Conformational Energy Calculations for Dinucleotide Molecules

A STUDY OF THE COMPONENT MONONUCLEOTIDES ADENOSINE 5'-MONOPHOSPHATE, NICOTINAMIDE MONONUCLEOTIDE AND ADENOSINE 3'-MONOPHOSPHATE

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Semi-empirical conformational energy calculations were performed for the mononucleotides 5'-AMP, NMN⁺ and 3'-AMP. Only intramolecular forces are considered. Essentially all conformational states were explored to investigate the population distribution likely to be found in a non-crystalline environment. The calculations suggest that 5'-AMP and 3'-AMP are relatively flexible and a mixture of conformational states is expected. In contrast, the results for NMN⁺ suggest that a strong electrostatic interaction between the positively charged nicotinamide nitrogen atom and negatively charged phosphate oxygen is possible, stabilizing a few specific states. This interaction will be most significant in a solvent-free situation or an apolar environment.

It is the purpose of this work to investigate by theoretical energy calculations the conformations of certain dinucleotides. Two categories of molecules are distinguished, namely (i) the dinucleoside 5'-pyrophosphates, consisting of two nucleosides joined by a 5'-5'-pyrophosphate linkage, e.g. NAD⁺ and its analogue diadenosine 5'-pyrophosphate, and (ii) the dinucleoside 3'-5'-phosphates, consisting of nucleosides joined by a 3'-5'-phosphate link, e.g. diadenosine 3'-5'-phosphate. Knowledge of the conformational properties of the nucleotide coenzyme NAD⁺ is of interest in itself as well as providing the basis for studying its complexes with dehydrogenase enzymes. The dinucleoside 3'-5'-phosphates exhibit many of the structural features of the polynucleotides and constitute model systems for the study of important nearest-neighbour interactions (Brahms *et al.*, 1966; Arnott & Hukins, 1969; Cantor *et al.*, 1966).

Within this context the component mononucleotides are important structural units, in addition to their biochemical role in the cell. Several previous theoretical studies into the conformations of 5'-AMP and 3'-AMP have been made by using energy calculations (Lakshminarayanan & Sasisekharan, 1969*a,b,c*; Olson & Flory, 1972*a,b*; Berthod & Pullman, 1973*a,b*). All methods have shown that the crystal structure for each molecule corresponds to a low-energy state. It has been suggested from a survey of X-ray-crystallographic results (Sundaralingam, 1973) that nucleotides are conformationally rigid molecules. Recent experimental work on 5'-AMP,

in particular by n.m.r., gives conflicting reports with regard to the state of the molecule in solution. Barry *et al.* (1971), using the lanthanide-ion-probe technique, found that a small region of conformational space is sufficient to explain their data, suggesting a relatively rigid conformation. In contrast, coupling-constant results (Sarma *et al.*, 1973) cannot be interpreted by a single state.

The work presented here extends previous calculations by examining the problem of multiple conformations which may be present in a non-crystalline environment. A qualitative picture of the conformational distribution is derived for the mononucleotides. This study is the first stage in evaluating the conformational properties of the dinucleotides, and considers the molecules 5'-AMP and NMN⁺ (which together constitute NAD⁺) and 3'-AMP.

Methods

All computations were performed on the Honeywell DDP516 digital computer system at Mill Hill with 16K core storage, and visual display system (Perkins *et al.*, 1971).

Energy calculations

The semi-empirical energy approach was adopted (Scheraga, 1968), in which the energy is evaluated as the sum of physically distinct terms:

$$V_{\text{tot.}} = V_{\text{non-bonded}} + V_{\text{electrostatic}} + V_{\text{torsional}} \quad (1)$$

The non-bonded energy V_{nb} between two atoms is calculated with the Lennard-Jones 6-12 potential:

$$V_{\text{nb}} = -\frac{A}{r^6} + \frac{B}{r^{12}} \quad (2)$$

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where r = separation of the atoms, and A and B are constants for given atoms. The Slater–Kirkwood equation (Slater & Kirkwood, 1931) is used to evaluate the constant A, in terms of the experimental quantities polarizability (α), mostly taken from Ketelaar (1953), and the effective number of valence electrons, N_{eff} , derived by using the method of Scott & Scheraga (1965). The polarizability of phosphorus was calculated, from refraction data (Pauling & Simonetta, 1952), to be $3.447 \times 10^{-24} \text{ cm}^3$, whereas that of a charged oxygen atom (O^-) was taken from α_{OH^-} to be $1.47 \times 10^{-24} \text{ cm}^3$. The value of the constant B is chosen such that the minimum of the potential curve falls at the sum of the Van der Waals radii, taken from Bondi (1964).

The electrostatic potential V_{es} between two atoms is calculated by using the simple monopole interaction:

$$V_{\text{es}} = 1394.4 \frac{e_i e_j}{\epsilon r_{ij}} \text{ kJ/mol} = 332.0 \frac{e_i e_j}{\epsilon r_{ij}} \text{ kcal/mol} \quad (3)$$

where $e_i e_j$ are partial charges on atoms i, j in units of electronic charge, r_{ij} is separation of charges in units of 0.1 nm ($\text{\AA}$) and ϵ is the dielectric constant. The value of the dielectric constant is set equal to 4 (Brant & Flory, 1965). The partial charges are calculated as the sum of the sigma and pi charges, found by using Del Re's method (Del Re, 1958, 1964; Del Re *et al.*, 1963) and the Simple Hückel Method (Streitwieser, 1961) respectively.

The total non-bonded and electrostatic contributions are found by summing overall atom–atom interactions for those atoms separated by three bonds or more.

The torsional potential is represented as:

$$V_{\text{tor}} = \frac{1}{2} V_0 [1 + \cos 3(\theta - \theta_0)] \quad (4)$$

where V_0 is barrier height, θ is torsion angle and θ_0 is phase displacement. The size of the potential V_0 is found by analogy with simple molecules, with minima at the staggered orientations. The torsional potential arises partly from non-bonded interactions, which may thus be included twice. Different methods disagree on the extent of the non-bonded contribution (Scott & Scheraga, 1965) and some allowance has been made for this contribution in the values of V_0 used. In the dinucleotides there are four types of bonds to consider. The values adopted for V_0 are 0.0 kJ/mol (0.0 kcal/mol) for the glycosidic N–C–1 bond, 10.5 kJ/mol (2.5 kcal/mol) for the exo cyclic C–4'–C–5' carbon–carbon bond, 8.4 kJ/mol (2.0 kcal/mol) for the C–5'–O–5' bond (Lakshminarayanan & Sasisekharan, 1969a) and 4.2 kJ/mol (1.0 kcal/mol) for the phosphate P–O_e bond (Semlyen & Flory, 1966).

In this relatively simple treatment, bond angles and bond lengths have fixed values; contributions to V_{tot} .

from interactions with solvent are ignored. Within these approximations, the potential energy of a given conformation may be evaluated. The limitations of this approach and other quantum-mechanical methods have been discussed in detail (Scheraga, 1968; Ramachandran, 1975). For the present problem the semi-empirical approach offers a conceptually simple and rapid method for a systematic study of molecules of moderate size and complexity.

Procedures

The mononucleotides possess several degrees of conformational freedom represented by the dihedral angles θ_i characterizing rotation about single bonds, e.g. 5'-AMP (Fig. 1a). The computational problem is that of finding that conformation ($\theta_1, \theta_2 \dots \theta_n$) with the lowest energy, E . The following strategy was adopted.

(a) *Single and adjacent bonds.* The energy E was calculated for small (10°) increments of single variables, $E(\theta_i)$, and pairs of adjacent variables $E(\theta_i, \theta_{i+1})$. Only those atoms whose positions depend solely on rotation about the bonds under study were included in the energy summation, e.g. only first-order interactions for single-bond rotations. Graphs of $E(\theta_i)$ and contour plots $E(\theta_i, \theta_{i+1})$ were thus constructed to show the interactions of adjacent sections of the molecule. These are fully applicable to the complete molecules, where nearest-neighbour effects will be of prime consideration, and provide information about completely forbidden high-energy areas, which need not be considered further, and low-energy regions, close to local minima.

(b) *The mononucleotides.* The mononucleotides were subjected to a systematic study covering all three or four variables. The allowed ranges of each variable, as defined by the energy calculations of the previous section, were divided into 30° intervals incorporating energy minima where possible. All possible conformations produced by a combination of the various angles were generated and their energies calculated. The output was analysed as follows.

(i) A plot of frequency versus energy was drawn for the lower energy conformations from $E_{\text{min.}}$ to $[E_{\text{min.}} + \text{approx. } 46.0 \text{ kJ/mol } (E_{\text{min.}} + 11 \text{ kcal/mol})]$, to show the distribution of energy states.

(ii) A Boltzmann distribution was calculated to show the population of states at room temperature. The statistical weight W_i of any state i and energy E_i is given by

$$W_i = \frac{e^{-\frac{(E_i - E_{\text{min.}})}{RT}}}{\sum_i e^{-\frac{(E_i - E_{\text{min.}})}{RT}}}$$

Fig. 1. Mononucleotide structures and nomenclature

Dihedral angles are shown as θ_1 – θ_4 (structures *a* and *b*) and ϕ_1 – ϕ_3 (structure *c*). (*a*) 5'-AMP; (*b*) NMN⁺; (*c*) 3'-AMP.

Table 1. Definition of the zero conformations for the mononucleotides

The dihedral angles θ_1 – θ_4 are defined by the *trans*-planarity and ϕ_1 – ϕ_3 by the *cis*-planarity of the atoms indicated. Alternative notations are given for comparison.

		Sundara- lingam's (1969) notation	Olson & Flory's (1972 <i>a,b</i>) notation
5'-AMP <i>trans</i>-planar atoms			
θ_1	C-8-N-9-C-1'-O-1'	$\chi+180$	$\chi+180$
θ_2	C-3'-C-4'-C-5'-O-5'	$\chi+180$	ϕ''
θ_3	C-4'-C-5'-O-5'-P	$\phi+180$	ϕ'
θ_4	C-5'-O-5'-P-O _e	$\omega+180$	χ''
NMN			
θ_1	C-2-N-1-C-1'-O-1'	—	—
3'-AMP <i>cis</i>-planar atoms			
ϕ_1	C-8-N-9-C-1'-O-1'	χ	χ
ϕ_2	C-4'-C-3'-O-3'-P	ϕ'	$\omega''+180$
ϕ_3	C-3'-O-3'-P-O _e	ω'	$\psi'+180$

where Σ represents a summation over all the possible states. $E_{\min.}$ is the lowest energy state located.

(iii) To find the distribution of angles in the 'lowest' energy states, the populated conformations with energies $E_i < E_{\min.} + 12.6 \text{ kJ/mol}$ ($E_{\min.} + 3 \text{ kcal/mol}$) were analysed further. Histograms were constructed by plotting the frequency of occurrence of a given value of θ_i within these conformations. If the structure is relatively rigid all the low-energy conformations should correspond to a small distribution of angles.

Energy minimization

Energy minimizations were performed for some structures by using the simplex minimization technique called 'NELMIN' (O'Neill, 1971), a non-derivative stepping algorithm which has been found particularly powerful in dinucleotide calculations (Thornton, 1973). Initial values of the angular variables were taken from low-energy structures, with a maximum number of four variables treated simul-

taneously. Convergence in the energy function was observed as further adjustment of the variables became insignificant.

Nomenclature and convention

The co-ordinates for 5'-AMP were taken from the crystal structure (Kraut & Jensen, 1963) with the C-3'-*endo* sugar. The co-ordinates for NMN⁺ were generated by using the crystal data for nicotinamide (Wright & King, 1954) and adding the ribose phosphate section. Ribose co-ordinates (C-2'-*endo* and C-3'-*endo*) were taken from the average dimensions given by Lakshminarayanan & Sasisekharan (1970). Co-ordinates for 3'-AMP were taken from the crystal structure (Sundaralingam, 1966) with the C-3'-*endo* sugar. The nomenclature is shown in Figs. 1(a), 1(b) and 1(c). 5'-AMP and NMN⁺ have four angular variables denoted by θ_1 – θ_4 , whereas 3'-AMP has only three, ϕ_1 – ϕ_3 . The zero conformations are defined by the planarity of the atoms shown in Table 1. Positive angles correspond to a clockwise rotation of the far group relative to the near one.

Results and Discussion

5'-AMP

The results of the single and adjacent bond-angle calculations are summarized in Table 2, which gives the preferred values of all the angles in order of increasing energy. The high-energy forbidden regions are also shown. Graphs and contour plots were drawn to illustrate the energy surfaces; Fig. 2 shows the θ_3 – θ_4 energy surface (Thornton, 1973). It is found that the glycosidic bond angle θ_1 shows a preference for the *anti* (190°) and *syn* (40°) states, with a forbidden high-energy region from 50 to 160°. The exocyclic C-4'-C-5' bond displays an equal preference for the classical staggered orientations, although no region is completely forbidden. θ_3 , the C-5'-O-5' bond, exhibits an extensive high-energy region (Fig. 2), caused by steric interactions between the sugar

Table 2. Values of dihedral angles corresponding to energy minima and forbidden regions for partial structures of 5'-AMP and NMN⁺

Conformational energy was calculated for adjacent angles $E(\theta_i, \theta_{i+1})$: minima are listed in order of increasing energy: parentheses denote equal energy.

	Minima	Forbidden region
5'-AMP θ_1	190, 40	50–160
θ_2	(0, 120, 240)	—
θ_3	0, 100, 260	140–220
θ_4	(0, 120, 240)	—
NMN ⁺ θ_1	(220, 40)	90–110, 270–290

Fig. 2. Conformational-energy surface for 5'-AMP, $E(\theta_3, \theta_4)$

The energy E , calculated as a function of θ_3 and θ_4 for atoms C-4' to O_e, is shown at contour intervals of 4.2 kJ/mol (1 kcal/mol) from the minimum value at $\theta_3 = 260^\circ$, $\theta_4 = 0^\circ$. Local minima are designated X.

atoms and the phosphorus atom. Energy minima occur at the *trans* (*t*) state ($\theta_3 = 0^\circ$) with subsidiary minima ($\theta_3 = 100, 260^\circ$) close to the forbidden region. Rotation about the θ_4 is relatively free with minima (0, 120, 240°) corresponding to the staggered *t*, *g*⁺, *g*[−] positions (Olsen & Flory, 1972b).

The results are very similar to those published by Olson & Flory (1972b), who include all atoms in their calculation by setting the other rotatable bonds in fixed orientations. Forbidden regions appear more extended in their work owing to higher-order interactions, especially for the $E(\theta_2, \theta_3)$ energy surface. Also the inclusion of higher-order terms differentiates between the preferred staggered states *tt*, *tg*⁺, *tg*[−] for θ_2, θ_3 , which here appear equivalent.

Fig. 3. Conformational-energy distribution for 5'-AMP

Plot of frequency versus energy for the lowest energy states $E < E_{\text{min.}} + 46.0 \text{ kJ/mol}$ ($E_{\text{min.}} + 11 \text{ kcal/mol}$), and (shaded) the percentage population showing the occupied states at 20°C, calculated from the Boltzmann distribution function (see the text).

However, the general similarity emphasizes the importance of first- and second-order interactions in determining favourable or unfavourable states. Extending the calculations beyond adjacent variables will inevitably increase the extent of the forbidden regions and define the low-energy regions more closely.

The results of the calculations for the complete molecule are shown in Fig. 3. The plot of frequency versus energy shows that there is a fairly continuous distribution of 'energy states', the highest frequencies occurring at approx. $E_{\text{min.}} + 25.1 \text{ kJ/mol}$ ($E_{\text{min.}} + 6 \text{ kcal/mol}$). The Boltzmann distribution reveals that only the energy states below about -71.1 kJ/mol (-17.0 kcal/mol) will be significantly populated. The analysis of the lowest energy states (110 conformations) is shown in Fig. 4. These angular distributions indicate that the low-energy states do not correspond to a single group of conformations; the angles θ_1 – θ_4 have a fairly wide distribution:

(i) θ_1 adopts values between 180 and 220°, i.e. *anti*. The *syn* state is scarcely populated.

(ii) θ_2 shows a strong preference for the 120–240° range.

(iii) θ_3 , except for the large forbidden central region, is well spread with most conformers in the 0 and 100° region.

Fig. 4. Angular distribution for the 110 lowest-energy states of 5'-AMP

The frequency of occurrence of a given value is plotted for each variable for the unweighted population from $E < E_{\min.} + 12.5 \text{ kJ/mol}$ ($E_{\min.} + 3 \text{ kcal/mol}$) (see the text).

(iv) θ_4 occurs most frequently in the *trans* 0° state (owing to the electrostatic forces), but almost any other value can give a low-energy conformer.

Comparison of Table 2 and Fig. 4 shows that the most notable effects of including high-order interactions are the restriction of θ_1 to the *anti* state and the preference of θ_2 for the g^+ and g^- conformations.

It should be stressed that these histograms are simply unweighted frequencies of those low-energy states that are qualitatively considered to be significantly populated. Further weighting was not applied, since the states as defined in terms of the incremented θ_i values do not necessarily correspond to local energy minima.

The five lowest-energy individual structures are given in Table 3; they correspond to two main

families, (A1, A2 and A4) and (A3 and A5). In all cases, the non-bonded and electrostatic energy contributions are approximately equal. Structures A1 and A3 (Fig. 5) exhibit no specific intramolecular features. These calculations were performed with large angular increments (30°), which may be too coarse to locate a specific low-energy structure. To test this possibility energy minimizations were performed by using the 12 lowest-energy structures as starting points. For all minimizations the total changes in energy and θ_i values were very small, being less than 4.2 kJ/mol (1 kcal/mol) and 10° respectively. Typical results for two structures (A1-M, A3-M) are given in Table 3. The absence of further significant minimization indicates that there are many local minima of similar energies. These states are evidently determined primarily by first- and second-order interactions; long-range interactions are important in defining the *anti* conformer. There is no evidence that the molecule as a whole is confined to a single conformation by the intramolecular forces. Calculations for the C-2'-*endo* sugar show that the glycosidic angle θ_1 adopts a conformational range centred on 240° , rather than 180° . The *syn* state also becomes more populated. These conclusions are generally substantiated by crystal-structure determinations for other nucleosides (Sundaralingam, 1969). Angular variables θ_2 , θ_3 and θ_4 show a similar distribution to the C-3'-*endo* structure, indicating that the C-2'-*endo* nucleotide is also flexible. For this sugar conformation there is the possibility of a hydrogen bond between the adenine N-3 and the ribose O-2'-H. This can only occur when the glycosidic bond adopts the *anti* state, and would slightly stabilize this conformer. Calculations by Pullman & Berthod (1973) indicate that the effect on the conformational distribution is negligible.

It is interesting to compare these results with the experimental data for θ_1 – θ_4 for 5'-AMP obtained from X-ray-crystallography and n.m.r. work (Table 4). The energies calculated for these conformations are also shown. The crystal structure is a very-low-energy one with angular values corresponding to well-populated states. It compares closely with structures A3 and A5 (Table 3). Conversely, the time-averaged n.m.r. solution conformation (Barry *et al.*, 1971), obtained by using the lanthanide-ion-probe technique, exhibits a relatively high energy [approx. 25.1 kJ/mol (6 kcal/mol) greater than $E_{\min.}$] essentially due to the change in θ_2 . Possible differences in this case are that the solvent or metal ion is instrumental in fixing 5'-AMP in a conformation which is not a low-energy state for the mononucleotide alone. Alternatively, 5'-AMP may exist in a conformational equilibrium, as suggested by these calculations. The lanthanide ion may change the nature of this equilibrium, as has been inferred for diadenosine 5'-pyrophosphate (Bayley & Debenham, 1974).

Table 3. *Individual lowest-energy structures for 5'-AMP*

Energy E is shown for lowest-energy structures found from the conformational-energy distribution. The non-bonded and electrostatic energies V_{nb} and V_{es} are given. The values in parentheses are in kcal/mol.

Structure no.	θ_1	θ_2	θ_3	θ_4	E (kJ/mol)	kJ/mol	
						V_{nb}	V_{es}
A1	180	120	100	0	-84.0 (-20.0)	-39.06 (-9.3)	-47.46 (-11.3)
A1-M	190	119	102	10	-86.5 (-20.6)		
A2	220	120	100	0	-83.2 (-19.8)	-38.64 (-9.2)	-46.2 (-11.0)
A3	180	240	0	0	-81.9 (-19.5)	-38.64 (-9.2)	-43.26 (-10.3)
A3-M	191	239	357	359	-83.2 (-19.8)		
A4	180	120	100	30	-81.0 (-19.3)	-38.22 (-9.1)	-47.04 (-11.2)
A5	220	240	0	0	-80.6 (-19.2)	-37.38 (-8.9)	-42.84 (-10.2)

Fig. 5. *Two low-energy structures of 5'-AMP*

(a) Structure A1 (θ_1 : 180°, 120°, 100°, 0°). (b) Structure A3 (θ_1 : 180°, 240°, 0°, 0°).

Table 4. *Experimental data for 5'-AMP*

The calculated conformation energy is also shown. The values in parentheses are in kcal/mol.

Method ...	X-ray	N.m.r.
Reference ...	Kraut & Jensen (1963)	Barry <i>et al.</i> (1971)
θ_1	205	194
θ_2	220	320
θ_3	357.3	354
θ_4	357.5	190
Energy (kJ/mol)	-81.6 (-19.5)	-66.9 (-16.0)

The computer technique used in the n.m.r. approach, whereby the experimental shift and broadening data are fitted to calculated values, in fact, searches for agreement with a single state. If the molecule is rather flexible the technique is not strictly applicable,

although one might expect to find no solution in this case. Additional n.m.r. data from Nuclear Overhauser Enhancement (Son & Chachaty, 1974) suggests that approx. 30% of the molecules will adopt the *syn* conformation ($\theta_1 \approx 0^\circ$). This estimate seems rather high compared with the energy distribution, which suggests less than 10% *syn*. N.m.r. coupling-constant data for 5'-AMP (Sarma *et al.*, 1973) indicate that approx. 65% of the molecules adopt the state with $\theta_2 = 240^\circ$, $\theta_3 = 0^\circ$, which is essentially the crystal structure.

Thus a consistent conclusion of calculation and experiment for this nucleotide is that the crystal structure represents a molecular conformation which is well populated, even in the absence of crystal-lattice forces. Several other states may also be populated. A relatively unhindered interconversion between these states is suggested by the features of

the potential-energy surfaces (e.g. Fig. 2) indicating a dynamic equilibrium of conformers. It is thus predicted that the molecule will be able to adapt its conformation to the environmental conditions, as encountered, for example, in different solvent

Fig. 6. Conformational-energy surface for NMN^+ , $E(\theta_1, \theta_2)$

The energy E , calculated as a function of θ_1 and θ_2 for nicotinamide and ribose (C-2'-endo conformation) up to atom C-5', is shown at contour intervals of 4.2 kJ/mol (1 kcal/mol) from the minimum value at $\theta_1 = 50^\circ$, $\theta_2 = 120^\circ$. Local minima are designated $\times$.

Fig. 7. Conformational-energy distribution for NMN^+

Plot of frequency versus energy for the lowest-energy states $E < E_{\min.} + 46.0$ kJ/mol ($E_{\min.} + 11$ kcal/mol) and (shaded) the percentage population showing the occupied states at 20°C , calculated from the Boltzmann distribution function (see the text).

conditions and in enzyme-cofactor-substrate-type interactions.

NMN^+

NMN^+ was investigated by using the same procedures as for 5'-AMP. In this case the conformation of the sugar is unknown. $E(\theta_1, \theta_2)$ energy surfaces were therefore calculated for the C-2'-endo, C-3'-endo and C-3'-exo ribose conformations. The results show that the C-2'-endo state exhibits the lowest energy by about 8.4 kJ/mol (2 kcal/mol) and this state is used hereafter. The contour plot of $E(\theta_1, \theta_2)$ for this conformer is shown in Fig. 6. The glycosidic bond angle θ_1 may adopt the anti or syn state, being equivalent energy minima. θ_2 as for 5'-AMP shows a preference for the staggered states. The other energy surfaces, $E(\theta_2, \theta_3)$ and $E(\theta_3, \theta_4)$ are the same as for 5'-AMP.

Fig. 8. Angular distribution for the 12 lowest-energy states of NMN^+

The frequency of occurrence of a given value is plotted for each variable for the unweighted population from $E < E_{\min.} + 12.5$ kJ/mol ($E_{\min.} + 3$ kcal/mol) (see the text),

Table 5. *Individual lowest-energy structures for NMN⁺*

The energy is shown for the three distinct lowest-energy structures. The non-bonded and electrostatic energies V_{nb} and V_{es} are given. The values given in parentheses are kcal/mol.

Structure no.	θ_1	θ_2	θ_3	θ_4	E (kJ/mol)	kJ/mol	
						V_{nb}	V_{es}
N1	130	330	90	60	-178.9 (-42.6)	-42.84 (-10.2)	-149.9 (-35.7)
N1-M	135	331	94	66	-197.8 (-47.1)		
N2	130	240	270	270	-197.8 (-41.1)	-24.36 (-5.8)	-154.6 (-36.8)
N2-M	130	226	273	277	-200.7 (-47.8)		
N3	40	240	330	240	-169.7 (-40.4)	-44.52 (-10.6)	-129.4 (-30.8)
N3-M	50	239	339	236	-177.2 (-42.2)

Fig. 9. *Three minimized low-energy structures of NMN⁺*

(a) Structure N1-M (θ_1 : 135°, 331°, 94°, 66°). (b) Structure N2-M (θ_1 : 130°, 226°, 273°, 277°). (c) Structure N3-M (θ_1 : 50°, 239°, 339°, 236°).

The plot of frequency versus energy and corresponding Boltzmann population distribution are shown in Fig. 7. They differ markedly from those for 5'-AMP, with far fewer states in the very-low-energy region. This implies that a specific interaction may be occurring, which drastically lowers the energy of certain states. The 12 conformations with energy $E < E_{\min.} + 12.5$ kJ/mol ($E_{\min.} + 3$ kcal/mol), which represent more than 95% of the populated states, were analysed to find the angular distributions, as shown in Fig. 8. The general conclusions from this are: (i) θ_1 : the region $\theta_1 = 130^\circ$ is occupied by half of the low-energy states. This corresponds to neither a true *anti* (220°) nor *syn* (40°) state, but will be called 'pseudo-*anti*', being within the same potential trough. In the $E(\theta_1, \theta_2)$ plots this region is of low energy but displaced from the minimum, and must therefore be stabilized by high-order interactions; (ii) θ_2 : a definite preference for the 240° (*g*⁺) state is shown; (iii) θ_3 : the 90° and 270° states are most populated; (iv) θ_4 : all values are populated.

Thus high-order interactions are important for NMN⁺, changing the angular distribution markedly from that expected from adjacent-angle calculations. A study of the individual states is most revealing. In all the 12 low-energy states in the analysis there exists close interaction between the nicotinamide ring and the phosphate oxygen atoms. Table 5 lists the three distinct conformations of lowest energy, N1, N2 and N3, and shows that the electrostatic stabilization energy is much larger than for 5'-AMP (Table 3). Structures N1 and N2 exhibit N⁺-O⁻ interactions, and structure N3, which is the common nucleotide crystal structure (cf. 5'-AMP) exhibits an NH₂-O⁻ interaction. Minimizations have been performed for the 12 lowest energy states of NMN⁺. Much larger decreases in energy [approx. 20.9 kJ/mol (5 kcal/mol)] were observed than for 5'-AMP. Angular changes were, however, small, usually being less than 10°. Table 5 shows the results of three such minimizations (N1-M, N2-M, N3-M) and the minimized structures are shown in Fig. 9.

A dominant stabilization force in these structures is clearly the electrostatic interaction between the positively charged nicotinamide nitrogen and the negatively charged phosphate oxygen atoms. This interaction is absent from the common uncharged nucleotides, including the reduced derivative NMNH.

In discussing these results there is no crystal-structure information available for NMN⁺ with which to make a comparison. There have been extensive n.m.r. investigations, which are summarized below.

(a) Data on NMN⁺ from Nuclear Overhauser Enhancement experiments (Egan *et al.*, 1973) indicate that neither the *anti* nor the *syn* state of the glycosidic rotation is occupied exclusively. They suggest an equal distribution with fast exchange between the two.

(b) Coupling-constant data, $J_{H4'-H5'}$ (Sarma & Mynott, 1973), show that 93% of the conformers exhibit the *gauche* (240°) state for θ_2 . Independent evidence from phosphorus ($J_{P-H5'}$) values suggest that most molecules adopt the $\theta_3 = 0^\circ$ conformation. Coupling constants for the ribose ring protons (Birdsall *et al.*, 1975) suggest that a mixture of the C-2'-*endo* and C-3'-*endo* states is present, although the C-2'-*endo* conformer is predominant.

(c) Lanthanide-ion shift and broadening data (Birdsall *et al.*, 1975) have been interpreted, in the light of the other results (Sarma & Mynott, 1973), as a mixture of conformational states with C-2'-*endo*: *syn* and C-3'-*endo*: *anti* structures.

(d) Blumenstein & Raftery (1972, 1973) have found that titration of the phosphate group in NMN⁺ results in shifts of the nicotinamide resonances. This effect is not seen in NMNH. They explain their observations by postulating an interaction between the negatively charged phosphate and positively charged nicotinamide ring nitrogen.

Thus the n.m.r. results indicate an equal *syn-anti* distribution and appear to favour a backbone conformation similar to the predominant crystal nucleotide structure ($\theta_2 = 240^\circ$, $\theta_3 = 0^\circ$) (Sundaralingam, 1969). This, however, would not accommodate a direct N⁺-O⁻ interaction.

The energy calculations show that the *syn* and 'pseudo-*anti*' states may occur with low energies and that the common nucleotide backbone state is relatively favourable (Table 5). However, they also indicate the occurrence of states (N1 and N2) in which electrostatic forces are of great importance in determining conformation. Since such forces would be affected by the presence of solvent, and cations would significantly modify the electrostatic interactions involving the phosphate oxygen atoms, it is possible that the calculations may overestimate the electrostatic stabilization for the molecule in free solution. Notwithstanding this, a significant intramolecular interaction is clearly indicated. Indeed, experimental evidence suggests an interaction

between the nicotinamide and phosphate moieties in NMN⁺, which is absent from NMNH (Blumenstein & Raftery, 1972, 1973). Although no single structure accommodates all of the inferential evidence from n.m.r., structures N1, N2 and N3 jointly could account for all of the observed features. Of these, structure N2 is closest to satisfying the major considerations, namely the N⁺-O⁻ interaction and the values of θ_2 and θ_3 .

These predictions may be compared with the conformation of the NMN⁺ moiety in NAD⁺ from the known structure of the lactate dehydrogenase-NAD⁺ complex (Chandrasekhar *et al.*, 1973). In this case the coenzyme adopts a relatively extended conformation with approximate dihedral angles for the NMN⁺ moiety of $\theta_1 \approx 270^\circ$, $\theta_2 \approx 93^\circ$, $\theta_3 \approx 358^\circ$, $\theta_4 \approx 289^\circ$, based on favourable conformations for nucleotides (Arnott & Hukins, 1969). Interaction between the coenzyme phosphate and charged enzyme residues precludes the N⁺-O⁻ intramolecular interactions.

3'-AMP

Calculations were performed for 3'-AMP with the 3'-*endo* ribose conformation as found in the crystal structure (Sundaralingam, 1966). Energy surfaces $E(\phi_1, \phi_2)$ and $E(\phi_2, \phi_3)$ were calculated. The results are summarized in Table 6. The glycosidic bond preferentially adopts the common *syn* and *anti* states, the latter being of lower energy. Rotation about the C-3'-O-3' bond is sterically very restricted with two very small favoured regions. ϕ_3 prefers the classical t, g⁻, g⁺ states.

Treatment of the whole molecule involves fewer combinations of variables, since only three bonds are involved. Analysis of the results shows (Fig. 10) that there are many states in the low-energy regions, as found with 5'-AMP. The angular distributions (Fig. 11) show that these states represent many different conformations: (i) ϕ_1 may adopt the *syn* or *anti* state; (ii) ϕ_2 is restricted to the region about 200°; (iii) ϕ_3 may adopt a wide range of values.

Minimizations applied to the lowest energy structures did not decrease the energy significantly. The lowest energy state (30°, 240°, 120°) is shown in Fig. 12. There is no evidence for intramolecular interaction.

Table 6. Analysis of adjacent-angle results for 3'-AMP

Conformation energy was calculated for adjacent angles, $E(\phi_i, \phi_{i+1})$; minima are listed in order of increasing energy.

	Minima	Forbidden regions
3'-AMP ϕ_1	0, 220, 280	300-330
ϕ_2	190, 270	330-180
ϕ_3	180, 60, 300	—

Fig. 10. Conformational-energy distribution for 3'-AMP

Plot of frequency versus energy for the lowest-energy states $E < E_{\min.} + 50.2 \text{ kJ/mol}$ ($E_{\min.} + 12 \text{ kcal/mol}$) and (shaded) the percentage population at 20°C calculated from the Boltzmann distribution function (see the text).

In comparing this result with the crystal structure ($\phi_1 = -3.9^\circ$, $\phi_2 = 236^\circ$, $\phi = 289^\circ$) (Sundaralingam, 1966), the latter is a low-energy well-populated *anti* state (see Fig. 11). N.m.r. data (Son & Chachaty, 1974) suggest that 3'-AMP in solution may adopt the *syn* conformation in preference to the *anti*. The energy calculations show that both *syn* and *anti* should be similarly populated (approx. 1:1), in contrast with 5'-AMP. The orientation about the C-3'-O-3' bond is expected to be fixed even in solution, on account of local steric restrictions.

General discussion

The methods used in calculating the conformational energy are semi-empirical and at best approximate. Limitations are inherent in the assumed potential functions, neglect of solvent contributions, assignment of dielectric-constant values and the further assumptions of fixed geometry and a single

sugar conformation. Interpretation of the results must therefore be restricted to the qualitative level. However, by using the simple analysis of population distribution which is described here, several predictions may be made.

For both 5'-AMP and 3'-AMP the crystal structure conformation is found to be a very-low-energy state. This is important, as at least a partial validation of the form of the conformational-energy calculation. However, in addition, other conformations of equally low energy have been found, which may also be expected to be populated. Thus intramolecular forces do not suggest a single rigid nucleotide conformation but rather one which is free to adapt to a given environment. Indeed when 5'-AMP is bound to lactate dehydrogenase it adopts the $\theta_2 \approx 108^\circ$ conformation (Chandrasekhar *et al.*, 1973) rather than other states which are thought to occur in solution (Sarma *et al.*, 1973). For the glycosidic bond it appears certain that the *anti* state is much preferred for 5'-

Fig. 11. Angular distribution for the lowest-energy states of 3'-AMP

The frequency of occurrence of a given value is plotted for each variable for the unweighted population from $E < E_{\min.} + 12.5 \text{ kJ/mol}$ ($E_{\min.} + 3 \text{ kcal/mol}$).

Fig. 12. A low-energy structure for 3'-AMP

Angles ϕ_1 : 30° , 240° , 120° .

AMP, owing to base-phosphate interaction; in contrast the *syn* and *anti* states appear equally favourable for 3'-AMP.

The behaviour of NMN⁺ is considerably different, being dominated by electrostatic interactions. It is

notable that the additional feature of a formal positive charge consistently leads to low-energy states involving such interactions. The absence of such dominating influences in the 5'-AMP and 3'-AMP structures gives further credence to the greater multiplicity of molecular conformations and their likely persistence in solution. For NMN⁺, the methods probably overestimate the importance of this intramolecular electrostatic interaction. Even so, it is judged to be an important factor in situations when solvent is excluded, for example in a crystalline environment or at an enzyme active site. The greater significance of V_{as} relative to V_{ab} due to the single positive charge implies that the conformational distribution for the NMN⁺ and NMNH moieties will be different. By extension the lowest energy conformations of NAD⁺ and NADH will also be different, and this may be significant for the enzyme-substrate interaction. Indeed for NAD⁺ itself there is some experimental evidence for such an N⁺-O⁻ interaction (Blumenstein & Raftery, 1973).

To investigate such effects a more detailed knowledge of the conformational properties of the complete dinucleotide molecules themselves is required. This may be approached by the application of the information gained on the properties of the component mononucleotides. The present work has shown the importance of treating the molecules as a single multi-variable system so as to explore adequately the higher-order interactions.

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