Free Energy Calculations Involving Internal Coordinate Constraints to Determine Puckering of a Six-Membered Ring Molecule

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Abstract: The free energy of boatlike puckering is examined for dihydropyridine (DHP) and dihydronicotinamide mononucleoside (NMSH) by thermodynamic simulations with an empirical force field. The interest in the conformation of the dihydropyridine ring stems from the question of a nonplanar ring conformation to facilitate hydride transfer between the cofactor NADH (reduced nicotinamide adenine dinucleotide) and the substrate in enzyme complexes. To investigate this question we secure a set of empirical force field parameters for the nonaromatic six-membered ring molecule dihydropyridine. A reaction coordinate comprising two dihedral angles is used to constrain the ring in a given boat conformation to calculate the free energy of ring puckering. In agreement with small molecule crystallographic structures, the free energy minimum lies close to a planar ring, but importantly, a broad minimum exists which would allow a range of boat conformations. In addition to the bonding interactions of the dihydropyridine ring, the NMSH free energy profile for puckering depends on nonbonded interactions between the ribose and base as determined by the anti or syn orientation of the glycosidic bonds and pyramidalization of the ring nitrogen. The parameters and computational approach reported here will be useful in calculations on any of the wealth of enzyme complexes involving the cofactor NADH.

Introduction

A description of the conformational equilibrium of molecules is necessary for a complete understanding of the function of biological systems. Multiple conformations with similar energies contribute to the thermodynamic properties of the system, and a description in terms of the average conformation may not be adequate. Even proteins with a well-ordered 3-dimensional structure exhibit conformational flexibility in active-site loops[1,2] and the binding of ligands.

We report a molecular dynamics study on the conformational equilibrium of the flexible-ring molecules dihydropyridine (DHP) and dihydronicotinamide mononucleoside (NMSH). DHP and NMSH are fragments of the cofactor reduced nicotinamide adenine dinucleotide (NADH). The pyridine cofactors NADH and NADPH are ubiquitous in living systems and are involved in a larger number of enzymic reactions than any other cofactor.[3] Their function is in stereospecific hydride transfer by reduction and oxidation of C4 of the pyridine/dihydropyridine ring (Figure 1a). Glycolysis, the tricarboxylic acid cycle, ethanol metabolism, and purine synthesis are a few of the many essential processes requiring pyridine nucleotide cofactors.

There is much interest in trying to understand the significance of conformational factors of NADH in enzyme catalysis and stereospecificity.[3-8] The ring conformation is one such factor; it has been proposed that a boatlike conformation is the transition-state structure for hydride transfer from C4 with the pseudoaxial hydrogen being the transferring hydrogen.[5,7,9] In a boatlike conformation the pseudoaxial positions for the hydrogen and N1 lone pair electrons facilitate transfer by allowing the appropriate overlap of π orbitals on opposite sides of the ring.

Although experiments play an invaluable role in studying structure, the information they provide is generally limited to a description of the average conformation. A more complete understanding can be gained from additional information provided by thermodynamic simulations[10-13] on the range of energetically accessible conformations. As such, the tendency toward boatlike puckering of the flexible dihydropyridine ring was investigated by calculating the free energy of ring pucker with thermodynamic simulation methods and internal-coordinate-constrained molecular dynamics. For systems separated in configuration space, the free energy difference as a function of an internal coordinate is determined by configurational perturbations and simulations in which the internal coordinate is held constant by holonomic constraints.[10,12,14] This procedure has been successfully applied to dihedral angle coordinates in a dipeptide[10] and an enzyme-bound inhibitor.[12]

In the first step toward studies on the conformational flexibility of the dihydronicotinamide ring in an enzyme active site, we investigate the ring puckering of these isolated fragments of NADH. Our investigation finds no tendency toward stabilization of the putative transition-state structure for hydride transfer in the isolated DHP and NMSH. However, the free energy profile as a function of ring pucker is nearly flat over a 20°-30° range in pucker angles, and the energy for N1 inversion is small.

In the next section we describe the parameterization of the CHARMM molecular force field. A description of the reaction coordinate and protocol for the free energy profile calculation follow. In Results, we examine the accuracy and convergence of the constraint algorithm for flexible ring molecules and free energy profiles for ring puckering and discuss structural features related to hydride transfer, including ring nitrogen pyramidalization.

Parameters

The parameters required for the potential function of the CHARMM program[15] are atomic partial charges q_i, van der Waals constants E_{min} and R_{min}, and the equilibrium geometry values for bond lengths, bond angles, and dihedral angles with their associated force constants. No explicit hydrogen-bond term was

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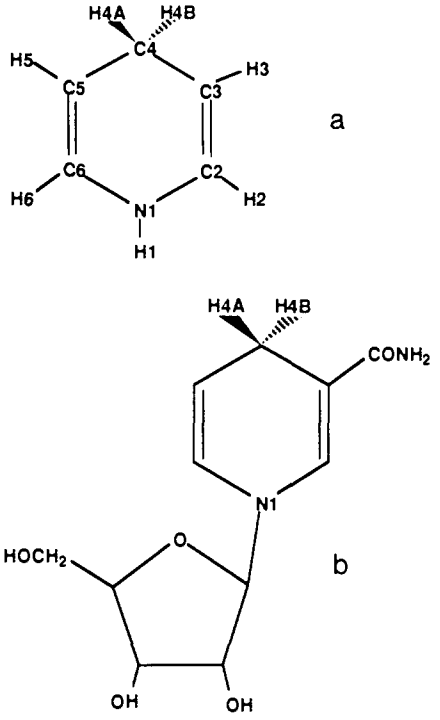

Figure 1. (a) Structure of dihydropyridine (DHP). (b) Structure of dihydronicotinamide mononucleoside (NMSH).

Table I. Partial Charges and van der Waals Constants for Dihydropyridine

atom	charge	E_{min}	R_{min}	atom	charge	E_{min}	R_{min}
N1	−0.48	−0.20	3.7	H4A	0.02	−0.0078	2.936
H1	0.28	−0.046	0.499	H4B	0.02	−0.0078	2.936
C2	0.06	−0.09	3.6	C5	−0.02	−0.09	3.6
H2	0.04	−0.0078	2.936	H5	0.05	−0.0078	2.936
C3	−0.02	−0.09	3.6	C6	0.06	−0.09	3.6
H3	0.05	−0.0078	2.936	H6	0.04	−0.0078	2.936
C4	−0.10	−0.1562	3.6				

included since the attraction between hydrogen-bonding atoms is adequately modeled by appropriate Coulombic and van der Waals potential terms. Partial charges and dihedral angle terms were determined for the base DHP, as described below. Other parameters are from crystallographic results or the CHARMM22 all-hydrogen potential function.[16]

Partial charges, q_i, of dihydropyridine were found by fitting the molecular dynamics intermolecular potential energy for a DHP–water dimer to corresponding ab initio intermolecular energies. Ab initio calculations were performed with the program GAUSSIAN 88[17] and a 6-31G* basis set. This basis set was chosen because of previous success in describing similar interactions[18] and to be consistent with other work on the development of the CHARMM22 force field.[16] To decrease the size of the calculation, C_s symmetry was imposed for DHP, the mirror plane being perpendicular to the ring plane and through atoms N1 and C4. The structures of DHP and water were obtained by full geometry optimization of the isolated molecule and were not varied in the dimer interaction. Optimization of DHP began from the crystal coordinates of an N-substituted dihydronicotinamide.[19] Mini-

Table II. Interaction Energies for DHP–Water Dimers[a]

complex[b] (water···DHP)	6-31G* ΔE	empirical ΔE
O···H1	−3.84	−3.66
H···N1	−3.64	−4.42
O···H2	−1.40	−0.65
H···C2	−2.62	−3.32
O···H3	−0.70	−0.62
H···C3	−2.71	−2.63
O···C4	−0.16	−0.03

[a] Interaction energies in kcal/mol. [b] Atoms used to define the direction of approach in the complex.

Table III. Bond Parameters

bond	equilibrium length	force constant	bond	equilibrium length	force constant
N1–H1	1.01	474.0	N1–C2	1.372	302.0
C2–H2	1.09	350.0	C2–C3	1.337	552.0
C3–H3	1.09	350.0	C3–C4	1.516	214.8
C4–H4A	1.11	317.0	C4–C5	1.516	214.8
C4–H4B	1.11	317.0	C5–C6	1.337	552.0
C5–H5	1.09	350.0	C6–N1	1.372	302.0
C6–H6	1.09	350.0			

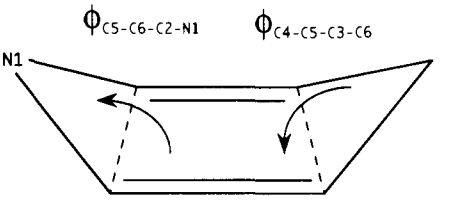

Figure 2. Diagram of the dihedral angles, $\phi_{C4-C5-C3-C6}$ and $\phi_{C5-C6-C2-N1}$, used to define the reaction coordinate S for ring pucker.

mization of the intermolecular ab initio energy for the DHP–water dimer was done for 7 of 11 selected orientations of the water relative to the geometry of DHP. (Four of these orientations are redundant due to symmetry.) The orientations chosen corresponded to the strongest intermolecular interactions. The intermolecular distance along a direction of approach and, in some cases, the angular orientation of the water molecule were allowed to vary in the ab initio optimization.

The intermolecular empirical energy was minimized as a function of the DHP–water distance using the simplex method. The minimum interaction energy values from the empirical potential were fit to the ab initio interaction energies by varying q_i. Of the nonbonded energy terms, only q_i was varied to reach agreement, while E_{min} and R_{min} are from the CHARMM22 parameters.[16] The resulting charges are listed in Table I. Listed in Table II are the energies for the seven interactions from the 6-31G* calculation and from the nonbonded terms of the empirical force field.

The force constants for the dihedral angles about N1–C2, N1–C6, C3–C4, and C4–C5 were varied to obtain an energy minimum for a near-planar structure of DHP in agreement with the optimized ab initio geometry and with other results. A near planar conformation of DHP is obtained by ab initio full geometry optimization by this work and others.[7,8,20,21] Furthermore, the dihydropyridine ring in the crystallographic structure of N-substituted dihydronicotinamides[19] is nearly planar, although crystal forces can be significant to determining the conformation observed in the crystal state. The values for the pseudodihedral angles $\phi_{C4-C5-C3-C6}$ and $\phi_{C5-C6-C2-N1}$ (see Figure 2) in the empirical energy optimized structure are 176° and 174°. The corresponding values in the crystallographic structure for dihydropyridine are 179.1° and 179.7°, and ab initio geometry optimization of DHP yields 174.4° and 171.5°. Thus, the minimum energy structure for the parameters reported here has a small degree of ring puckering

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Table IV. Angle Parameters

angle	equilibrium value	force constant
H1-N1-C2	121.0	32.0
N1-C2-H2	114.7	30.0
H2-C2-C3	120.5	30.0
C2-C3-H3	120.5	30.0
H3-C3-C4	119.5	30.0
C3-C4-H4A	108.7	36.0
C3-C4-H4B	108.7	36.0
H4A-C4-H4B	108.4	36.0
C4-C5-H5	119.5	30.0
C5-C6-H6	120.5	30.0
C6-N1-H1	121.0	32.0
N1-C2-C3	123.3	125.0
C2-C3-C4	122.6	125.0
C3-C4-C5	110.0	50.0
C4-C5-C6	122.6	125.0
C5-C6-N1	123.3	125.0
C6-N1-C2	116.9	70.0
H4A-C4-C5	108.7	36.0
H4B-C4-C5	108.7	36.0
H5-C5-C6	120.5	30.0
H6-C6-N1	114.7	30.0

Table V. Dihedral and Improper Dihedral Parameters

dihedral	period	phase	force constant
H1-N1-C2-H2	3	0.0	0.4
H2-C2-C3-H3	2	180.0	5.6
H3-C3-C4-H4A	3	0.0	0.4
H3-C3-C4-H4B	3	0.0	0.4
H4A-C4-C5-H5	3	0.0	0.4
H4B-C4-C5-H5	3	0.0	0.4
H5-C5-C6-H6	2	180.0	5.6
H6-C6-N1-H1	3	0.0	0.4
C6-N1-C2-C3	3	0.0	0.4
N1-C2-C3-C4	2	180.0	5.6
C2-C3-C4-C5	3	0.0	0.4
C3-C4-C5-C6	3	0.0	0.4
C4-C5-C6-N1	2	180.0	5.6
C5-C6-N1-C2	3	0.0	0.4

improper	force constant	improper	force constant
C5-C4-C6-H5	75.0	C6-C5-N1-H6	75.0
C3-C2-C4-H3	75.0	C2-N1-C3-H2	75.0

which is intermediate to that from the crystallographic and the ab initio structures.

Equilibrium values for bond lengths and angles are those of the crystal structure.[19] Other parameters listed in Tables III-V are from CHARMM22.[16]

Free Energy Profile

Although thermodynamic simulation techniques have been applied to numerous systems involving chemical mutations, fewer reports on procedures for calculation of conformational free energy differences have appeared. To determine conformational equilibria by thermodynamic perturbation methods, the reaction coordinate is specified in terms of internal coordinates. This report concerns the free energy change associated with structural changes in a closed-ring system. Conformational changes in the six-membered ring of dihydropyridine (Figure 1a) and the nucleoside dihydronicotinamide mononucleoside (NMSH) (Figure 1b) were made using internal coordinate constraints on dihedral angles in the ring. The reaction coordinate includes planar and boat configurations with puckering in both directions. (The chair conformation is not considered for reasons discussed below.)

Details of free energy perturbation calculations and the holonomic constraint method are in the literature,[22,23] so that only a brief outline of the theory is provided here. The free energy profile for ring pucker is calculated from the exponential form of the perturbation simulation method.[22,24] For states 0 and 1,

$$\Delta A = A_1 - A_0 = -kT \ln\langle e^{-\beta(E_1 - E_0)}\rangle_0 \qquad (1)$$

is the free energy of state 1 relative to state 0. k is Boltzmann's constant; T, the absolute temperature; $\beta = 1/kT$; and E_i is the potential energy of the ith conformational state. The expression $\langle e^{-\beta(E_1 - E_0)}\rangle_0$ is the statistical mechanical ensemble average evaluated from the simulation representative of state 0.

To calculate the free energy profile for ring pucker in DHP and NMSH, the full range of pucker was divided into small conformational changes and the individual values of ΔA combined to construct the complete profile. The ring pucker reaction coordinated is defined in terms of dihedral angle internal coordinates which were constrained during the dynamics by using holonomic constraints according to Tobias and Brooks.[10] The trajectory structures were "perturbed" by 4°-12° increments (see Results) in the ring pucker to determine ΔA (eq 1) for small pucker changes. The free energy profile for the full range of pucker was therefore calculated by perturbations from 5 to 7 simulations referenced to a value of the reaction coordinate.

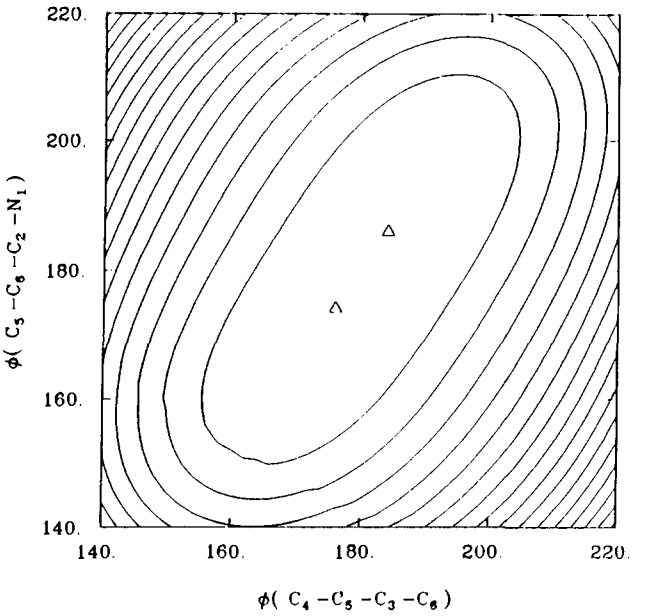

Figure 3. Potential energy contour surface for the two dihedrals composing S. The triangles are not part of the contour but are placed at the energy minima points (176,174) and (184,186). The energy spacing is 1.5 kcal/mol.

Ring Puckering Coordinate

A structure coordinate (S) used to constrain the ring in various degrees of pucker is defined as a function of two dihedral angles: $\phi_{C4\text{-}C5\text{-}C3\text{-}C6}$ and $\phi_{C5\text{-}C6\text{-}C2\text{-}N1}$ (Figure 2). S is a linear combination of these angles determined from the $\phi_{C4\text{-}C5\text{-}C3\text{-}C6}$ versus $\phi_{C5\text{-}C6\text{-}C2\text{-}N1}$ potential energy surface obtained by energy minimization with harmonic constraints on the dihedral angles. Figure 3 is the resulting contour map, with the contours representing 1.5 kcal/mol spacing of the energy values. The actual minima are marked with triangles, and the increase in energy between the minima is equal to 4.6×10^{-2} kcal/mol. The values for $\phi_{C4\text{-}C5\text{-}C3\text{-}C6}$ and $\phi_{C5\text{-}C6\text{-}C2\text{-}N1}$ at the minima are (176,174) and (184,186); (180,180) represents the flat ring.

Figure 3 represents all possible combinations of the two dihedral angles ranging from a symmetrical boat conformation (both angles equal and bent toward each other) to a symmetrical chair conformation (both angles equal relative to the ring plane but bent

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away from each other) and possibilities in between. The two symmetrical boat conformations, differing in the direction of pucker, are represented in the extreme by (140,140) and (220,220), and the chair conformations are represented by (140,220) and (220,140).

Since the lines of steepest descent are in the region representing the chair conformation, the free energy perturbation calculations were limited to a profile involving the boat conformation. The large energy of the chair is due to the double bonds C2–C3 and C5–C6. The planarity of the six atoms of each double bond is maintained by rotating about an axis through C4 and N1 to yield a boat conformation, while the energetically unfavorable twisting of the double bonds is required for a chair conformation. (We note that in both the X-ray structure and the ab initio optimized structure, the dihedrals about these double bonds may deviate slightly (<2.4°) from planar.)

S is the linear combination of $\phi_{C4-C5-C3-C6}$ and $\phi_{C5-C6-C2-N1}$ determined by the line passing through the two minima marked by the triangles in Figure 3. The slope is 1.5. If we let $S = 0$ correspond to (140,120) and $S = 1$ correspond to (220,240), then

$$S = -1.667 \times 10^{-2}\phi_{C4-C5-C3-C6} + 1.944 \times 10^{-2}\phi_{C5-C6-C2-N1} \quad (2)$$

defines the structure coordinate.

Molecular Dynamics Simulations

Seven simulations were carried out for S_i over the range 0.0–1.0 to allow adequate sampling for the average in eq 1. We perform the calculation using the double-wide sampling method,[22] in which the trajectory calculated at S_i is perturbed both forward ($+\delta S_i$) and backward ($-\delta S_i$) to give two ΔA values for one simulation. The entire free energy curve is then obtained from the combined ΔA_i. The S_i are chosen such that the window edges meet ($S_i + \delta S_i = S_{i+1} - \delta S_{i+1}$), except at the limits $S_i - \delta S = 0.0$ and $S_i + \delta S = 1.0$.

Calculations were carried out with the program CHARMM[15] including internal coordinate constrained dynamics[14] running on a CYBER 205 and DECStation 3100. For each S value, energy minimization was performed fixing S_i with harmonic constraints. Before the dynamics calculation, S_i was reset to the exact value, as the dihedral angles may deviate a few degrees from the target value with the harmonic constraints. Trajectories were calculated with an integration step of 0.5 fs with the Verlet algorithm and a temperature of 300 K.

Equilibration was carried out for 5 ps, scaling the velocity every 200 steps if the temperature fell outside a ±25° window. In most simulations, the energy was stable after approximately 1 ps, so during the analysis period no rescaling of the temperature was necessary. When the ring was constrained to a puckered conformational state more than about 30° from planar, stabilization of the energy required almost 2 ps. The analysis period was 10 ps. The trajectory ensemble was used to calculate the free energy differences corresponding to $S_i \pm \delta S_i$ (eq 1).

The starting coordinates for DHP simulations were from the ab initio calculation with full geometry optimization. For NMSH in the anti conformation, the initial coordinates for the nicotinamide base and ribose were from the crystallographic structure for the ternary complex of dogfish lactate dehydrogenase with NADH and oxamate.[25] The syn conformation about the glycosidic bond was generated by a 180° rotation about N1–C1′. The conformation of the ribose was C_3′-endo. Simulations were carried out without constraints on the glycosidic angle. Thus, the calculations involving syn and anti conformers differed only in the glycosidic dihedral angle of the starting structures.

Results

1. Conformationally Constrained Molecular Dynamics of 6-Membered Rings. Internal-coordinate-constrained molecular dynamics was used to evaluate the free energy profile for ring puckering in DHP and NMSH. In the case of NMSH, results were obtained for initial torsion angles of the N1–C1′ glycosidic bond in either anti or syn configuration. Of the 13 atoms in DHP, only the positions of the hydrogens are not part of a constrained internal coordinate. The configurations composing the ensemble therefore do not have large fluctuations and remain similar to the starting structure. In the case of NMSH (34 total atoms, 18 heavy atoms), the number of freely moving atoms is larger and includes 12 heavy atoms and all hydrogen atoms. Calculation of the free energy profile of this molecule includes changes in conformation of the ribose ring and amide side chain.

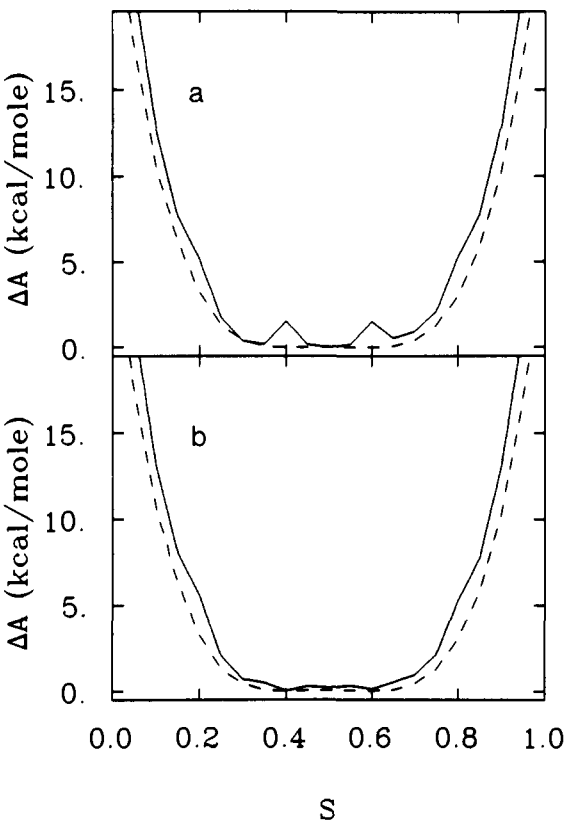

Figure 4. (a) Free energy curve (solid) as a function of ring pucker for dihydropyridine calculated from five simulations at $S = 0.1, 0.3, 0.5, 0.7$, and 0.9. Conformational perturbations extend midway between simulation points, i.e. $\delta S = 0.1$. The potential energy curve (dashed) is shifted for purposes of comparison. (b) The maxima in part a are not present when the free energy curve for dihydropyridine (solid) is calculated with smaller $\delta S = 0.05$ near the planar region; two simulations at $S = 0.4$ and 0.6 are included with those for part a to obtain the ΔA values composing this curve. The potential energy curve (dashed) is shifted for purposes of comparison.

The coordinate S is a linear function of the two dihedral angles defined in Figure 2. If, in addition to $\phi_{C4-C5-C3-C6}$ and $\phi_{C5-C6-C2-N1}$, constraints are specified for $\phi_{N1-C2-C6-C3}$ and $\phi_{C2-C3-C5-C4}$, the holonomic constraint algorithm does not converge. Nor did the algorithm converge when the constraints were defined in terms of four or six bond torsion angles around the ring.

Initially, simulations were calculated for five values of S, corresponding to 8° and 12° perturbations, similar in magnitude to the 15° perturbations used by Tobias and Brooks[10] for dihedral angles of an extended chain: $S_i = 0.1$ (148,132), 0.3 (164,156), 0.5 (180,180), 0.7 (196,204), and 0.9 (212,228). However, this size of δS proved too large for ΔA evaluated near the planar state; values for ΔA are too large, and erroneous maxima appear as "bumps" at $S = 0.4$ and 0.6 in the free energy profile shown with the solid curve in Figure 4a. The minimum energy curve is the dashed curve.

The maxima in the free energy curve do not result from a large ΔA value greater than $2kT$ (1.5 kcal/mol).[22] The error in ΔA results from imposing a conformational perturbation which produces a nonequilibrium conformational state; rigid rotation about

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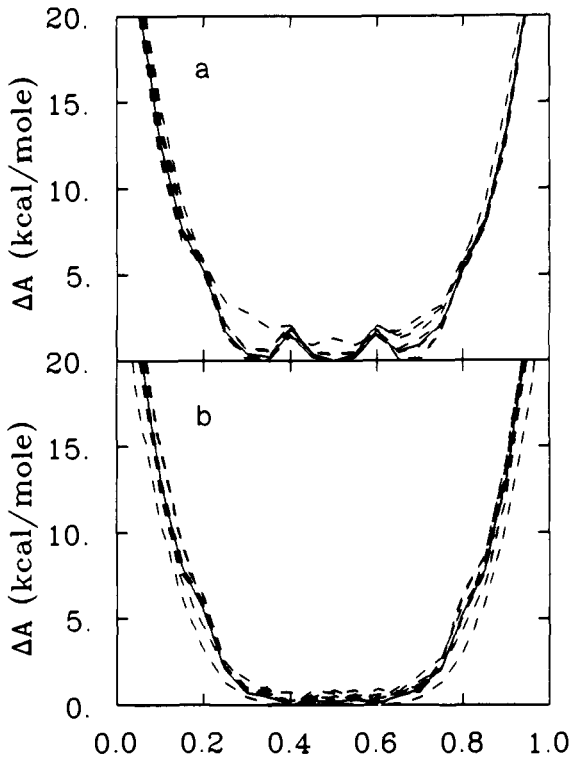

Figure 5. (a) Free energy curve (dashed) for ring pucker of dihydropyridine calculated from five simulations with a different set of starting velocities. The solid curve is the average of the dashed curves. (b) As in part a except that each profile was obtained by using smaller δS values from seven simulations.

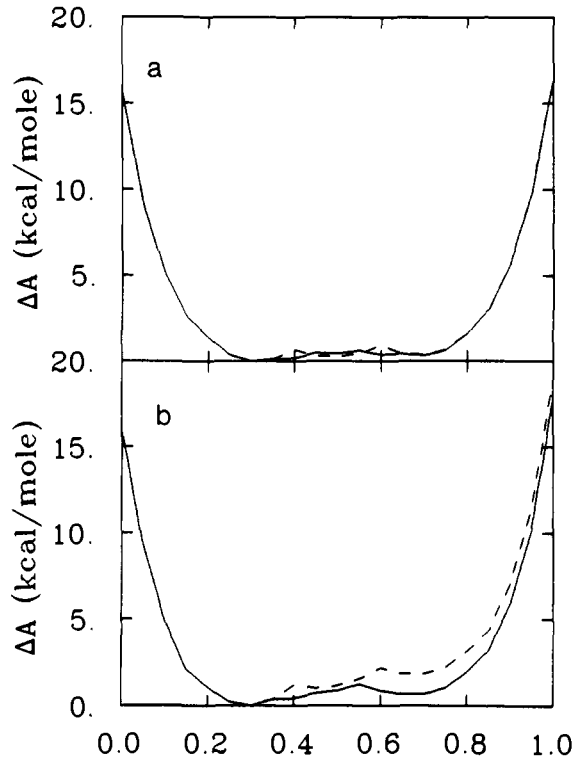

Figure 6. Free energy curve for NMSH with the (a) anti or (b) syn configuration about the N1–C1′ glycosidic bond. The dashed curve is the result from five simulations, and the solid curve is from seven simulations (see caption to Figure 4).

$\phi_{C5-C6-C2-N1}$ produces unfavorable positions of H1. From the simulation referenced to the planar ring at $S = 0.5$, perturbations to puckered states $S = 0.4$ and 0.6 generate geometries of N1 with higher energy than found for the structures in simulations referenced at these S values. The higher-energy geometry is due to the inversion of the ring N1. That is, for a given direction of pucker, there is a minimum energy configuration for orientation of the N1–H1 bond. (See Pyramidalization of the Ring Nitrogen, below.) In the simulation referenced to the planar ring, the H1 fluctuates to out-of-plane positions on both sides of the ring. Perturbation by rigid rotation toward a direction of pucker opposite that corresponding to the energy minimum for the actual orientation of H1 gives an energy difference which is too large.

To reduce the inaccuracy of rigid H1 rotation, two more simulations invoking a smaller δS are carried out referenced to S at the energy maxima. That is, simulations with $S_i = 0.4$ (172,168) and 0.6 (188,192) allow the smaller perturbation value of $\delta S_i = 0.05$ (4,6) near the planar conformation, which is necessary to avoid significant errors in ΔA resulting from rigid dihedral rotations. Joining the two sets of data together yields Figure 4b, a noticeably smoother profile.

Because the small molecule systems of DHP and NMSH are almost harmonic and because energy relaxation is slow, different starting velocities were used to ensure precision in the calculations. The results of using several velocity sets for DHP are shown in Figures 5a and 5b (dashed curves), respectively, for either five or seven S_i values analogous to Figures 4a and 4b. In Figure 5 the solid curve is the average of the dashed values. The dashed curves closely resemble each other, although the discrepancy between profiles is greater in the case of fewer S_i values. Hence, the error in the curves results from too large δS_i and not from initial bias in slowly relaxing systems.

2. Dihydropyridine. The minimum of the free energy profile for DHP (Figure 4b) occurs near the planar conformation. It is a broad minimum; the increase in free energy is less than 1.0 kcal/mol for $S = 0.3$ or 0.7, corresponding to 16° and 24° rotations out-of-plane for N1 and C4, respectively. A free energy minimum near a planar structure is consistent with the crystal structure of *N*-(methoxymethyl)-1,4-dihydropyridine and *N*-propyl-1,4-dihydronicotinamide,[19] while the small energy penalty for distortion of the ring is consistent with the puckering induced by base stacking measured with NMR coupling constants of nicotinamide adenine dinucleotide.[26]

The adiabatic energy profile is also shown in Figure 4, dashed curve. From the similarity of the two curves, the thermal fluctuations at 300 K do not significantly alter the energy, and the entropic component associated with the conformational changes is small. The free energy calculation results in a slight narrowing of the pucker profile.

3. Dihydronicotinamide Nucleoside. The free energy profiles for ring pucker of NMSH in the anti and syn configurations were calculated following the protocol established with DHP. No constraints were placed on the glycosidic dihedral angle during the simulations, so the anti and syn calculations differed only in the initial dihedral angle value. ΔA values from five simulations with different initial velocities at each S_i were averaged to obtain the profiles shown in Figures 6a and 6b for anti and syn configurations, respectively. The dashed curve results from simulations referenced to $S_i = 0.1$, 0.3, 0.5, 0.7, and 0.9, while the solid curve includes additional sampling at $S_i = 0.4$ and 0.6. Structures averaged over the last picosecond of dynamics for simulations at $S_i = 0.3$, 0.5, and 0.7 are shown in the top, middle, and bottom, respectively, of Figure 7 for anti and Figure 8 for syn conformations.

The error due to rigid rotational perturbations is reduced for the mononucleoside compared to that observed for DHP. Profiles calculated for NMSH by using five simulations (dashed curves in Figure 6) are nearly equivalent to those determined with additional simulations at $S = 0.4$ and 0.6 (solid curves in Figure 6). C1′ in NMSH has smaller out-of-plane fluctuations than H1

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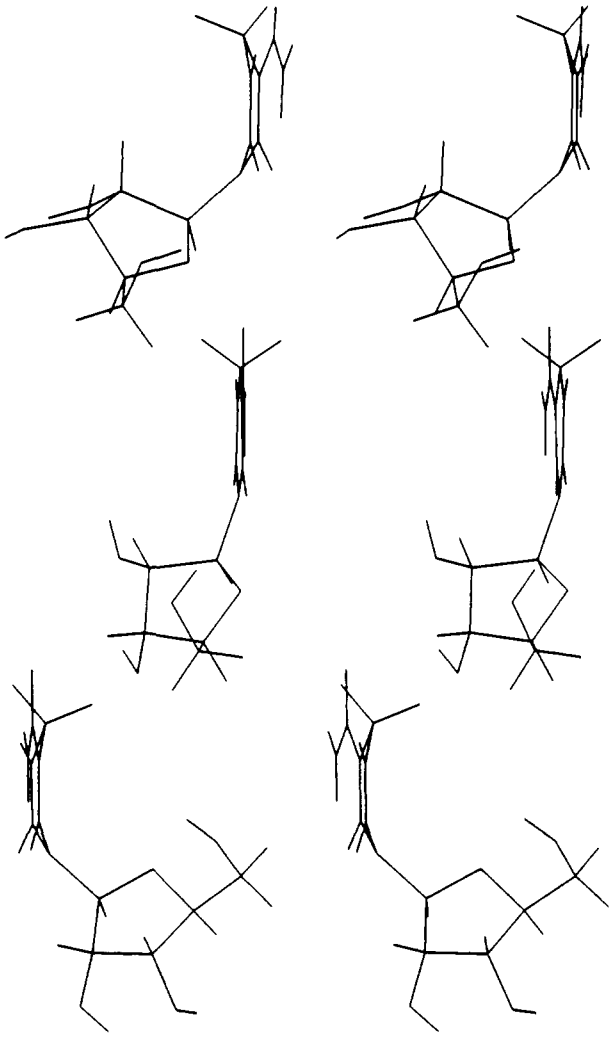

Figure 7. Stereo drawings of NMSH (anti) averaged over the last picosecond of dynamics for S_i = 0.3, 0.5, and 0.7 (top, middle, and bottom, respectively).

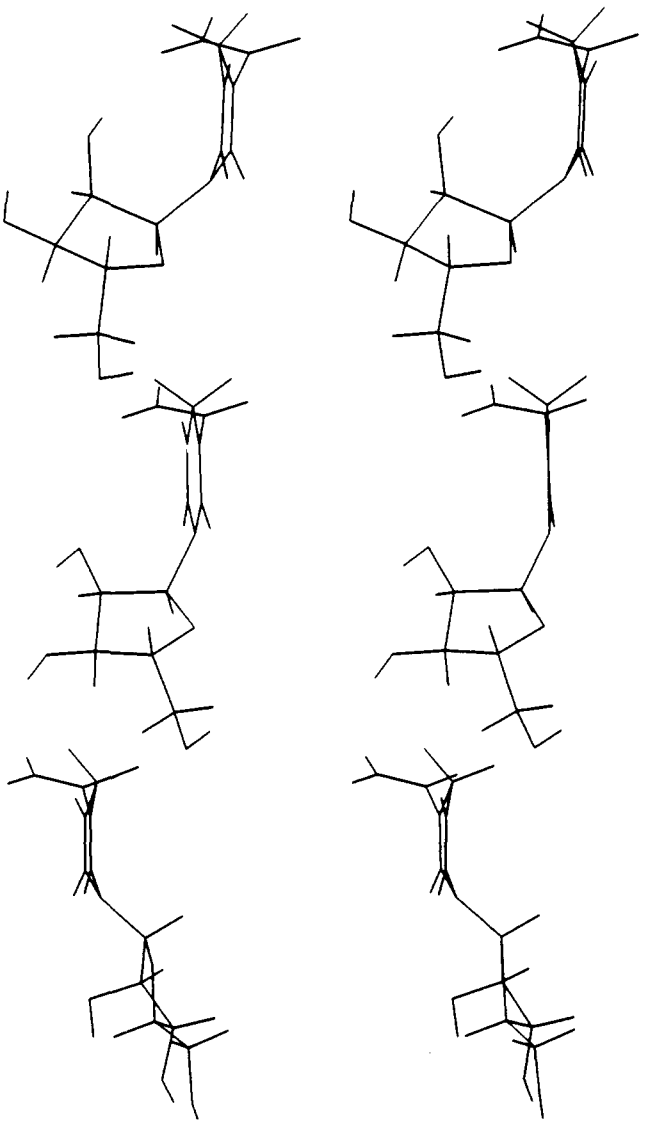

Figure 8. Stereo drawings of NMSH (syn) averaged over the last picosecond of dynamics for S_i = 0.3, 0.5, and 0.7 (top, middle, and bottom, respectively).

in DHP, and the deviation from the minimum energy value as structures are rigidly perturbed along S is reduced. As a result, the irregularities in the profile determined with larger ΔS values are diminished for NMSH (dashed curves in Figure 6) relative to those for DHP (solid curve in Figure 4a). However, it should be noted that in the case of syn NMSH (Figure 6b) the free energy at S_i = 0.7 is overestimated since the error for perturbing forward along S is not compensated by that for perturbing backward along S. The result is that the free energy for S_i = 0.7 relative to S_i = 0.3 is too large, unlike the behavior seen with anti NMSH.

The profiles for NMSH have a broader minimum than that found with DHP; for NMSH the free energy increases less than 1 kcal/mol for $0.23 < S < 0.77$ ($\Delta\phi_{C5\text{-}C6\text{-}C2\text{-}N1} = \pm 33°$, $\Delta\phi_{C4\text{-}C5\text{-}C3\text{-}C6} = \pm 22°$), while for DHP the range is $0.30 < S < 0.70$ ($\Delta\phi_{C5\text{-}C6\text{-}C2\text{-}N1} = \pm 24°$, $\Delta\phi_{C4\text{-}C5\text{-}C3\text{-}C6} = \pm 16°$). In the case of the syn configuration (Figure 6b), the free energy profile shows that the boat conformation around (164,156) is slightly favored. The reason for the minimum in the curve is seen in the top of Figure 8; at S = 0.3 the ribose O2′ is within hydrogen-bonding distance (3.0 Å) of the amide.

4. Pyramidalization of the Ring Nitrogen. The direction of pyramidalization of N1 in the transition state for hydride transfer proposed by Benner and co-workers[5,6] places C1′ pseudoequatorial and the N1 lone pair electrons pseudoaxial. However, using the force field reported here, C1′ is pseudoaxial in the minimum free energy configurations along the ring pucker coordinate (Figures 7 and 8). An examination of the energetics of N1 pyramidalization, described in this section, finds that the preference for the pseudoaxial position of C1′ is due to van der Waals contacts among atoms in the ribose and the dihydronicotinamide rings.

An adiabatic potential profile for N1 pyramidalization of NMSH was calculated for three fixed dihydropyridine ring conformations: planar and puckered in either direction. Energy minimization was carried out as a function of the pyramidalization of N1 by constraining the improper dihedral angle N1–C2–C6–C1′ to values in the range ±40°. (The configuration of the first atom named is specified by an improper dihedral angle, as described by Brooks, et al.[15]) The pucker of the dihydronicotinamide ring was held constant at S = 0.3, 0.5, or 0.7, while all other degrees of freedom were unconstrained. The adiabatic profiles for the puckered dihydronicotinamide ring shown in Figure 9a indicate a slight preference for the pseudoaxial position of C1′. That is, C1′ is pseudoaxial for negative values of the improper dihedral N1–C2–C6–C1′ when S = 0.3 (dashed curves) and for positive values when S = 0.7 (dotted curves). In the planar configuration (S = 0.5), an out-of-plane direction for the N1–C1′ bond is also favored (e.g., Figure 7 or 8, middle), although both sides of the ring are of nearly equal energy. Profiles almost identical to those in Figure 9a, with the pseudoaxial position for C1′ being lower energy, are obtained for either the anti or syn conformations of the ribose ring. A pseudoaxial C1′ position is energetically favored because of van der Waals contacts between atoms one or more bonds away from N1 or C1′. Which pairs of atoms are involved depends on several factors, including the torsion angle of the glycosidic bond (an unconstrained degree of freedom

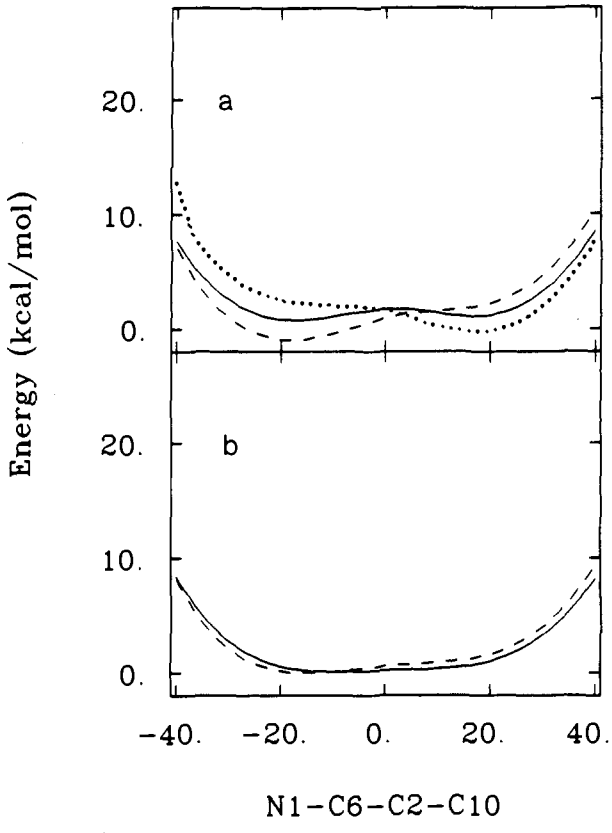

Figure 9. Adiabatic potential energy profile for N1 pyramidalization of (a) NMSH and (b) *N*-(hydroxymethyl)-DHP. The improper dihedral angle N1–C6–C2–C1′ was constrained to the value plotted at the abscissa during energy minimization. The dihydronicotinamide ring was also constrained for each curve to a given pucker state. The ring pucker states are planar (solid), and puckered (dashed S = 0.3; dotted S = 0.7).

in the calculation) and the pucker state of the dihydronicotinamide ring. It is clear that the van der Waals interactions between the two rings can induce the pseudoaxial position of C1′. Nonetheless, in all cases the pseudoequatorial position is attained without a large energetic cost.

To further examine N1 pyramidalization, the adiabatic profile was also calculated for *N*-(hydroxymethyl)-1,4-dihydronicotinamide for which the long-range, nonbonded interactions involving the ribose atoms are not present. The adiabatic profiles for the puckered and planar states of the ring (Figure 9b) show little preference for either direction of pyramidalization, in contrast to those obtained with NMSH. The energies for pseudoaxial and pseudoequatorial positions of C1′ are predicted to be nearly equivalent.

Wu and Houk[7] have examined ring nitrogen pyramidalization using ab initio methods in similar molecules: *N*-(hydroxymethyl)-1,4-dihydropyridine and *N*-(ethyl)-1,4-dihydropyridine. They report both pseudoequatorial and pseudoaxial positions for the carbon bonded to the ring nitrogen depending on the rotation about the N–C bond (see Table IV, Wu and Houk[7]). Their structures have small pucker angles (≤5°) and are therefore most similar to our planar structures, for which the adiabatic profile in Figure 9 (solid) is nearly flat for small degrees of pyramidalization. Although it is not possible to make an exact comparison of pyramidalization between the optimized structures reported by Wu and Houk with those reported here because of variation in other conformational degrees of freedom, we find no significant discrepancies between our model and the ab initio model.

Conclusion

Conformationally constrained molecular dynamics was used to determine the free energy profile for ring puckering in dihydronicotinamide with thermodynamic perturbation methods, demonstrating the usefulness of this technique for ring systems. It should be noted that with this approach a rigid conformational perturbation can lead to a value of ΔA for an interval of the reaction coordinate which is too large due to deviation from the minimum energy path. The propagation of such an error in the free energy profile will depend on the nature of the summation over the reaction coordinate intervals.

Of the many degrees of freedom in the conformational state of NMSH, we have focused primarily on the base ring pucker, but we have examined as well N1 pyramidalization and dihedral rotation about the N1–C1′ bond in the ground state of isolated NMSH. The ring is nearly planar at the free energy minimum, but transitions to boat conformers with 20° and 30° out-of-plane displacements of C4 and N1, respectively, require only small increases in free energy (<1.0 kcal/mol). A somewhat broader free energy profile exists for NMSH than for DHP due to favorable dispersion interactions between the base and ribose. In the configuration of N1 at the free energy minimum, the lone pair electrons are pseudoequatorial. However, the energy barrier for N1 inversion and the energy difference of the two configurations are small (<3 kcal).

The conformation of the ground state as determined here differs from the conformation indicated for the transition state of hydride transfer; ab initio studies find a preference for the axial position of the transferring hydride,[27] and the syn stereochemistry of 1,4-elimination reactions suggests an axial lone pair for N1. A possible mechanism by which an enzyme utilizing NADH might gain a kinetic advantage is by stabilizing the reactive form of dihydronicotinamide relative to the distribution of conformations accessible to the isolated cofactor at room temperature. How the interactions with the active site of an enzyme influence the conformational equilibrium of NADH and the consequences of that influence on hydride transfer by dehydrogenases is being investigated.

Acknowledgment. The authors thank Professor Charles L. Brooks III and Dr. Douglas J. Tobias for the internal coordinate constraint and perturbation subroutines, as well as helpful discussions, Professor Martin Karplus, who made available the CHARMM22 parameters before publication, and Dan Severance, who provided software for charge refinement. This work was supported by a grant from the Lucille P. Markey Charitable Trust for the development of structural studies at Purdue University.

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