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Theoretical study of stereoselective reduction controlled by NADH analogs

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Abstract

The potential energy surfaces (PES) of 2-methyl-4-(*R*)-methyl-1,4-dihydropyridine-3-carboxamide (4*R*-DM, 1), 2-methyl-4-(*S*)-methyl-1,4-dihydropyridine-3-carboxamide (4*S*-DM, 2) and 2-methyl-1,4-dihydropyridine-3-carboxamide (MM, 3) have been explored with ab initio calculations at the RHF/6-311G** and MP2/6-311G** levels of theory. In agreement with previous experimental and computational results, the PES provides three minima for each of the above molecules. The calculations reported herein indicate that the *cisoid* conformation is most favorable in gas phase and hydrophobic environments. Nevertheless, the preference of the *cis* conformation can be controlled by different solvents. The most favorable conformation in methanol, water, and probably in the polar (or water medicated) enzyme active sites, however, would be the one in which the carbonyl group is in a *transoid* position and is *syn* to H*syn*. In addition, our calculations suggest that the carbonyl group in the *syn*, rather than *anti*, position relative to H*syn* is preferred. These observations are in very good agreement with previous computational and experimental results. Our computational studies have provided an explanation as to why the *transoid* conformation is preferred in enzyme active sites as well as in many other NADH mimics. Furthermore, these new data imply that the stereoselectivity of NADH analogs can be controlled by means of changing solvents in which the reaction is carried out.

Keywords: Stereoselectivity; NADH; Solvent effect; NADH mimics; Conformational search

1. Introduction

Nicotinamide adenine dinucleotide (NAD+), its phosphate derivative (NADP+) and their reduced forms NADH and NADPH (Fig. 1) are major cofactors in a large number of oxido-reductases. In many dehydrogenase and reductase reactions, one hydrogen of the prochiral C_4 methylene group in the dihydropyridine ring of NAD(P)H is transferred to a trigonal center of a carbonyl group, giving rise to a new chiral tetragonal center stereospecifically. Since the seminal stereoselective reduction by NADH mimics introduced by Ohno's group in 1975 [1], a variety of biomimetic NADH models have been synthesized. The goal has been the achievement of specific stereoselectivity, by imposing different chiral auxiliaries on the side chain of NADH [2,3]. Many of these synthesized NADH mimics prefer H*syn* (the reacting C_4–H and C=O bonds in the *syn*-configuration, Fig. 2) for the hydride transfer in the presence of magnesium or zinc ions.

Donkersloot and Buck [4] have reported the conformation of H*syn* corresponds to the reaction of low enthalpy of activation, while the configuration with C_4–H and C=O bonds in the *anti*-configuration (the dipoles of C_4–H and C=O bonds away from each other) is observed to have higher enthalpy of activation. Wu and Houk [5] have reported a theoretical study of NADH that shows the amide group favors a *cisoid* conformation (B in Fig. 3, i.e., the C=O dipole is pointed towards the N_1 atom, where the, C=O bond and C_2=C_3 bond are synperiplanar) by about 1.00 kcal/mol than the *transoid* conformation (A in Fig. 3, where the C=O bond and C_2=C_3 bond are antiperiplanar). Yet it fails to answer why the amide group adopts the *transoid* conformation in most of the enzyme active sites even though the *cisoid*

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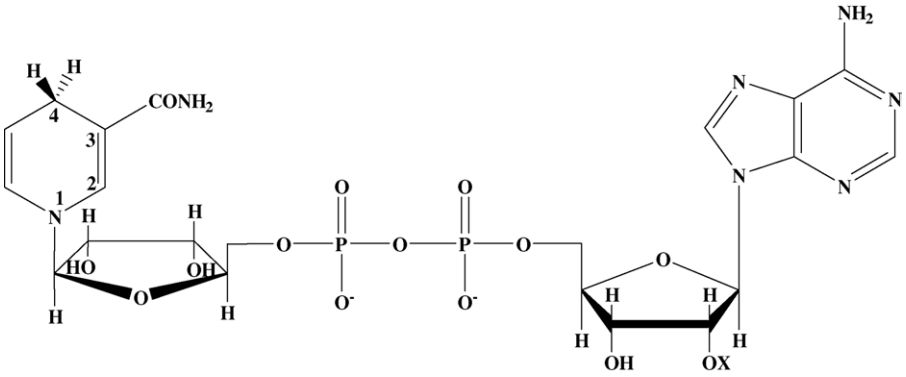
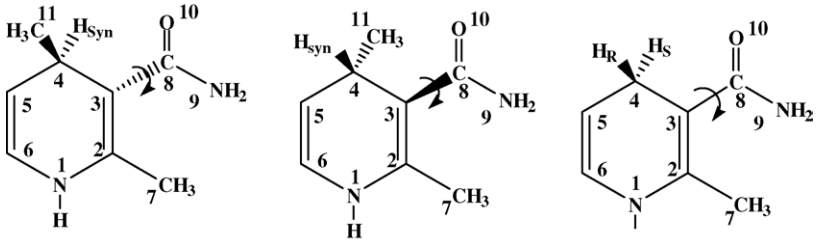


Fig. 1. The structures of NADH (X = –H) and NADPH (X = $–OPO_2H_2$).

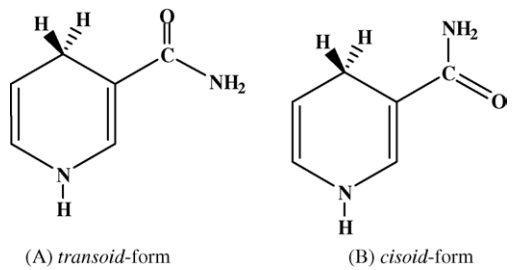


(A) 4R-DM, **1** (B) 4S-DM, **2** (C) MM, **3**

Fig. 2. The structures of the model molecules. Torsional scan for PES was performed on dihedral angle C_4–C_3–C_8–N_9: (A) 4*R*-DM, 1 (B) 4*S*-DM, 2 and (C) MM, 3.

conformation is more favorable in gas phase. The *transoid* conformation of the carboxamide group is observed in the active sites of many alcohol dehydrogenases, such as the *Drosophila* alcohol dehydrogenase (DADH) [6] and the *pseudomonas fluorescens* mannitol 2-dehydrogenase (MDH), a secondary alcohol dehydrogenase [7]. Presumably, the active site interior stabilizes the *transoid* conformation.

It has been suggested that the stereospecificity of the coenzyme in the active site originates from the out-of-plane distortion of the *transoid* amide group and the plane is defined by the two double bonds in the nicotinamide ring (Fig. 3). Employing AM1 semiempirical calculations, Almarsson and Bruice [8] show that the global energy minimum of NADH corresponds to a conformation in which the amide group and the nicotinamide ring are coplanar and in the *cisoid* orientation, while the *transoid* conformation is about 2.3 kcal/mol higher in energy. The barrier of rotation of the amide functional group is approximately 3.0 kcal/mol. Again it is still unclear why the *transoid* conformation is the most popular conformation in most enzyme active sites. Some speculations might attribute the *transoid* preference to the crystal packing forces. Using a synthesized NADH mimic, Vasse et al. [9] have shown that the hydride transfer occurs with H*syn* and that the dihedral angle C_4–C_3–C=O in the *transoid* conformation is about 10–15°.



(A) *transoid*-form (B) *cisoid*-form

Fig. 3. The *transoid*- and *cisoid*-conformations of the carboxamide group: (A) *transoid*-form and (B) *cisoid*-form.

In order to provide insight into why the *transoid* conformation is preferred in the enzyme active site and to better understand the stereoselective reduction of the NADH mimics, ab initio RHF/6-311G** and MP2/6-311G** calculations were performed on NADH analogs: 2-methyl-4-(*R*)-methyl-1,4-dihydropyridine-3-carboxamide (4*R*-DM, 1), 2-methyl-4-(*S*)-methyl-1,4-dihydropyridine-3-carboxamide (4*S*-DM, 2) and 2-methyl-1,4-dihydropyridine-3-carboxamide (MM, 3) (Fig. 2). A number of NADH mimics contain an alkyl group at the C_2 position. Therefore, a methyl group was introduced in the present studies to mimic the steric effect of the C_2 alkyl groups. A methyl group was also introduced at the C_4 position in order to investigate its possible steric effect on the stereoselective reduction in enzyme active sites and other NADH mimics.

2. Methods

All ab initio calculations were carried out using the Gaussian03 packages at the RHF/6-311G** and MP2/6-311G** levels of theory [10]. In all cases, the default convergence criteria were used. By default, the ''frozen-core'' (FC) option is used for the MP2 calculation mode. Complete exploration of the conformational space was carried out for all three NADH analogs based on the dihedral angle C_4–C_3–C_8–N_9 (Fig. 2) for a full 360.0° sweep in 10.0° increments. All reported minima along the potential energy surface were subject to full geometry optimizations without any constraints and were further confirmed through the analytical frequency calculations, which were calculated at RHF/6-311G** levels of theory. Transition structures were considered to be the highest point along the potential energy surfaces (PES) and were further confirmed with frequency calculations, which gave only one imaginary frequency. All stationary points reported were visualized using XChemEdit [11].

Solvent effects were calculated for the transition state structures and the optimized minima by single-point energy calculations using self-consistent reaction field (SCRF) theory with the isodensity surface polarized continuum model (IPCM) method in Gaussian03 at the RHF/6-311G** [12]. The SCRF-IPCM calculations were carried out at 298.15 K (the temperature of the applied observed dielectric constants) in chloroform, methanol and water, with solvent dielectric constants of 4.90, 32.63 and 78.39, respectively. The combination of 44 phi points and 22 theta points was used for current systems during the SCRF-IPCM calculations.

3. Results and discussion

Systematic searching of the conformational space for three NADH model structures yielded nine minima, three for each investigated analog (Figs. 4 and 5). The energies and characteristic torsion angles for all minima, optimized without any constraints, are given in Table 1. The differences in energies for the nine minima are all less than 3.00 kcal/mol. In each case, the *cisoid* conformation has the lowest gas phase energy. The degree of distortion of the carboxamide group relative to the nicotinamide plane, as defined by two double bonds, is expressed by the dihedral angle C_2–C_3–C_8=O_{10}. The out-of-plane distortions for all three *cisoid* minima are quite small, with absolute values ranging from 11.0° to 20.0°, while the out-of-plane orientations for all *transoid* minima have larger torsional angles, with absolute values ranging from 30.0° to 40.0° (Fig. 6). Fig. 6 demonstrates that the carboxamide group of the *cisoid* conformation is less distorted relative to the nicotinamide ring. The better localized conjugation between the carbonyl group and the double bond in the nicotinamide ring might help stabilize the *cisoid* conformation, the lowest energy one in gas phase. The amide group in the *transoid* conformation, however, is rotated substantially out-of-plane. The larger out-of-plane strains contribute to higher gas phase energy for the *transoid* conformations. These observations are in very good agreement with previous studies [4,5,8].

There is appreciable pyramidalization at N_1 and at amide nitrogen in all nine minima (Fig. 6), largely due to the steric effect of the C_2 and C_4 methyl groups. This phenomenon has been observed in previous computational [5] and experimental studies [13]. Similar to Wu and Houk's observations [5], the nicotinamide rings of all minima are slightly puckered into a boat conformation. It is noteworthy to point out that there is a C_2 symmetry between 4*S*-DM and 4*R*-DM, as it is obviously seen in Figs. 4 and 6, where the PES is symmetric between 4*S*-DM and 4*R*-DM and the minima are symmetric to each other as well.

The introduction of a methyl group in the C_4 position mimics the steric effect in the enzyme active site. For example, the *pseudomonas fluorescens* MDH contains residue Phe-37, which is stacked against the A side of the nicotinamide and therefore making only the B side accessible to the substrate, and generating the 4-*pro-S* hydride [7]. You listed 156 A-specific (*pro-R* hydride transfer) and 121 B-specific (*pro-S* hydride transfer) dehydrogenases [14] and found that both the A- and B-specific dehydrogenases carry out the H*syn* hydride transfer. In agreement with the above observations, our calculations show that both 4*S*-DM and 4*R*-DM prefer the carbonyl group

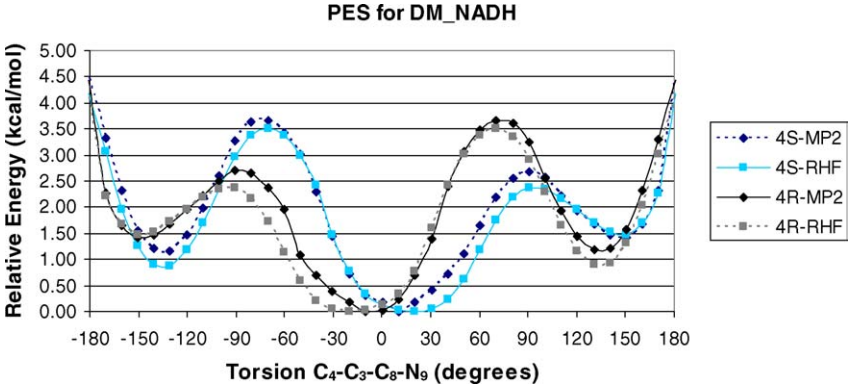


Fig. 4. Potential energy profiles of 4*S*-DM and 4*R*-DM at RHF/6-311G** and MP2/6-311G** levels of theory.

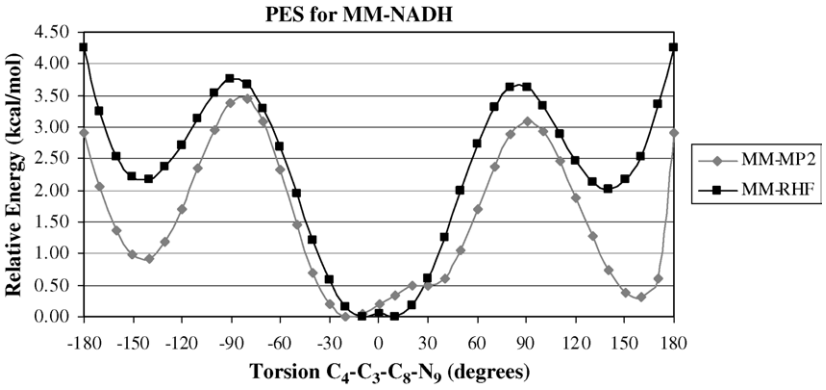


Fig. 5. Potential energy profiles of MM at RHF/6-311G** and MP2/6-311G** levels of theory.

in the *syn*, rather than *anti* position to H*syn*. It is also interesting to note that the relative energies of all three analogs are dependent upon the different theoretical model chemistries, although the trends are quite similar. For example, the lowest energy conformation for 4*R*-DM is at −20.0° for RHF/6-311G**, while the corresponding torsion angle for MP2/6-311G** is −10.0°.

The low energy *cisoid* conformations (Table 1) over their respective *transoid* counterparts suggests that the former would be most populated in the gas phase. The preference of the *cisoid* over the *transoid* conformation is also confirmed by the Gibbs free energy (Table 2), derived from the frequency calculations. Table 2 shows that, in the gas phase, after addition of the entropy term, the free energy difference between *cisoid* and *transoid* is magnified. The information suggest that the *cisoid* conformation is preferred in gas phase. All *cisoid* to *transoid* transitions involve a transition structure, whose carbonyl group is almost perpendicular to the nicotinamide ring. The rotational energies for the *cisoid* to the *trans*oid transition for 4*S*-DM, 4*R*-DM and MM are about 3.66, 3.66 and 3.09 kcal/mol, respectively (Table 1). The perpendicular arrangement of the carbonyl groups in TS 1, 2, 4, 5 and 7 (Fig. 7) results in the disruption of the localized conjugation between the carbonyl group and the vinyl group in the nicotinamide ring. This leads to the higher energy of the transition states.

In addition, the rotation of the carboxamide bond from the *cisoid* to the *transoid* conformation gave rise to two different conformations, dependent upon the *syn* or *anti* orientation of the carboxamide carbonyl group relative to the C_4–H bond. Fig. 6 shows the preference of 4*S*-n130 over 4*S*-150. The same is true for the preference of 4*R*-130 over 4*R*-n150, where the energetically preferred conformation has a *syn* orientation between carbonyl and C_4–H. Note, Table 1 lists torsion C_4–C_3–C–N from the scan whereas Fig. 6 lists C_2–C_3–C_8=O_{10} to define the out-of-plane torsion. Therefore, hydride transfer could occur for 4*S*-n130 and 4*R*-130 if the rotational barrier from the *cisoid* to *transoid* can be overcome. The

Table 1
The energetic data and characteristics of the stationary points for NADH analogs

Conformer	Name	C=O	C_4–C_3–C–N (degree)	Relative energy (Δ*E*, kcal/mol, MP2)	Relative energy (Δ*E*, kcal/mol, RHF)			
					Gas	$CHCl_3$	MeOH	H_2O
1	4*S*-n130	*transoid*	−134.1	1.04	0.86	0.66	−0.11	−0.13
2	4*S*-10	*cisoid*	17.8	0.00	0.00	0.00	0.00	0.00
3	4*S*-150	*transoid*	147.9	1.37	1.46	1.15	0.99	0.97
4	4*R*-n150	*transoid*	−147.9	1.36	1.46	1.15	0.99	0.97
5	4*R*-n20	*cisoid*	−17.8	0.00	0.00	0.00	0.00	0.00
6	4*R*-130	*transoid*	134.1	1.04	0.86	0.13	−0.11	−0.13
7	MM-n140	*transoid*	139.6	0.91	2.16	1.47	1.16	1.12
8	MM-10	*cisoid*	7.8	0.00	0.00	0.00	0.00	0.00
9	MM-140	*transoid*	−143.4	0.30	2.01	1.45	1.27	1.25
TS1	4*S*-90	–	93.8	2.68	2.38	1.63	1.36	1.32
TS2	4*S*-n70	–	−69.5	3.66	3.50	2.63	2.35	2.32
TS3	4*S*-n180	–	−179.5	4.47	4.16	3.56	3.22	3.18
TS4	4*R*-70	–	70.0	3.66	3.50	2.62	2.33	2.30
TS5	4*R*-n90	–	−89.8	2.71	2.36	1.57	1.29	1.25
TS6	4*R*-180	–	−179.8	4.43	4.13	3.49	3.20	3.16
TS7	MM-90	–	85.0	3.09	3.66	2.71	2.46	2.43
TS8	MM-n170	–	−169.7	2.90	5.44	4.37	3.93	3.88

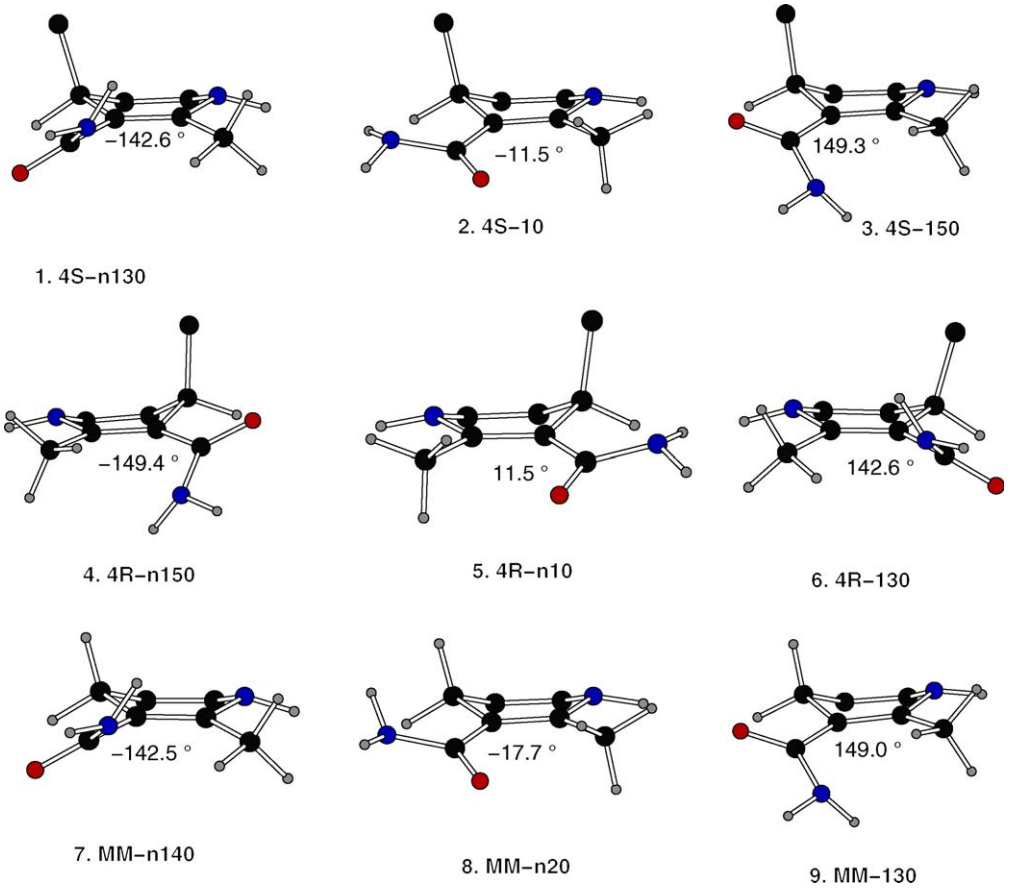


Fig. 6. The side views of structures of minima of 4*S*-DM, 4*R*-DM and MM minima. The degree of distortion of the carboxamide group relative to the nicotinamide plane is expressed by the dihedral angle C_2–C_3–C_8═O_{10}.

energy barrier for rotation from the *cisoid* conformation to 4*S*-n130 and 4*R*-130, however, is 3.66 kcal/mol. This would discourage the transition at room temperature. The transition barrier from *cisoid* to 4*S*-150 and 4*R*-n150, in contrast, is less than 3.00 kcal/mol. Hence, one would propose that the transition in the gas phase from *cisoid* to *transoid* would give rise to 4*S*-150 and 4*R*-n130. This would lead to the *anti* orientation as the one to perform the hydride transfer. This would be contradictory to many experiments, which have demonstrated that most of the hydride transfer conducted by NADH and its mimics occur at H*syn*. Nonetheless, the *syn* orientation of the carbonyl group is the preferred orientation in enzyme active sites and is the most frequently observed in most stereoselective reduction performed by various synthesized NADH mimics. What factors bring about the energy to overcome the energy barriers between *cisoid* conformation and 4*S*-n130, *cisoid* conformation and 4*R*-130? Is there any solvent effect during such a transition?

To provide an answer to this important question, we carried out the SCRF-IPCM calculations on all minima and

Table 2
The torsional angles, relative zero-point energies, enthalpies and free energies of minima for NADH analogs

Conformer	Name	C═O	C_4–C_3–C–N (degree)	Relative energy (ΔE, kcal/mol, MP2)	Relative energy[a] (kcal/mol, RHF)		
					ΔE (ZPE)	ΔH (Enthalpy)	ΔG (Free energy)
1	4*S*-n130	*transoid*	−134.1	1.04	0.86	0.82	1.11
2	4*S*-10	*cisoid*	17.8	0.00	0.00	0.00	0.00
3	4*S*-150	*transoid*	147.9	1.37	1.47	1.37	1.87
4	4*R*-n150	*transoid*	−147.9	1.36	1.47	1.37	1.88
5	4*R*-n20	*cisoid*	−17.8	0.00	0.00	0.00	0.00
6	4*R*-130	*transoid*	134.1	1.04	0.86	0.82	1.11
7	MM-n140	*transoid*	139.6	0.91	2.03	1.98	2.14
8	MM-10	*cisoid*	7.8	0.00	0.00	0.00	0.00
9	MM-140	*transoid*	−143.4	0.30	1.95	1.88	2.09

[a] Calculated with vibrational frequencies scaled by 0.890 at the RHF/6-31G** level at $T = 298.15$ K.

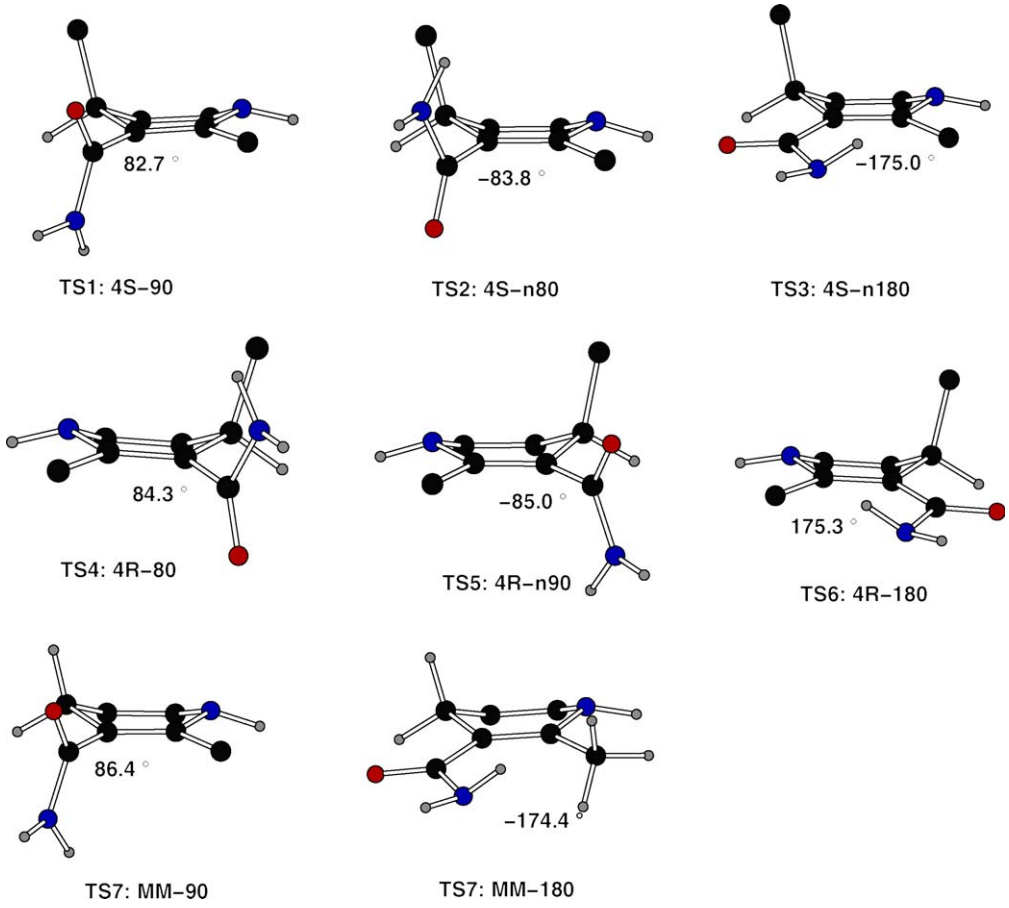


Fig. 7. The side views of structures of transition structures of 4*S*-DM, 4*R*-DM and MM. The degree of distortion of the carboxamide group relative to the nicotinamide plane is expressed by the dihedral angle C_2–C_3–C_8═O_{10}.

transition state structures at 298.15 K in three different solvent systems: chloroform, methanol and water, with solvent dielectric constants of 4.90, 32.63 and 78.39, respectively. The reason for our selection of the SCRF-IPCM method instead of the self-consistent isodensity polarizable continuum model (SCI-PCM) rests upon the report from Jhon and Kang [15] showed that results from the IPCM method are consistent with the experimental results on *N*,*N*-dimethylacetamide (DMA). Our calculations on butanone and butanimine also show that SCRF-IPCM can produce experimental date very well [16]. The solvent effect on different transition structures and the optimized minima

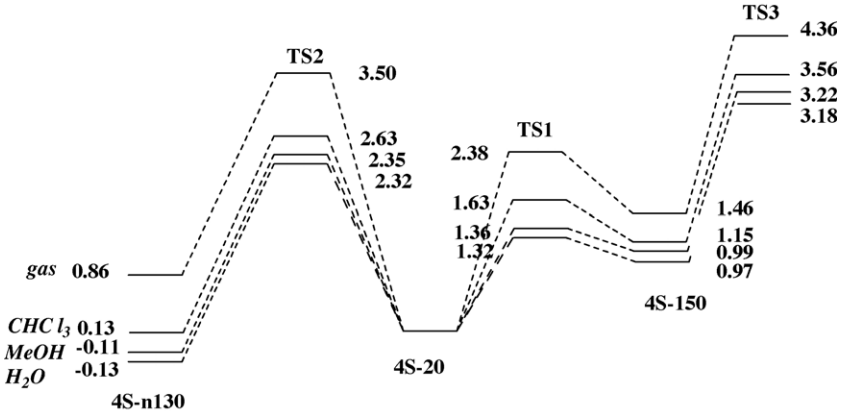


Fig. 8. The reaction pathway of 4*S*-DM NADH analog.

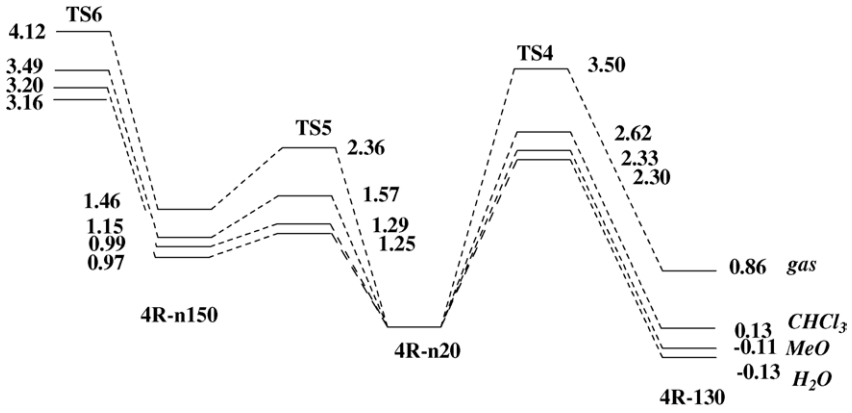


Fig. 9. The reaction pathway of 4*R*-DM NADH analog.

was calculated using the IPCM method, and the data are listed in Table 1. The energy difference between the *cisoid* and the *transoid* conformations decreases as the increase in solvent polarity in all three catalogs. This indicates that the population of the *transoid* conformation increases in more polar solvents, e.g. methanol and/or water. Interestingly, conformers 4*S*-n130 and 4*R*-130 become even more stable than the *cisoid* conformations in both methanol and water. Conformers 4*S*-n130 and 4*R*-130 correspond to the conformation in which the carboxamide group is in the *transoid* position and in the *syn* position to H*syn* (Fig. 2). This indicates that in polar environment, i.e., methanol, water or polar active sites, the hydride transfer would occur with the H*syn* atom.

The reaction pathways of 4*S*-DM and 4*R*-DM NADH analogs are illustrated in Figs. 8 and 9. As shown in both figures, the transition state structures are also stabilized as solvent polarity increases. The activation energy for the transition of 4*R*-n20 (for RHF and 4*R*-n10 for MP2) to 4*R*-130 decreases from 3.50 to 2.30 kcal/mol and the energy barrier for transforming 4*R*-n20 to 4*R*-n150 decreases from 2.36 to 1.25 kcal/mol when the medium changes from gas phase to water. These decreases in activation energies indicate that starting from the *cisoid* 4*R*-n20, the transition to 4*R*-n150 or to 4*R*-130 would both be likely to occur. However, the ground state of 4*R*-130 has much lower energy than 4*R*-n150. Given the low energy barrier (2.3 kcal/mol), it would be obvious that the conformation 4*R*-130 would be the most populated conformation. This is especially true in the enzyme active sites and other NADH mimic reaction sites where a magnesium or zinc ion is present. The lower energy of the carbonyl group arranged in the H*syn* position in combination with the coordination of a magnesium ion through carbonyl groups favors the *syn* orientation between the carbonyl group and H*syn* [2]. The activation energy for 4*R*-n150 rotating back to 4*R*-n20 is very small, only ca. 0.30 kcal/mol in either methanol or water and the activation energy for 4*R*-n150 to 4*R*-130 is 2.2 to 2.3 kcal/mol.

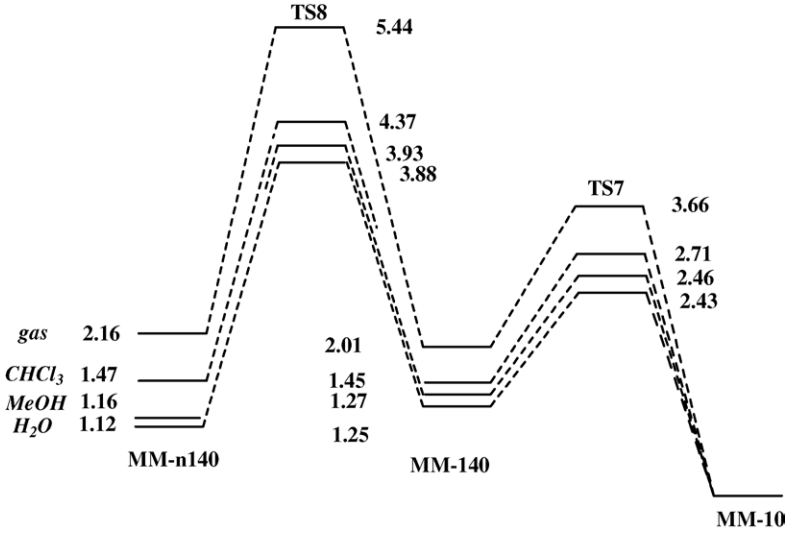


Fig. 10. The reaction pathway of MM NADH analog.

The activation energy for the transformation of 4*R*-130 back to 4*R*-n150 is 3.3 kcal/mol. All these forces combined drive the equilibrium toward 4*R*-130. Therefore, 4*R*-130 is the most stable conformation in a polar environment or in enzyme active sites. The same is true for 4*S*-DM, where the C_4 position is in the *S* configuration. Our results provide a very convincing explanation as to why the *transoid* conformation is the most heavily populated conformation in the active sites and in many NADH analogs. Our data also provide a basis for understanding why the H*syn* is the preferred hydrogen for hydride transfer.

The activation energy for the *transoid*/*cisoid* rotation (for 4*R*-130 and 4*S*-n130) is approximately 2.40–2.50 kcal/mol in methanol and water. This low activation energy indicates that given appropriate conditions, some NADH analogs will prefer the *cisoid* conformation, especially for those NADH mimics that have a more hydrophobic substituent at the N_1 position. The SCRF-IPCM calculations in chloroform show the preference of the *cisoid* conformation 4*R*-n20 and 4*S*-10 in less polar solvents (Table 1). These observations are in good agreement with known X-ray structures, such as *N*-(methoxymethyl)-1,4-dihydropyridine-3-carboxamide [13].

The activation barriers for the MM NADH model in Fig. 9 show the inequivalence of the C_4 hydrogen atoms. This was also observed by Tropp and Redfield in their NMR studies [17]. For the C_4 unsubstituted model, an increase in the solvent polarity similarly stabilizes the minima of both MM-140 and MM-n140 as well as the transition states TS7 and TS8. The magnitude of the decrease in activation energy between the *cis* MM-10 and MM-140 and between MM-140 and MM-n140 indicate that during the reduction process, the reaction may tilt the reaction pathway to a particular conformation and therefore induce the particular stereoselectivity, especially in enzyme active site and other Zinc ion coordinated NADH mimics (Fig. 10).

4. Conclusion

Our calculations have suggested that the *cisoid* conformation of the NADH analogs is more favorable in gas phase and hydrophobic environments. The *cisoid* preference, however, can be controlled by different solvents. Our calculations suggest that in methanol, water and probably in the polar (or crystal water medicated) active sites, the *transoid* conformation would be preferred. In addition, our data suggest that it is energetically favorable for the carbonyl group to be oriented in the *syn* position to H*syn*, rather than the *anti* position. These observations are in very good agreement with previous computational and experimental results. Furthermore, our results have provided an explanation to why the *transoid* conformation is popular in enzyme active sites and in many other NADH mimics. The present studies also suggest that the stereoselectivity of NADH analogs can be controlled by means of changing different solvents in which the reaction is carried out.

References

[1] Y. Ohnishi, M. Kagami, A. Ohno, Reduction by a model of NAD(P)H. Effect of metal ion and stereochemistry on the reduction of alpha-keto esters by 1,4-dihydronicotinamide derivatives, J. Am. Chem. Soc. 97 (1975) 4766–4768.

[2] J.L. Vasse, V. Levacher, J. Bourguignon, G. Dupas, Chiral biomimetic NADH models in the benzo[b]-1,6-naphthyridine series. A novel class of stable, reactive and highly enantioselective NADH mimics, Tetrahedron 59 (2003) 4911–4921.

[3] S. Obika, T. Nishiyama, S. Tatematsu, K. Miyashita, T. Imanishi, Studies on novel and chiral 1,4-dihydropyridines. IV. Mechanistic aspects of the asymmetric reduction with chiral NADH model compounds, (S_S)-3-(*p*-tolylsulfinyl)-1,4-dihydropyridines, Tetrahedron 53 (1997) 3073–3082.

[4] M.C.A. Donkersloot, H.M. Buck, The hydride-donation reaction of reduced nicotinamide adenine dinucleotide. 2. MINDO/3 and STO-3G calculations on the role of the carbamoyl group in enzymic reactions, J. Am. Chem. Soc. 103 (1981) 6554–6558.

[5] Y.D. Wu, K.N. Houk, Theoretical study of conformational features of NAD+ and NADH analogs: protonated nicotinamide and 1,4-dihydronicotinamide, J. Org. Chem. 58 (1993) 2043–2045.

[6] J. Benach, S. Atrian, R. Gonzàlez-Duarte, R. Ladenstein, The catalytic reaction and inhibition mechanism of *Drosophila* alcohol dehydrogenase: observation of an enzyme-bound NAD-ketone adduct at 1.4 Å resolution by X-ray crystallography, J. Mol. Biol. 289 (1999) 335–355.

[7] K.L. Kavanagh, M. Klimacek, B. Nidetzky, D.K. Wilson, Crystal structure of *pseudomonas fluorescens* mannitol 2-dehydrogenase binary and ternary complexes. Specificity and catalytic mechanism, J. Biol. Chem. 277 (2002) 43433–43442.

[8] Ö. Almarsson, T.C. Bruice, Evaluation of the factors influencing reactivity and stereospecificity in NAD(P)H dependent dehydrogenase enzymes, J. Am. Chem. Soc. 115 (1993) 2125–2138.

[9] J.L. Vasse, V. Levacher, J. Bourguignon, G. Dupas, Tetrahedron: Asymmetry 13 (2002) 227–232.

[10] Gaussian 03, Revision C.02, M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, J.A. Montgomery Jr., T. Vreven, K.N. Kudin, J.C. Burant, J.M. Millam, S.S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G.A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J.E. Knox, H.P. Hratchian, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, P.Y. Ayala, K. Morokuma, G.A. Voth, P. Salvador, J.J. Dannenberg, V.G. Zakrzewski, S. Dapprich, A.D. Daniels, M.C. Strain, O. Farkas, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J.V. Ortiz, Q. Cui, A.G. Baboul, S. Clifford, J. Cioslowski, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, C. Gonzalez, J.A. Pople, Gaussian Inc., Wallingford CT, 2004.

[11] D.C. Lim, W.L. Jorgensen, in: P.V.R. Schleyer (Ed.), The Encyclopedia of Computational Chemistry, vol. 5, John Wiley & Sons Ltd., Athens/USA, 1998, p. 3295.

[12] J.B. Foresman, T.A. Keith, K.B. Wiberg, J. Snoonian, M.J. Frisch, Solvent effects. 5. Influence of cavity shape, truncation of electrostatics, and electron correlation on ab initio reaction field calculations, J. Phys. Chem. 100 (1996) 16098–16104.

[13] A. Glasfeld, P. Zbinden, M. Dobler, S.A. Benner, J.D. Dunitz, Crystal structures of two simple *N*-substituted dihydronicotinamides: possible

implications for stereoelectronic arguments in enzymology, J. Am. Chem. Soc. 110 (1988) 5152–5157.

[14] K.S. You, Stereospecificity for nicotinamide nucleotides in enzymatic and chemical hydride transfer reactions, CRC Crit. Rev. Biochem. 17 (1985) 313–451.

[15] J.S. Jhon, Y.K. Kang, Imide *cis-trans* isomerization of *N*-acetyl-*N*′-methylprolineamide and solvent effects, J. Phys. Chem. A 103 (1999) 5436–5439.

[16] H. Zhong, E.L. Stewart, M. Kontoyianni, J.P. Bowen, Ab initio and DFT conformational studies of propanal, 2-butanone, and analogous imines and enamines, J. Chem. Theory Comput. 1 (2005) 230–238.

[17] J. Tropp, A.G. Redfield, Proton magnetic resonance of NADH in water–methanol mixtures. Conformational change and behavior of exchangeable proton resonances as a function of temperature, J. Am. Chem. Soc. 102 (1980) 534–538.