Tetrahedron Vol. 44, No. 24, pp 7601 to 7610, 1988
Printed in Great Britain

0040-4020/88 $3.00 + .00

THEORETICAL STUDIES ON THE HYDRIDE TRANSFER BETWEEN 1-METHYL-1,4-DIHYDRONICOTINAMIDE AND ITS CORRESPONDING PYRIDINIUM SALT

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(*Received in USA 27 April* 1988)

Abstract: MNDO and AM1 studies were performed to investigate the degenerate hydride transfer reaction between 1-methyl-1,4-dihydronicotinamide and the 1-methylnicotinamide cation, a model system for a novel brain-targeted delivery system and for the $NAD^+ \rightleftharpoons NADH$ interconversion. Four initial approach vectors were selected. These involved an endo orientation in which the carbamoyl groups are syn with respect to one another (cis H_S-re), an endo orientation with the carbamoyl groups anti (cis H_S-si), an exo configuration with the carbamoyl groups syn (trans H_S-re) and an exo configuration with the carbamoyl groups anti (trans H_S-re). The cis H_S-re approach generated the transition state with the lowest energy. The optimized structure indicated that a linear hydride transfer occurred. A more detailed study examined the cis H_S-re approach from a 100Å separation to the transition state. The data indicated the formation of an intermediate induced dipole-charge complex which altered the trajectory of the two species. Closer approach yielded the transition state. The energy of activation for this reaction was calculated to be 30.7 kcal/mol using the MNDO approximation and 9.3 kcal/mol using the AM1 method. Finally, while the linear transition state was found to be the most stable conformation, bending of the C-H-C bond by ± 30° only modestly increased (3-4 kcal/mol) the energy of the system.

Introduction:

The oxidation of dihydropyridines have been extensively studied since this reaction is the crucial step in the operation of biologically essential coenzymes such as NADH and NADPH[1,2]. In the in vivo circumstance, a hydride is transferred from the 1-substituted-1,4-dihydronicotinamide moiety of NADH to an appropriate substrate or to the oxidized form of the coenzyme, $NAD(P)^+$. The enzymatically mediated reactions are exceedingly stereospecific i.e., enzymes transfer either the H_S or the H_R prochiral hydrogens from NADH or transfer a hydride to only the re or si face of the nicotinamide cation[3]. The uncatalyzed reactions between 1-substituted-1,4-dihydronicotinamides and various hydride receptors are useful models for the enzyme system although these reactions are not stereospecific.

In addition to examining these model reactions to gain insight into endogenous biological mechanisms, they are also useful in studying brain-targeted chemical delivery systems (CDS)[4,5]. These CDS are derivatives of

known drugs and endogenous hormones or neurotransmitters which act to selectively increase brain levels of the active principle while at the same time sparing the periphery of excess drug, thus lowering dose related toxicities. In this approach, for example, hydroxy or amino containing drugs are synthetically converted to the 1-methyl-1,4-dihydronicotinates or 1-methyl-1,4-dihydronicotinamides. Systemic administration of these derivatives lead to extensive tissue distribution due to the increased lipophilicity of the conjugate. The critical step in this scheme is the oxidation of the dihydropyridine group to a pyridinium salt. This polar conjugate is readily lost from the periphery but is trapped behind the lipoidal blood-brain barrier resulting in selective retention of the drug-oxidized carrier conjugate. With time, this ester or amide can be hydrolyzed to give the parent drug which can subsequently exert central pharmacologic action. This system provides for the elimination of the drug rapidly from the systemic circulation and for the deposition of a drug precursor in the brain, where the active agent is slowly released. This process is summarized in figure 1. This CDS has been shown to be nontoxic[6] and applicable to numerous drugs including sex steroids such as estradiol[7-11], ethinyl estradiol[12], testosterone[13,14] and norethindrone[15], neurotransmitters such as dopamine [16-18], tryptamine[19] and γ-aminobutyric acid[20], anticancer agents such as chloroethylnitrosoureas[21], antiviral agents such as acyclovir[22], trifluorothymidine[23,24] and azidothymidine[25] and others.

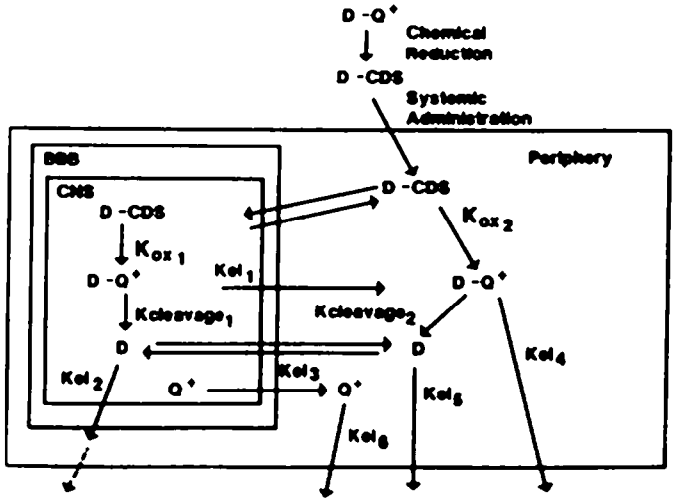

Figure 1. A schematic representation of the chemical delivery system (CDS). In this system, a drug is synthetically modified so that it contains a trigonellinate ester, amide or other functionality ($D\text{-}Q^+$). Chemical reduction of this quaternary salt yields the 1,4-dihydropyridine derivative or the D-CDS. Systemic administration of this conjugate lead to extensive distribution into both the brain and into the periphery. With time, the enzymatically labile CDS oxidizes, forming the corresponding quaternary salt. This polar combination is readily lost from the periphery due to its hydrophilicity but is retained behind the blood-brain barrier (BBB) because of this same property (i.e. $Kel_4 >> Kel_1$). With time, the inactive $D\text{-}Q^+$ conjugate hydrolyses regenerating the biologically potent drug (D) and the polar carrier which is readily lost from the CNS (i.e. $Kel_3 >> Kel_1$).

As indicated, the oxidation of the CDS to its quaternary nicotinamide salt is required for brain selectivity and rapid systemic clearance. Homogenate studies show that the K_m's generated for oxidation of these species are within the range of those produced by NADH transhydrogenases and may therefore suggest an enzymatically selective oxidation. An understanding of the in vivo conversion of these CDS therefore requires an understanding of the biologically relevant dihydropyridine ⇌ pyridinium salt interconversion.

Various model systems have been developed to study the chemistry of dihydronicotinamide oxidation. Aside from the degenerate reactions, the oxidation of dihydronicotinamides by N-methylacridinium salts[26-28], substituted trifluoroacetophenones, acetophenones and thiobenzophenones[31-32], substituted quinolinium salts[33-35], potassium ferricyanide[36-39] and other hydride acceptors has been studied. Numerous hypotheses concerning the mechanism of hydride transfer were developed based on these studies and as of now controversy continues to rage. The classical work by Abeles and Westheimer indicated that hydride transfer between substituted 1,4-dihydronicotinamides and thiobenzophenones was a concerted, single step process[40]. Subsequently, differences in the kinetic isotope effects and isotope contents in the reaction products suggested that intermediates were involved in the hydride transfer[26,27]. Several multi-step mechanisms were forwarded including e-, H transfer[41] and e-, H^+, e- "hydride-like" oxidation[42,43]. Recently, reanalysis of existing data, illustration of side reactions in the redox system and demonstration of various quantum mechanical effects such as tunneling have argued that hydride transfer is synchronus[44,45].

Another important mechanistic point, assuming concerted hydride transfer, which requires clarification in the reaction of dihydronicotinamides with hydride acceptors such as 1-substituted nicotinamides is the nature of the transition state. Verhoeven examined the structure for a transition state produced by hydride transfer between 1,4-dihydropyridine and protonated pyridine[46,47]. He found that a linear transition state was formed and that the two pyridine moieties could freely rotate around the C-H-C axis producing exo (parallel) and endo (non-parallel) configuration at nearly the same energies. This result was in contrast to those of Bruice who suggested that a bent transition state which resulted from a face to face π charge-transfer complex was more likely[44].

This communication summarizes several theoretical studies which were performed to examine the degenerate reaction of 1-methyl-1,4-dihydronicotinamide and the 1-methylnicotinamide cation. It was felt that while the carbamoyl group may not greatly interact electronically with the dieneamine structure, it may in some way act to affect the approach of the two molecules.

Methods:

The calculations were carried out on an IBM 3081 Model K dual processor computer operating at 15 MIPS using the MNDO[48,49] and AM1[50] molecular orbital approximation. The MNDO program was obtained through quantum chemical program exchange (QCPE), converted to VS Fortran and adapted to run on the IBM 3081

computer. The structural input was generated using the SYBYL/MOPAC interface, and the geometries were found by minimizing the total energy with respect to all structural variables using the standard Davidon-Fletcher-Powell optimization procedure. In this study, the MNDO method was used to calculate the energies (heats of formation) and geometries of various transition states for the hydride transfer between 1-methyl-1,4-dihydronicotinamide and the 1-methylnicotinamide cation. In addition, this technique was used to examine the approach of these two molecules from infinite distance (100A) to the transition state and to examine various effects of certain perturbation on the transition states. The AM1 method was used to examine the lowest energy transition state as well as the infinitely separated (100A) species.

Results and Discussion:

In the first set of experiments, the effect of the initial molecular approach of 1-methyl-1,4-dihydronicotinamide (1) towards 1-methylnicotinamide (2) was investigated and the resulting transition states calculated. Figure 2 gives the molecular numbering scheme for the interacting molecules while Figure 3 illustrates the four initial geometries. In the cis (H_S-re) case, the molecules were positioned front to back so that the prochiral H_S hydrogen could interact at the C21 site on the re face of the pyridinium salt. In this conformation, the C7 and C24 methyl groups, the C8 and C28 carbonyl carbons and the N10 and N30 amide nitrogens were juxtapositioned. Rotation of the pyridinium component 180° in the z-plane generates the trans (H_S-re) conformation in which the H_S hydrogen is again transferred to the re face of the pyridinium ion. In this conformation the amides are no longer on the same side of the supermolecule. The cis (H_S-si) structure results from a 180° rotation of the pyridinium moiety of the cis (H_S-re) system in the y-plane. In this conformation the prochiral H_S is transferred to the si face. The carbamoyl groups do not face each other but are on opposite sides of the interacting molecules, i.e. C22 faces C3, C26 faces C2 and C5 is across from C23.

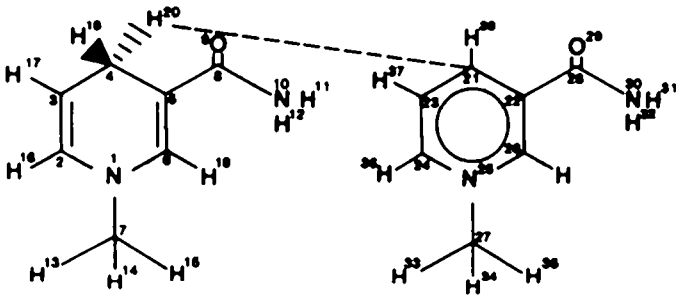

Figure 2. Numbering scheme for the 1-methyl-1,4-dihydronicotinamide ⇕ 1-methylnicotinamide cation interaction.

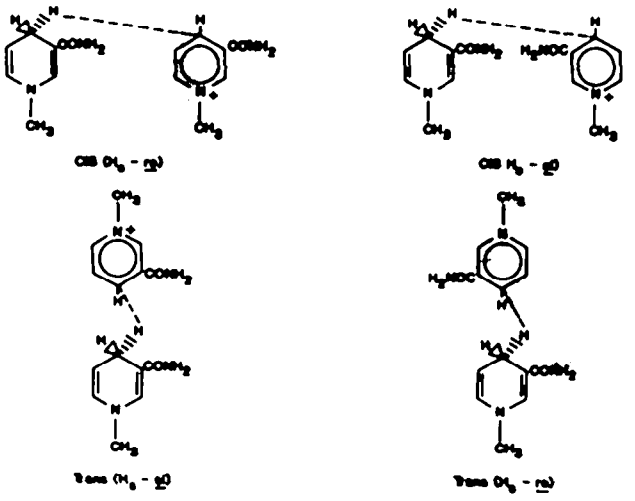

Figure 3. Initial approach conformations for the interaction of 1-methyl-1,4-dihydronicotinamide and 1-methylnicotinamide cation.

Rotation of the pyridinium moiety 180° in the z-plane gives the trans (H_S-si) conformation. In this structure, the carbamoyl groups are on the same side. In the structural optimization, all parameters varied except that the C4-H20 bond was made equal to the H20-C21 bond. The initial approach vector resulting in the transition state with the lowest energy was the cis (H_S-re) scenario. This conformation was 8, 5.5 and 1.4 kcal/mol more stable than the transition state produced by cis (H_S-si), trans (H_S-re) and trans (H_S-si) geometries, respectively. In the most stable transition state, the C4-H20-C21 bond angle is 180.2° indicating a linear hydride transfer. In this configuration, the two partially reduced pyridine rings have moved relative to each other to maximize the separation between the attached carbamoyl groups. Thus, in the "real" situation, the free rotation which was suggested in the case of the unsubstituted dihydropyridine-pyridine interaction is unlikely. This is illustrated in figure 4 which is a structural representation of the cis (H_S-re) generated transition state. In addition, the two carbamoyl groups have oriented out of the plane of their respective pyridine rings. The cis (H_S-re) approach vector apparently allows the two annular portions of the molecule the most latitude in finding an energy well. The high energy of the cis (H_S-si) initiated transition state may result from the fact that reorientation of the system to maximize the separation between the methyl groups (C7 and C27) exacerbates congestion around the amides and vice versa.

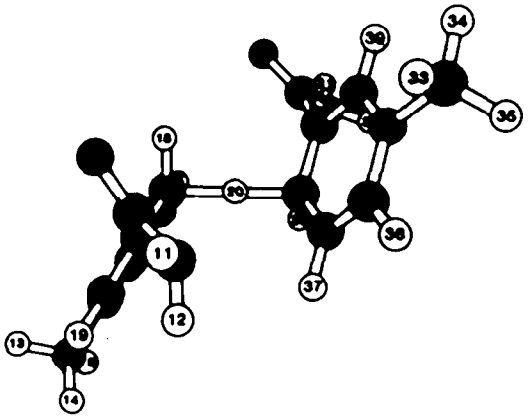

Figure 4. Structural representation of the transition state obtained by optimization of the Cis (H_S-re) starting geometry using the MNDO approximation.

Given this information, the approach of the two structures (1) and (2) towards each other from infinite distance (100A) to transition state and beyond was studied. The cis (H_S-re) configuration was selected for the starting geometry. In this experiment all molecular parameters were allowed to vary with the exception of the bond length between H20 and C21. This distance was set, the supermolecule optimized, the distance decreased and reoptimized. The initial molecular separation was set at 100A. This was then decreased to 25, 20, 15, 10, 8, 4, 3.5, 3.0, 2.5, 2.0, 1.9, 1.8, 1.7, 1.6, 1.5, 1.4, 1.35, 1.3, 1.2, and 1.1A. Figure 5 shows that at separation greater than 10A, the molecular approach of the two species towards each other does not dramatically alter the energy of the system. When the molecules come within 4A, there is a drop of 8.3 kcal/mol. Past this point, the energy of the system, as measured by semiempirical heats of formation, rises until the transition state is reached. MNDO predicted an energy of activation (Ea) of 30.7 kcal/mol while the value found by using the AM1 approximation was 9.3 kcal/mol. The AM1 value corresponds well with experimentally derived values. For example, the degenerate hydride transfer reaction between 1,3-bis(3-carbamoyl-1,4-dihydropyridin-1-yl)propane and its corresponding quaternary salt, a model system for the NAD $\overset{+}{\bullet}$NADH$^+$ interaction, gave an Ea of 9.75 kcal/mol[47]. In addition, the non-degenerate reaction between 1-(4-methylphenyl)-3-carbamoyl-1,4-dihydropyridine and the 1-methylacridinium cation required 8.19 kcal/mol for activation[44]. Other hydride transfer reactions gave similar values for the energies of activation[42]. The closer approximation of the Ea by AM1 compared to MNDO may be related to the tendency of the latter method to exaggerate molecular repulsions[50]. In the approach of the two species (1) and (2), the carbonyl oxygen 09 begins to reorient at 8A (Figure 6).

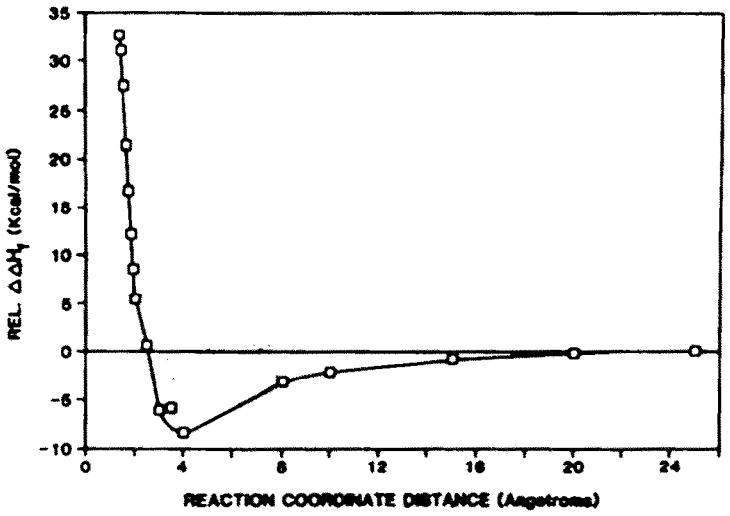

Figure 5. System energies for the approach of 1-methyl-1,4-dihydronicotinamide and the 1-methylnicotinamide cation from 25Å to the transition state (1.4Å). The reaction coordinate is defined by the H20-C21 axis and all calculations were performed using the MNDO method.

This is mirrored by a change in the atomic charge on 09 which became significantly more negative at H20-C21 separation of 3.5Å, dropping from -0.35 at infinite separation to -0.42. This geometry is associated with stabilization of the supermolecule compared to the isolated system. These data suggest some electrostatic interaction as the positively charged pyridinium salt approaches the electronegative carbonyl oxygen. Upon closer approach, the interaction of 09 and the pyridinium species decreases and the atomic charge returns to the values observed at infinite separation (100Å). At the transition state, the 09 atomic charge becomes less negative as the hydride is lost. The migrating hydrogen (H20) becomes significantly more negative (40-fold) as the transition state forms, consistent with a single step hydride transfer. The bond angle C21-H20-C4 which indicates the linearity of hydride migration is initially acute (250°) but as the molecules approach each other this value falls and at the transition state is 180°. At a interatomic (H20-C21) separation of less than 1.35Å, the molecular complex reorganizes: the C21-H20-C4 bond angle increases and the energy of the system rapidly falls.

These data suggest that a linear transition state occurs. The energy required to distort this linear conformation was next investigated. The optimized transition state was perturbed by fixing the bond angle C21-C22-C4 at various values and then this structure was reoptimized. The results of these calculations indicate that the molecule can tolerate a moderate amount of bending without significant energetic costs. Bending the angle ±30° requires 3-4 kcal/mol while a ±60° distortion requires 19-24 kcal/mol.

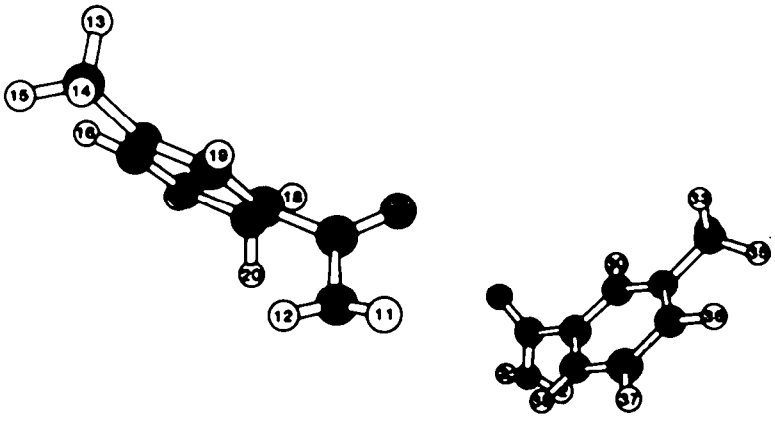

Figure 6. Molecular orientation which gives rise to the charge-dipole complex. This configuration was produced assuming a Cis (H_S-re) initial geometry and was generated using the MNDO method.

These results indicate that the amide and methyl moieties alter the properties of the unsubstituted pyridine-dihydropyridine interaction. Verhoeven showed that the transition state for the hydride transfer between 1,4-dihydropyridine and protonated pyridine was linear and that there was free rotation around the axis of the transferring hydride. The present data would indicate that significant energy barriers are associated with this rotation in this biologically more relevant model. This can be shown by the data obtained from the first series of experiments which suggested a barrier of at least 5.5 and 7.1 kcal/mol for the rotation of a H_S-re transition state and H_S-si transition state, respectively. Certainly other higher barriers could be present. In addition, the amide and methyl substitutions clearly affect the approach of the two species. As Donkersloot and Buck have reported in the reaction of 1,4-dihydronicotinamide and the cyclopropenium cation, the carbamoyl group orients towards the approaching positively charged species which lowers the energy of the system and favors the approach[51,52]. We have similarly demonstrated that an electronically favorable interaction takes place as the pyridinium species approaches the dihydropyridine. The significant increase in the magnitude of the negative atomic charge on O9 and the increased magnitude of the positive charge on C23 indicate that an induced charge dipole complex may form and that this energetically more stable form may influence the geometry of the transition state. Interestingly the majority of enzymes that catalyze the NADH $\rightleftharpoons$ NAD interconversion i.e. $NAD(P)^+$ transhydrogenase (E.C. 1.6.1.1), are of the B type i.e. a H_S-re interaction.

In summary, semiempirical molecular orbital calculations were used to study the reaction between 1-methyl-1,4-dihydronicotinamide and the 1-methylnicotinamide cation. Results demonstrated that an initial molecular

approach in which the pyridine species were endo and in which the carbamoyl groups faced each other produced a transition state of lowest energy. In this model, a prochiral H_S hydrogen atom and associated electron were transferred to C21 on the re face. In the optimized structure, the carbamoyl groups had moved relative to each other to maximize separation. In a subsequent study, the reaction between 1-methyl-1,4-dihydronicotinamide and the 1-methyl-nicotinamide cation was monitored as the H20-C21 interatomic distance was decreased from 100A to transition state. As the molecules approached each other an energetically favorable charge-dipole interaction occurred which was associated with changes in supermolecular conformation and atomic charge densities. This molecular alteration is apparently important in determining the final molecular approach of the two species and may, when attached to an enzyme, induce structural changes which result in stereospecific reactivity. As the molecules approach the transition state, the C4-H20-C21 bond angle straightens to 180°. While MNDO indicated an Ea of 30.7 kcal/mol, calculations using the AM1 approximation suggest the value was 9.3 kcal/mol, which is consistent with previously reported experimental values for similar systems[47,44].

Acknowledgments: The authors would like to thank Ned Phillips for his help with this project. The expert editorial skills of Joan Martignago are also acknowledged. Financial support was provided by the NIH (GM 27167).

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