electrically neutral (the notation NAD$^+$ refers to the redox pair NADH/NAD$^+$ and does not mean that the molecule holds a positive charge).

The structure of Li$^+$NAD$^+$ obtained from orthorhombic crystals has been reported by Saenger *et al.* (1977) and Reddy *et al.* (1981). In the triclinic crystals, the two ribose rings occur in a C4-*endo* (adenine moiety), C14-*endo* (nicotinamide moiety) envelope conformation (C2′-*endo* in the classical nucleic acid atom numbering scheme), which is the most favourable conformation allowed. NAD$^+$ (I) can also adopt a C3′-*endo* conformation as in the orthorhombic crystals of Li$^+$NAD$^+$. The other usual conformations, characteristic of nucleic acids, are also represented: the nicotinamide and adenine planes are in an *anti* orientation. The C1—C2 and C11—C12 bonds are *gauche* for both residues, with torsion angles O4—C1—C2—O5 and O10—C11—C12—O11 of −64.9 (1) and −71.3 (1)° for adenine and nicotinamide nucleotides, respectively.

The C15—N6 bond length in the nicotinamide moiety is 1.502 (2) Å, while the equivalent C5—N1 bond of the adenine moiety is 1.466 (2) Å. This significant difference in the two parts of the molecule is probably a consequence of the presence of the positive charge on N6.

Despite the local pseudo symmetry across the two sides of the pyrophosphate linkage, the lengths of the P—O1 bonds are very different: 1.580 (2) Å for P1—O1 and 1.630 (2) Å for P2—O1. This is also the case in the Li$^+$NAD$^+$ salt complex (Saenger *et al.*, 1977) and in most holoenzyme complex structures. The wide range of O_2P—O bond lengths seems to be a characteristic of the NAD$^+$ molecule.

The overall conformation of NAD$^+$ in the free acid form is compact and quite different from that usually found in holoenzyme complexes (Carugo & Argos, 1997). The torsion angles O4—C1—C2—O5, C2—C1—O4—P1, C1—O4—P1—O1 and O4—P1—O1—P2, and the corresponding angles in the nicotinamide moiety, allow a large variety of conformations for the molecule. The distance between the centroids of the adenine and nicotinamide generally approaches 15 Å when the molecule is complexed with an enzyme but is about 9.5 Å in the case of (I). In the same way, the two rings tend to

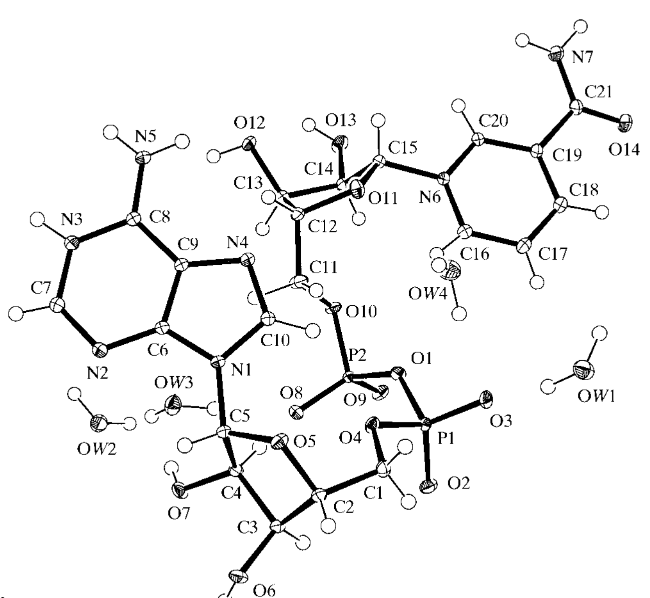


Figure 1
The molecular structure of NAD$^+$ (I) and the water molecules (*ORTEP*III; Burnett & Johnson, 1996). The displacement ellipsoids are shown at the 50% probability level and H atoms are shown as open circles.

be perpendicular in holoenzyme complexes and in the lithium salt crystal but are nearly parallel in the triclinic crystal form.

Despite this fact, there is no clear intramolecular or intermolecular ring–ring stacking in the crystal structure of (I). The N2, C7 and N3 atoms of the adenine ring deviate slightly from planarity: the torsion angles N2—C6—C9—C8 and C9—C6—N2—C7 are −2.4 (1) and 3.6 (1)° respectively, while C6—N2—C7—N3 is −1.0 (1)°. The O14—C21—N7 amide moiety makes a dihedral angle of −11.9 (1)° with the nicotinamide ring.

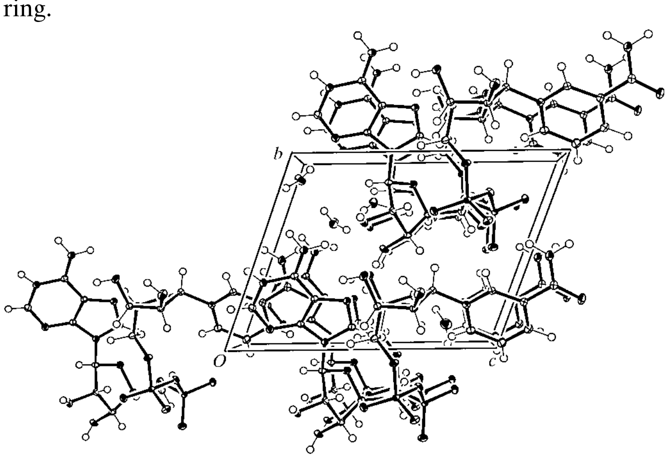


Figure 2
A view of the NAD^+ (I), including the water molecules, along the *a* axis (*ORTEP*III; Burnett & Johnson, 1996).

There is a strong hydrogen-bonding network which stabilizes the crystal structure (Table 2). The water molecules *W*3, *W*2, $W1^{viii}$ and $W4^{viii}$ fill the largest solvent void of the crystal structure and are arranged in a linear way, *W*3, *W*2 and $W1^{viii}$ being linked together by hydrogen bonds. The case of *W*3 is particularly noteworthy. H*W*3*B* is hydrogen bonded with O8 of the nicotinamide moiety and atom O*W*3 with HO7 of the adenine moiety. Thus, this water molecule clearly maintains the pyrophosphate link in its compact conformation by simultaneously holding the two sides of the molecule together.

Experimental

NAD was purchased in lyophilized form from SIGMA (St Louis, USA). Parthasarathy & Fridey (1984) have described the obtention of triclinic NAD^+ crystals at acidic pH. The crystallization solution was prepared by dissolving 1 g of NAD in 1 ml water and then adding 1 ml methanol. The pH was adjusted to 4.5 by addition of LiOH (1 *M*) and the solution placed in a sealed volume. Crystals appeared after 48 h at room temperature.

Crystal data

$C_{21}H_{27}N_7O_{14}P_2 \cdot 4H_2O$
M_r = 735.50
Triclinic, $P1$
a = 8.592 (10) Å
b = 8.845 (10) Å
c = 11.192 (10) Å
α = 109.64 (5)°
β = 90.56 (5)°
γ = 103.92 (5)°
V = 773.7 (14) Å^3
Z = 1
D_x = 1.579 Mg m^{-3}
Mo $K\alpha$ radiation
Cell parameters from 141 reflections
θ = 3.0–19.0°
μ = 0.233 mm^{-1}
T = 100 (5) K
Irregular, colourless
0.41 × 0.35 × 0.30 mm

Data collection

Nonius KappaCCD diffractometer
Oscillation scans
15736 measured reflections
7459 independent reflections
7439 reflections with $I > 2\sigma(I)$
R_{int} = 0.026
θ_{max} = 36.33°
h = 0 → 14
k = −14 → 14
l = −18 → 18

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)]$ = 0.019
$wR(F^2)$ = 0.052
S = 1.013
7459 reflections
573 parameters
All H-atom parameters refined
$w = 1/[\sigma^2(F_o^2) + (0.0371P)^2 + 0.0533P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$ = 0.001
$\Delta\rho_{max}$ = 0.32 e Å^{-3}
$\Delta\rho_{min}$ = −0.25 e Å^{-3}

Table 1
Selected geometric parameters (Å, °).

O1—P1	1.580 (2)	C9—N4	1.378 (2)
O1—P2	1.630 (2)	N4—C10	1.322 (2)
P1—O3	1.498 (1)	P2—O9	1.484 (2)
P1—O2	1.500 (2)	P2—O8	1.487 (1)
P1—O4	1.598 (2)	P2—O10	1.599 (2)
C5—N1	1.466 (2)	C15—N6	1.502 (2)
N1—C6	1.367 (2)	N6—C20	1.345 (1)
N1—C10	1.371 (1)	N6—C16	1.351 (2)
C6—N2	1.356 (2)	C16—C17	1.384 (2)
C6—C9	1.391 (2)	C17—C18	1.394 (2)
N2—C7	1.317 (2)	C18—C19	1.396 (2)
C7—N3	1.361 (2)	C19—C20	1.386 (2)
N3—C8	1.367 (2)	C19—C21	1.505 (2)
C8—N5	1.320 (2)	C21—O14	1.243 (2)
C8—C9	1.412 (2)	C21—N7	1.335 (2)
P2—O1—P1—O3	160.9 (1)	N2—C6—C9—C8	−2.4 (1)
P2—O1—P1—O2	32.7 (1)	P1—O1—P2—O9	−87.7 (1)
P2—O1—P1—O4	−84.3 (1)	P1—O1—P2—O8	45.2 (1)
O3—P1—O4—C1	−64.8 (1)	P1—O1—P2—O10	160.9 (1)
O2—P1—O4—C1	64.1 (1)	O9—P2—O10—C11	177.5 (1)
O1—P1—O4—C1	−177.5 (1)	O8—P2—O10—C11	44.3 (1)
P1—O4—C1—C2	−147.2 (1)	O1—P2—O10—C11	−69.9 (1)
O4—C1—C2—O5	−64.9 (1)	P2—O10—C11—C12	170.2 (1)
O5—C5—N1—C6	−144.2 (1)	O10—C11—C12—O11	−71.3 (1)
C9—C6—N2—C7	3.6 (1)	C20—C19—C21—N7	9.6 (1)

Table 2
Hydrogen-bonding geometry (Å, °).

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
O6—HO6···N4^{i}	0.83 (2)	1.99 (2)	2.764 (3)	154 (2)
O7—HO7···O*W*3	0.86 (2)	1.86 (2)	2.693 (3)	161 (2)
O12—HO12···O14^{ii}	0.86 (2)	1.90 (2)	2.719 (2)	161 (2)
O13—HO13···O12	0.86 (2)	2.05 (2)	2.629 (3)	124 (2)
O13—HO13···O6^{iii}	0.86 (2)	2.20 (2)	2.905 (3)	139 (2)
N3—HN3···O2^{iv}	0.91 (2)	1.72 (2)	2.615 (2)	165 (2)
N5—HN5*A*···O3^{iv}	0.86 (2)	1.99 (2)	2.830 (3)	166 (2)
N5—HN5*B*···O7^{v}	0.89 (2)	1.97 (2)	2.823 (2)	159 (2)
N7—HN7*A*···O*W*2^{vi}	0.92 (2)	2.06 (2)	2.971 (2)	174 (2)
N7—HN7*B*···O9^{v}	0.91 (2)	1.92 (2)	2.815 (3)	171 (2)
O*W*1—H*W*1*A*···O3	0.94 (1)	1.87 (1)	2.790 (3)	164 (2)
O*W*1—H*W*1*B*···N2^{vii}	0.937 (9)	2.04 (1)	2.975 (3)	176 (2)
O*W*2—H*W*2*A*···O*W*1^{viii}	0.95 (1)	1.86 (1)	2.806 (3)	175 (3)
O*W*2—H*W*2*B*···O6^{ix}	0.926 (9)	1.95 (1)	2.877 (3)	175 (2)
O*W*3—H*W*3*A*···O*W*2	0.94 (1)	2.02 (1)	2.947 (3)	168 (2)
O*W*3—H*W*3*B*···O8	0.941 (9)	1.82 (1)	2.746 (3)	168 (2)
O*W*4—H*W*4*A*···O3	0.95 (1)	1.95 (1)	2.900 (4)	173 (2)
O*W*4—H*W*4*B*···O13^{x}	0.97 (1)	1.81 (1)	2.773 (3)	172 (4)

Symmetry codes: (i) $x, y-1, z$; (ii) $x, y, 1+z$; (iii) $x-1, 1+y, z$; (iv) $x, 1+y, 1+z$; (v) $x, 1+y, z$; (vi) $x, 1+y, z-1$; (vii) $x, y, z-1$; (viii) $x-1, y, 1+z$; (ix) $x-1, y, z$; (x) $1+x, y, z$.

All H atoms were located in Fourier difference maps and freely refined [C—H 0.88 (2)–1.04 (2) Å]. Our analysis did not allow us to establish the absolute configuration, but this was already known from previous work.

Data collection: *COLLECT* (Nonius, 1998); cell refinement: *DENZO-SMN* (Otwinowski & Minor, 1997); data reduction: *DENZO-SMN*; program(s) used to solve structure: *SHELXS*97 (Sheldrick, 1990); program(s) used to refine structure: *SHELXL*97 (Sheldrick, 1997); software used to prepare material for publication: *SHELXL*97.

Supplementary data for this paper are available from the IUCr electronic archives (Reference: GS1072). Services for accessing these data are described at the back of the journal.

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