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Analysis of the interactions of NAD\(^+\) with Horse Liver Alcohol Dehydrogenase using molecular mechanics

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Abstract. The interactions of NAD\(^+\) in a ternary complex with Horse Liver Alcohol Dehydrogenase and DMSO were simulated using molecular mechanics calculations. Starting conformations were taken from X-ray crystallographic data as reported by Eklund and coworkers\(^3\). In this study, NAD\(^+\) was encaged by the constituent amino acids of the enzyme within a range of 6.0 Å apart from the NAD\(^+\)/DMSO/Zn\(^{2+}\) complex. Analysis of the calculational results show AMBER to be a useful tool for the evaluation of the essential NAD\(^+\)-enzyme/DMSO/Zn\(^{2+}\) interactions. Moreover, it is able to provide structural information additional to the initially used X-ray crystallographic data. For example, the inclusion of a water molecule near the nicotinamide mononucleotide phosphate group results in a significant improvement in the geometry of the phosphate bridge. Neither the conformation of the phosphate bridge, nor the position of the adeninemoiety affects the geometry of the nicotinamide group. It is shown that the overall best fit of the energy refined geometry with the initial X-ray geometry is obtained by fixing the adenine amino group at its initial positions, introducing a water molecule near the nicotinamide mononucleotide phosphate bridge and using negatively charged deprotonated Cys 46 and Cys 174 residues.

Introduction

Over the last decade, the interaction of Nicotinamide Adenine Dinucleotide (NAD\(^+\)) with dehydrogenases has been studied extensively in an attempt to understand the factors involved in the productive binding between NAD\(^+\) and the enzyme\(^1\). A number of X-ray crystallographic studies have provided a detailed insight into the conformation and orientation of the coenzyme and the substrate in the active site\(^2\). One of the enzymes for which the geometry of the ternary complex has been elucidated is Horse Liver Alcohol Dehydrogenase (LADH)\(^3\). This enzyme has received widespread attention since it accepts a broad structural range of substrates. We recently attempted, following the elegant work of Kollman\(^4\), to simulate the interactions of LADH and NAD\(^+\) in a ternary complex with DMSO. We report here the use of molecular mechanics calculations for modelling the enzyme/coenzyme/substrate interactions. It will be shown that the AMBER molecular mechanics package is a suitable tool for the evaluation of the individual interactions and, in fact, provides structural information additional to the data obtained from an X-ray crystallographic study (e.g. the inclusion of a water molecule, which is not detected by X-ray analysis as a result of limited resolution).

Procedure for calculational studies

Calculational method

Energy calculations and total energy minimizations were performed using the AMBER molecular mechanics package (version 3.0)\(^9\) on a VAX 11/785 computer. In order to obtain the energetically preferable conformation of NAD\(^+\) within the active site of an enzyme/substrate/Zn\(^{2+}\) complex, we used AMBER to minimize a total energy function consisting of separate terms covering bond-stretching (I), -bending (II) and torsional (III), as well as van der Waals, electrostatic (IV) and hydrogen bond (V) interactions (AMBER energies, Eqn. E).

\[
E_{\text{total}} = \sum_{\text{bonds}} [K_R (R - R_{eq})^2] + \sum_{\text{angles}} [K_\theta (\theta - \theta_{eq})^2] \\
+ \sum_{\text{dihedrals}} \left[ \frac{\nu_\sigma}{2} [1 + \cos (n\phi - \gamma)] \right] \\
+ \sum_{i<j} \left( \frac{A^\mu}{R_{ij}^{12}} - \frac{B^\mu}{R_{ij}^6} + \frac{q_i \cdot q_j}{R_{ij}} \right) \\
+ \sum_{\text{H bonds}} \left( \frac{C_{\mu\nu}}{R_{ij}^{12}} - \frac{D_{\mu\nu}}{R_{ij}^6} \right) \\
+ \sum_{\text{H bonds}} \left( \frac{C_{\mu\nu}}{R_{ij}^{12}} - \frac{D_{\mu\nu}}{R_{ij}^6} \right)
\]

(1)

Over the last decade, the interaction of Nicotinamide Adenine Dinucleotide (NAD\(^+\)) with dehydrogenases has been studied extensively in an attempt to understand the factors involved in the productive binding between NAD\(^+\) and the enzyme\(^1\). A number of X-ray crystallographic studies have provided a detailed insight into the conformation and orientation of the coenzyme and the substrate in the active site\(^2\). One of the enzymes for which the geometry of the ternary complex has been elucidated is Horse Liver Alcohol Dehydrogenase (LADH)\(^3\). This enzyme has received widespread attention since it accepts a broad structural range of substrates. We recently attempted, following the elegant work of Kollman\(^4\), to simulate the interactions of LADH and NAD\(^+\) in a ternary complex with DMSO. We report here the use of molecular mechanics calculations for modelling the enzyme/coenzyme/substrate interactions. It will be shown that the AMBER molecular mechanics package is a suitable tool for the evaluation of the individual interactions and, in fact, provides structural information additional to the data obtained from an X-ray crystallographic study (e.g. the inclusion of a water molecule, which is not detected by X-ray analysis as a result of limited resolution).
Energy refinement and minimization using analytical gradients were performed until the root-mean-square gradient of the energy was less than 0.1 kcal/Å. Additional semi-empirical parameters, which would not be obtained from the AMBER parameter set, are listed in the supplementary material. Standard bond lengths and bond angles were employed and harmonic force constants were either obtained from the literature or extrapolated from available data. We used the MNDO semi-empirical molecular orbital method to calculate atomic charges. All groups, containing hydrogen atoms not essential for hydrogen bonding, are treated as united atoms, i.e., atomic charges of the hydrogen atoms are added to the charge of the adjacent atom to which they are bonded. AMBER energy minimization procedures were performed using the distance dependent dielectric constant \( \varepsilon = R_0^6 \), damping long-range interactions in favour of short-range polarization interactions. This is likely to be the best simulation of the distance dependency of the dielectric constant within proteins.

Examination of the resulting conformations was achieved using the Anal module of AMBER in order to calculate the interaction energies and an interactive computer graphics program (Chem-X, July 1987 update) was used to generate stereodiagrams.

Initial conformations

AMBER calculations require starting geometries of the structures to be calculated. For this, we employed the X-ray crystallographic data for the ternary complex of LADH/NAD\(^+\) /DMSO (2.9 Å resolution, crystallographic R factor of 0.22) as reported by Eklund et al.\(^4\), which were readily retrievable from the Brookhaven Protein Database.

Enzyme

Since AMBER can only perform its calculations with a limited number of atoms, the core of the enzyme can only be represented by a "cage" which is constructed of a relatively small number of amino acids, each fixed at its initial (X-ray) position. In order to obtain a "sealed" construction, 44 amino acids within a range of 6.0 Å from the coenzyme/Zn\(^2+\)/DMSO complex were taken into account.\(^6\) Enlargement of the cut-off distance did not result in any improvement in the results of the calculations. All parameters required could be obtained from the standard AMBER data base.

Coenzyme

Since AMBER does not have all parameters needed in its initial data base, we have introduced the parameters by creating additional data bases.\(^3\) Parameters for adenine, ribose and the phosphate groups were retrieved directly from the AMBER data base, whereas parameters used for the nicotinamide moiety were obtained from MNDO calculations (bond lengths, bond angles, torsion angles and charges) or estimated in accordance with data reported in the literature (harmonic force constants).\(^9\) The geometry of NAD\(^+\) was taken from the X-ray data. Hydrogen atoms which can be involved in hydrogen bonding were added with the computer graphics modelling programme Chem-X (ribose OH's, nicotinamide NH's and adenine NH's).

DMSO

The starting conformation was taken from the X-ray data. AMBER parameters were added to the AMBER data base and charges were calculated using MNDO.

Zinc

The charge of the zinc ion in the catalytic site of the ternary complex is plus two. AMBER parameters were estimated according to procedures reported in the literature.\(^6\) Initial studies, in which the zinc ion is not restricted to its initial position, showed highly unlikely movements of DMSO bonded zinc upon energy refinement. The origin of this shift can easily be denoted; zinc is bonded in the active site of the enzyme by two negatively charged deprotonated cysteine residues. In the AMBER parameter set, however, parameters for the cysteine residues are available only for the neutral state of the amino acid. This means that interactions between zinc and the core of amino acids are neglected. We devised two approaches to overcome this problem. The first comprises fixation of the zinc ion at its initial (X-ray) position, whereas the second approach includes negatively charged cysteine residues via modification of the AMBER data base. Both methods have been applied and the results are discussed below.

Results and discussion

The first case studied was the energy refinement of the NAD\(^+\) coenzyme molecule within the core of amino acids, employing the initial AMBER parameter set for all constituent amino acids of the "cage" (including those of the neutral cysteine residues). The zinc ion was fixed at its initial position. Conformational restraints on the position of the coenzyme molecule were not included. In order to visualize the effects of energy minimization of the geometry of NAD\(^+\), both the initial NAD\(^+\) (X-ray) conformation as

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* Positional restraints during energy minimization (Zn represents a restraint on the zinc ion, Ad a restraint on the exocyclic adenine amino group).\(^7\) The range of variation of the torsion angles detected for the four subunits is presented by listing the minimal and maximal values, respectively.\(^8\) Sym conformation of the nicotinamide group in NAD\(^+\) bound to GAPDH.
Fig. 1. Stereodiagram of the energy refined NAD$^+$ geometry (white). The structure represented in green is the geometry of the NAD$^+$ coenzyme molecule prior to energy refinement (X-ray). Neither the amino acids nor the zinc ion are drawn since these atoms are not allowed to move energy refinement.

Fig. 3. Conformations of NAD$^+$ prior to (green) and after (white) energy refinement with the adenine amino group fixed at its initial position.

Fig. 4. Region of the phosphate bridge of the initial NAD$^+$ geometry (green), the energy refined geometries without (red) and with (white) an additional water molecule. The AMP phosphate group is situated at the right-hand side. The position of both ribose units is not significantly affected.

Fig. 5. Energy refined geometry of NAD$^+$ (white) bound to LADH with negatively charged cysteine 46 and 174 (yellow) residues, restraining the zinc ion and the adenine amino group at their initial positions. The structure represented in green is the initial NAD$^+$ geometry, that in red is the neutral cysteine energy geometry of Figure 3.

Fig. 6. Energy refined geometries of NAD$^+$ (white) bound to LADH with negatively charged cysteine 46 and 174 (yellow) residues, maintaining only the restraints on the adenine amino group and the core of amino acids. The structure represented in green is the initial NAD$^+$ geometry, that in red is the neutral cysteine energy refined geometry of Fig. 3.
Table II  Interaction energies (kcal/mol) of NAD$^+$ bound to LADH with neutral and negatively charged cysteine 46 and 174 residues; 1 represents NAD$^+$, 2 the core of amino acids, 3 the zinc ion and 4 DMSO.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Restraints*</th>
<th>1-1</th>
<th>1-2</th>
<th>1-3</th>
<th>1-4</th>
<th>2-2</th>
<th>2-3</th>
<th>2-4</th>
<th>3-3</th>
<th>3-4</th>
<th>4-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>LADH neutral cys</td>
<td>X-ray energy refined</td>
<td>40.2</td>
<td>-127.4</td>
<td>6.4</td>
<td>-3.8</td>
<td>920.6</td>
<td>324.3</td>
<td>-7.2</td>
<td>0.0</td>
<td>-44.4</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>-7.5</td>
<td>-198.8</td>
<td>-2.8</td>
<td>-2.9</td>
<td>920.6</td>
<td>324.3</td>
<td>-11.2</td>
<td>0.0</td>
<td>-45.2</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Ad + Zn</td>
<td>-4.3</td>
<td>-197.8</td>
<td>-2.1</td>
<td>-2.7</td>
<td>920.6</td>
<td>324.3</td>
<td>-11.2</td>
<td>0.0</td>
<td>-45.3</td>
<td>0.1</td>
</tr>
<tr>
<td>LADH neg cys</td>
<td>X-ray energy refined</td>
<td>40.2</td>
<td>-124.2</td>
<td>6.4</td>
<td>-3.8</td>
<td>1073.1</td>
<td>-205.9</td>
<td>7.8</td>
<td>0.0</td>
<td>-44.4</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Ad + Zn</td>
<td>-7.2</td>
<td>-193.6</td>
<td>6.8</td>
<td>-4.2</td>
<td>1073.1</td>
<td>-205.9</td>
<td>4.3</td>
<td>0.0</td>
<td>-44.7</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Ad</td>
<td>-7.8</td>
<td>-190.2</td>
<td>9.8</td>
<td>-5.0</td>
<td>1073.1</td>
<td>-205.9</td>
<td>2.9</td>
<td>0.0</td>
<td>-43.8</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* Positional restraints during energy minimization (Zn represents a restraint on the zinc ion, Ad a restraint on the exocyclic adenine amino group).

Fig. 2. Nomenclature of the coenzyme torsion angles and the atoms used in the text (adopted from Eklund et al. (ref. 3b) in accordance with the IUPAC-IUB convention of 1983).

Fig. 3 shows the energy refined structures of restrained NAD$^+$ relative to the initial NAD$^+$ conformation.

In contrast to the situation depicted in Fig. 1, the most relevant discrepancies are in this case observed in the phosphate bridge. A relatively strong perturbation of $\alpha_N$ is compensated by a change of $\beta_A$ and $\gamma_A$. Analysis of the resulting structure shows that the AMP phosphate group is shifted towards Lys 228 at the expense of the phosphate-Arg 47 interaction (Table IV).

This shift is caused by interaction between the positive charge of the amino group of the Lys 228 side-chain and the negative charge of the phosphate group. The distance between the phosphate oxygen atom and the Lys nitrogen atom in the X-ray structure is 5.69 Å. This distance, resulting in neglectable hydrogen bonding between Lys 228 and the AMP phosphate group, is large enough to accommodate a water molecule. This water molecule could probably not be detected owing to insufficient resolution of the X-ray analysis. Skarzynski and co-workers$^{12}$, on the other hand, showed in their high-resolution crystallographic study of a
binary complex of NAD\(^+\) and Glyceraldehyde-3-phosphate
Dehydrogenase (GAPDH) from *Bacillus stearothermophilus*
(resolution of 1.8 Å, crystallographic R factor of 0.177) that
several water molecules are included in the region of the
phosphate bridge. In view of the fact that the coenzyme
binding domains of GAPDH and LADH show a close
resemblance, resulting in similar binding conformations of
NAD\(^+\) (Table I), it is safe to assume that at least one water
molecule is involved in hydrogen bonding with the
phosphate bridge of NAD\(^+\) in LADH. In order to evaluate its
effect, a single water molecule was introduced into the initial
Eklund X-ray structure just between the adenosine
phosphate group and the amino group of the Lys 228 side-
chain. The system obtained was submitted to energy
refinement, restraining all atoms at their initial positions, except
for those of the added water molecule. Subsequently, the entire structure, i.e. coenzyme, DMSO and the water
molecule, was allowed to minimize energy while retaining
restraints only on the position of the core of amino acids,
the zinc ion and the adenosine amino group. Analysis of the
final geometry\(^\dagger\) revealed that the conformational change of the phosphate group was essentially reduced. (The shift of the AMP phosphorus atom upon energy refinement is 0.665 Å instead of 1.254 Å found in absence of the water molecule). The results (Fig 4 and Table IV) indicate that the position of the phosphate group is largely governed by H-bonding interactions in which at least one water molecule is involved.

It can be expected that determination of the exact position of the water molecule (or molecules) may reduce the conformational changes even more. At any rate it should be noted that the conformation of the phosphate bridge does not affect the position of the nicotinamide moiety in the active site.

Apart from the relatively large perturbations mentioned above, Fig 3 also displays minor changes of the nicotinamide glycosidic bond torsion angle \(\chi_3\) (rotation of 13\(^\circ\), vide supra) and the decrease of the carbonyl out-of-plane torsion angle of the nicotinamide moiety (34\(^\circ\) changing into 6\(^\circ\)).

In order to explain these conformational changes, we tabulated the relevant interaction energies between NAD\(^+\) and the individual amino acids as well as the zinc ion (Table V). From the top half of Table V, it appears that only the interaction energies of NAD\(^+\) with the zinc ion and Val 292 are significantly changed upon energy refinement. Apparently, the electrostatic repulsion between the positive charge of the zinc ion and the positively charged C5\(_h\) atom (Fig 2) of the nicotinamide group induces a slight torsion of the glycosidic bond (Table I). This movement is accompanied by an additional rotation around the C3\(_h\)-C7\(_h\) bond, resulting in a diminished out-of-plane torsion angle of the amide group. The latter rotation enables the amide oxygen (O7\(_N\)) and the hydrogen atom (\(\text{syn relative to the carboxyl dipole}\)) to remain in close contact with Phe 319 and Ala 317, respectively. Combination of the above described interrelated rotational movements results in a rather large displacement of one of the amide hydrogen atoms (anti position to the carboxyl group), bringing it significantly closer to the main-chain carboxyl group of Val 292. From this it is evident that the magnitude of the carboxyl dipole out-of-plane rotation is very much dependent upon the exact positioning of Phe 319 and Val 292 and the extent of repulsion exerted by the zinc ion. The observed repulsion effect stimulated us to investigate the influence of introducing the negative charge on the cysteine 46 and 174 residues to which the zinc ion is bound. This modification will diminish the zinc-nicotinamide repulsion and, at the same time, meets with the necessity of artificially restraining the zinc ion (vide supra). AMBER parameters for the negatively charged cysteine residues were taken directly from the AMBER parameter set except for the atomic charges. These charges were derived from MNDO calculations of neutral cysteine adding one formal negative charge to the sulfur atoms, changing its value from +0.07 to -0.93. This method was adopted rather than using the calculated charges of deprotonated cysteine.

\[\begin{array}{|c|c|c|c|c|c|c|c|c|}
\hline
\text{Enzyme} & \text{Restrains}^a & \text{cys} & \text{thr} & \text{val} & \text{val} & \text{ala} & \text{ile} & \text{phe} & \text{Zn} \\
\hline
\text{LADH neutral} & \text{X-ray} & -3.6 & -1.5 & 0.8 & -9.0 & -3.1 & -2.7 & -3.3 & 6.4 \\
\text{cys} & \text{energy} & -3.4 & -1.5 & 1.0 & -12.8 & -3.7 & -2.5 & -3.3 & -2.8 \\
\text{refined} & \text{Zn} & -2.9 & -1.4 & 1.2 & -12.5 & -8.0 & -4.0 & -3.3 & -3.0 & -2.1 \\
\text{LADH neg} & \text{X-ray} & 3.3 & -5.1 & 0.8 & -9.0 & -3.1 & -2.7 & -3.3 & 6.4 \\
\text{cys} & \text{energy} & 3.2 & -5.1 & 0.3 & -12.9 & -8.3 & -4.0 & -3.3 & -3.0 & 6.8 \\
\text{refined} & \text{Zn} & 1.9 & -5.9 & 0.3 & -12.5 & -8.0 & -3.4 & -3.2 & -3.1 & 9.8 \\
\hline
\end{array}\]

\(^a\) Positional restraints during energy minimization (Zn represents a restraint on the zinc ion, Ad a restraint on the exocyclic adenine amino group).

---

Table IV: Interatomic distances (Å) between the AMP phosphate oxygen atoms and the (nearest) side-chain-terminating nitrogen atoms of arg 47 and lys 228, respectively (values in parentheses indicate differences relative to the initial (X-ray) structure).

<table>
<thead>
<tr>
<th></th>
<th>OP1(_A)</th>
<th>OP2(_A)</th>
<th>OP1(_B)</th>
<th>OP2(_B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-ray</td>
<td>2.91</td>
<td>4.68</td>
<td>6.18</td>
<td>5.96</td>
</tr>
<tr>
<td>Energy refined</td>
<td>3.86(0.95)</td>
<td>6.34(1.66)</td>
<td>5.56(0.62)</td>
<td>5.99(0.30)</td>
</tr>
<tr>
<td>without H(_2)O</td>
<td>3.26(0.35)</td>
<td>5.46(0.78)</td>
<td>6.12(-0.06)</td>
<td>5.87(0.18)</td>
</tr>
</tbody>
</table>
cysteine itself, since, in this case, the extra negative charge is neither stabilized by the S–H bond nor by the S–Zn²⁺ bond. The absence of a proton or zinc ion in the MNDO calculation will lead to a redistribution of the additional negative charge over all atoms, including the main-chain atoms (the charge of the sulfur atom in deprotonated cysteine in this case is –0.75). Figs. 5 and 6 depict the energy refined NAD⁺ geometry with and without the restraint on the position of the zinc ion, respectively.

Torsion angles, interaction energies, AMBER energies and specification of interaction energies of NAD⁺ with the relevant individual amino acids are inserted in Tables I, II, III and V, respectively. Figs. 5, 6 and 7 depict the final geometries of NAD⁺ using neutral and negatively charged cysteine, demonstrates the shift of the nicotinamide group towards Cys 174 (Table V also indicates a favourable Cys 174-NAD⁺ interaction energy). The same effect, expected for Cys 46, is, however, totally overshadowed by the enhanced repulsion between the sulfur atom of the cysteine residue and the nicotinamide-linked ribose oxygen atoms. This feature, illustrated by the increase of the Cys 46–NAD⁺ interaction energy (Table V), induces a perturbation of the position of the ribose unit at issue, increasing the distance between the sulfur atom and O₂ₛ and O₃ₛ, respectively, simultaneously rotating O₄ₛ towards the sulfur atom (Table VI).

Table VI Interatomic distances (Å) between the nicotinamide-linked ribose oxygen atoms and the sulfur atom of neutral (I) and negatively charged (II) Cys 46 residues (fixing the zinc ion and the adenine amino group).

<table>
<thead>
<tr>
<th></th>
<th>O₂ₛ</th>
<th>O₃ₛ</th>
<th>O₄ₛ</th>
</tr>
</thead>
<tbody>
<tr>
<td>neut. cys</td>
<td>4.84</td>
<td>7.13</td>
<td>6.62</td>
</tr>
<tr>
<td>neg. cys</td>
<td>5.57</td>
<td>7.55</td>
<td>6.41</td>
</tr>
</tbody>
</table>

In addition, Figs. 5 and 6 clearly show that the position of the nicotinamide group of the energy refined geometry with negatively charged cysteine residues matches the Eklund X-ray NAD⁺ geometry very well. In particular, the C₃ₛ and C₅ₛ atoms of the X-ray and the energy refined geometries are almost superimposable. The main discrepancies between these two geometries are a small shift of the glycoside bond, which is a direct consequence of the perturbed position of the ribose unit, and a slight torsion of the glycoside bond (6° in the case of the zinc ion being fixed and only 2° when the zinc ion is subjected to energy refinement, see Table I).

Conclusions

The results presented above show AMBER to be a useful tool in evaluating the essential interactions governing the geometry of NAD⁺ in the active site of the ternary complex of LADH/NAD⁺/DMSO. Furthermore, there are strong indications that this calculational method can even provide information additional to data obtained from X-ray crystallographic studies. For example, we have shown that the inclusion of a water molecule near the phosphate bridge of NAD⁺ leads to a better fit with the Eklund model. Energy refinement of NAD⁺ results in a geometry which is closely related to the actual structure of the NAD⁺ determined with X-ray analysis. The overall best fit is observed by fixing the adenine amino group at its initial positions, introducing a water molecule between the nicotinamide mononucleotide phosphate group and Lys 228 and using negatively charged deprotonated Cys 46 and Cys 174 residues. High-resolution X-ray data obtained for a binary complex of NAD⁺ and GADPH¹² (vide supra) reveal an NAD⁺ geometry which resembles the calculation geometry to an even better extent (Table I). This observation becomes quite relevant considering the fact that the coenzyme domains of several NAD⁺-dependent dehydrogenases (including LADH and GAPDH) are closely related.¹⁴

Comparing the NAD⁺ geometries in Table I, one should bear in mind that torsion angles are highly sensitive to small deviations in positional parameters of the atoms. Although one may expect errors in the X-ray data of the coenzyme to be corrected by the energy refinement procedure, inaccuracies in the positions of atoms within the core of amino acids inevitably lead to the introduction of systematic errors in the energy refined coenzyme geometry. Since resolution of the X-ray data is limited to 2.9 Å, the decrease of the out-of-plane torsion angle of the nicotinamide carbonyl dipole, for instance (LADH: 30°, GAPDH: 22° diminished to 6° upon energy refinement), is probably due to a relatively small perturbation of Val 292 or Phe 319.¹³

It can also be concluded that conformational changes of the phosphate bridge and the AMP subunit do not affect the geometry of the nicotinamide group. This finding is in accordance with the observations of Siesjö and coworkers¹⁵, who showed that several fragments of NAD⁺(H), nicotinamide mononucleotide and mononucleoside (in the presence of AMP) are functional in enzyme-catalyzed redox reactions. In addition, immobilization using the exocyclic amino group of adenine does not interfere with the activity of NAD⁺.

In view of all these considerations, AMBER would appear to be capable of giving a reasonably accurate description of the geometry of NAD⁺ bound in the active site of an enzyme. In a subsequent paper we will report the use of AMBER to calculate the geometry of NAD⁺ derivatives modified in their nicotinamide moieties. X-ray data for all ternary complexes with such NAD⁺ derivatives are not available. It will be shown that the geometry of the NAD⁺ analogues within the ternary complex, as calculated by AMBER, can be correlated with their reactivity in enzymatic redox reactions.

Acknowledgements

The use of the services and facilities of the Dutch CAOS/CA/MM Center under grant numbers SON-11-20-700 and STW-NCH-44.0703 is gratefully acknowledged.

Supplementary Material Available

Tables of additional AMBER parameters and cartesian coordinates of the initial and all the energy refined geometries of NAD⁺ are available. The interaction energies of the NAD⁺ structure minimized with one water molecule and the AMBER energies of Table III are also available.

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Information concerning the energy-refined geometry and interaction energies, as well as AMBER energies, are available in the supplementary material.
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