Structural Aspects and Rearrangement of Radical Cations Generated from NADH Analogues

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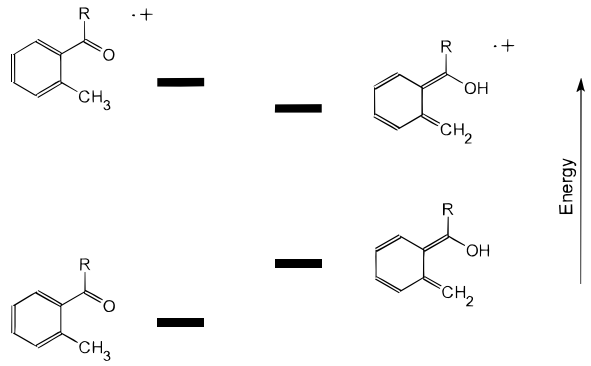
We showed recently that upon radiolytic ionization various ortho-substituted aromatic compounds undergo spontaneous hydrogen atom transfer to yield radical cations with *o*-quinoid structures.[1] It was shown that enolization is endothermic in the neutral molecules but exothermic in the radical cations. The inverse stability of enol radical cations is schematically presented in Scheme 1. Spontaneous hydrogen atom transfer and formation of enol radical cations is not limited to aromatic carbonyl compounds carrying *o*-alkyl substituents. The migrating hydrogen can also originate from a hydroxy or formyl group, and a nitro or nitroso group can act as acceptor.[2]

Since the observed spontaneous hydrogen atom transfer upon ionization turned out to be of surprisingly wide scope, we decided to apply this concept to other important reactions involving participation of radical cations. The first system selected for studies was the conversion of NADH to NAD^+. In spite of vast investigations there is still no general agreement concerning the reaction mechanism. Distinction between a direct one-step hydride transfer and a multistep electron−proton−electron transfer mechanism is still open to debate particularly for substrates containing a carbonyl group. Evidence is reported to support the electron−proton−electron mechanism for reactions involving thermal, photochemical, and electrochemical oxidation.[3,4] Our interest was focused on radical cations generated upon one-electron oxidation of the model synthetic NADH analogues to find out whether rearrangement involving the hydrogen atom shift will result in thermodynamic stabilization of radical cations similarly to other systems studied by us previously.

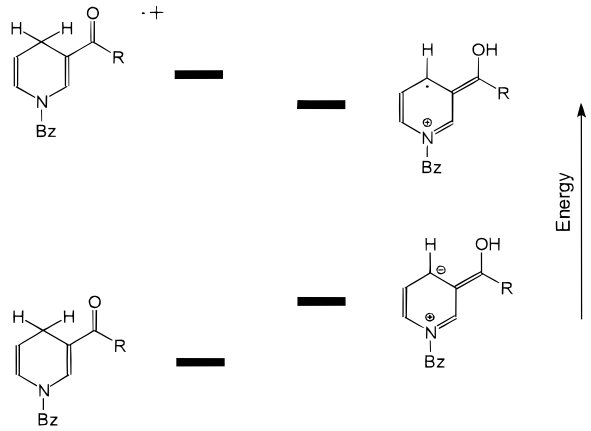
AM1 calculations on 1-benzyl-1,4-dihydronicotinamide (BNAH) and 1-benzyl-3-acetyl-1,4-dihydropyridine (BAPH) indicated the inverse stability of the hydrogen-transferred isomers (enol form) in the radical cations, as compared to the neutral molecules (Scheme 2). 1,4-Hydrogen atom transfer from C-4 to the carbonyl group in the neutral molecules leads to the enol of zwitterionic character which was calculated to be less stable by 29.5 and 22.4 kcal/mol than the non-hydrogen-transferred tautomer (keto form) of BNAH and BAPH, respectively. Reversely, AM1 predicts the enol radical cations to be more stable by −13.4 and −15.2 kcal/mol as compared to the keto radical cations of BNAH and BAPH.[5,6] The calculated

Scheme 1[a]



[a] R = H, CH_3, Ph.

Scheme 2[a]



[a] Bz = benzyl; R = NH_2 (BNAH), CH_3 (BAPH).

transition-state energy gives the activation barrier E_a = 26.8 kcal/mol for hydrogen atom transfer in $BNAH^{\bullet+}$.[7] If this prediction is correct, the substantial barrier for hydrogen atom transfer may create a problem in observation of the enol radical cations in cryogenic hydrocarbon glasses. In all examples of 1,5-hydrogen atom shifts in radical cations studied so far, the reaction was very fast even at cryogenic temperatures, allowing a direct detection of the keto radical cations only by time-resolved techniques (pulse radiolysis).[1,2] A substantial difference in activation energies for hydrogen atom transfer in $BNAH^{\bullet+}$ *vs o*-alkylphenyl ketones can intuitively be understood in terms of differences in the transition-state geometry (five-membered *vs* six-membered).

Radiolytic generation of radical cations combined with optical detection is a powerful technique that enjoys widespread use. A variety of radical cations of unusual structure and reactivity have been evaluated by means of this method.[8] However, the application of complementary time-resolved techniques like pulse radiolysis is essential to determine activation barriers and to elucidate interesting mechanistic details of radical cation interconversions.[9] In view of the above mentioned methodology, studies on radical cations generated from NADH analogues

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(3) Fukuzumi, S.; Tanaka, T. In *Photoinduced Electron Transfer*; Fox, M. A., Chanon, M., Eds.; Elsevier: Amsterdam, 1988; Part C, pp 578−635.

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(5) No gain in energy was calculated for $BNAH^{\bullet+}$ when the hydrogen atom at C-4 was transferred to the amide or ring nitrogen.

(6) A question concerning intramolecular hydrogen atom transfer has already been raised in discussion of the electronic absorption spectrum of $NADH^{\bullet+}$. Czochralska, B.; Lindqvist, L. *Chem. Phys. Lett.* **1983**, *101*, 297.

(7) Our experience is that AM1 tends to overestimate the transition-state energy for radical cations as compared with the measured values for 1,5-hydrogen atom shifts; nevertheless, a relative comparison seems to be meaningful.

(8) (a) Shida, T.; Haselbach, E.; Bally, T. *Acc. Chem. Res.* **1984**, *17*, 180. (b) Bally, T. In *Radical Ionic Systems*; Lund, A., Shiotani, M., Eds.; Kluwer: Dordrecht, 1991; pp 3−54.

(9) Gębicki, J. *Pure Appl. Chem.* **1995**, *67*, 55.

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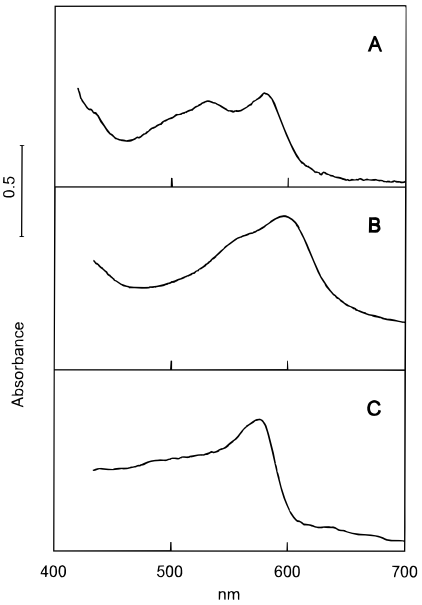


Figure 1. Absorption spectra of radical cations of BAPH (A), MCPH (B), and BCPH (C) generated radiolytically in a glassy matrix of *sec*-butyl chloride at 77 K (concentration, 0.02 mol/L; radiation dose, 9 kGy; sample thickness, 3 mm).

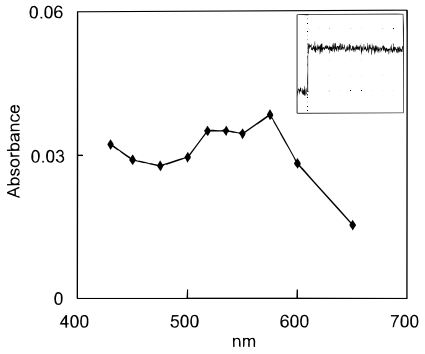
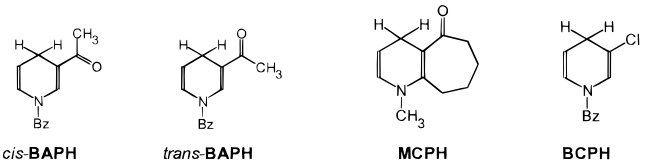


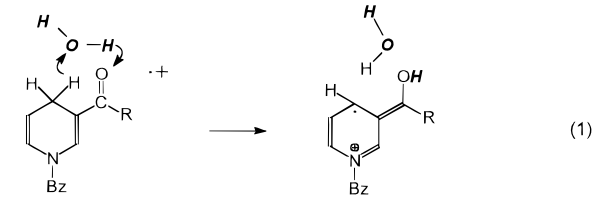
Figure 2. Transient absorption spectrum of BAPH$^{\bullet+}$ in *sec*-butyl chloride at 30 K detected 1 ms after the 4 μs electron pulse delivering a dose of 10^3 Gy. Inset: Scope trace at 575 nm (100 μs/division).

have been undertaken, and selected results are presented in Figure 1. The observed steady-state spectrum of BAPH$^{\bullet+}$ (Figure 1A) is similar to the spectrum detected by low-temperature pulse radiolysis (Figure 2), for which no time evolution on its formation is observed.[10] Even at temperatures as low as 30 K this spectrum was formed within a 4 μs electron pulse. In documented cases of 1,5-hydrogen atom shifts in radical cations the enol tautomers were observed to be formed with a delay even up to several seconds for some systems.[1] Deuteration of BAPH at the 4-position should slow down the hydrogen atom transfer. BAPH-$d_2^{\bullet+}$ was also seen to be formed at 30 K immediately, within an accelerator electron pulse. This observation seems to indicate that hydrogen atom transfer is not taking place; otherwise the activation barrier for hydrogen atom transfer would be extremely low (below 1 kcal/mol). This is far less likely since for a much more favorable six-membered transition state the activation barrier for intramolecular hydrogen atom transfer in radical cations was measured to be on the order of several kilocalories/mole.[1] These findings strongly support our assignment of the observed radical cations generated from BAPH and BAPH-d_2 to the keto form.

As the calculated population of the *cis*-conformer in neutral BAPH prior to ionization is overwhelming, it may happen that hydrogen atom transfer does not take place because of conformational reasons.[11] To avoid complications introduced by the



conformational mobility of the acetyl group in BAPH, a new model NADH analogue with the carbonyl group locked in the *trans*-conformation was synthesized, namely, 1-methyl-1,4-dihydro-6,7,8,9-tetrahydro-5*H*-cyclohepta[*b*]pyridin-5-one (MCPH).[12] The electronic absorption spectrum of MCPH$^{\bullet+}$



(Figure 1B) is similar to that observed for BAPH$^{\bullet+}$, and in addition it was also formed immediately after ionization. Since conformational requirements for hydrogen atom transfer in MCPH$^{\bullet+}$ seem to be fulfilled, the large activation barrier is the only feature which may prevent hydrogen atom transfer from occurring. Additional support for assignment of the spectra in Figure 1A,B to the keto radical cations comes from the study of the radical cation generated from 1-benzyl-3-chloro-1,4-dihydropyridine (BCPH). For this molecule hydrogen atom transfer is not feasible due to the lack of a hydrogen-accepting group in the 3-position. The spectrum of BCPH$^{\bullet+}$ (Figure 1C) is quite similar to those seen in Figure 1A,B. To conclude, we may say that the keto form of BAPH$^{\bullet+}$ and MCPH$^{\bullet+}$ is stabilized kinetically in cryogenic glasses, which does not seem to be so surprising in view of the substantial activation barrier calculated for the hydrogen atom transfer.

Hydrogen atom transfer leading to the thermodynamically more stable tautomer of the NADH radical cation can also be considered as a process taking place *via* assistance of the solvent molecules as shown in reaction 1.[13]

At present we are not in the position to comment on the significance of the thermodynamically favored hydrogen atom transfer in enzymatic reactions of NADH. However, it is known that the amide group adopts a *trans*-conformation in most of the enzyme active site even though the *cis*-conformation is intrinsically more favorable.[14,15] In addition the amide group in the *trans*-conformation is rotated out of plane by 20−30°, which suitably prepares the orientation of the carbonyl group to accept a hydrogen atom.[15]

Acknowledgment. This work was supported by grants from the State Committee for Scientific Research.

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(10) A description of our system and the methodology of the low-temperature pulse radiolytic studies of radical cations are given elsewhere. Karolczak, S.; Hodyr, K.; Łubis, R.; Kroh, J. *J. Radioanal. Nucl. Chem.* **1986**, *101*, 177. Gębicki, J.; Marcinek, A.; Rogowski, J. *Radiat. Phys. Chem.* **1992**, *39*, 41.

(11) By rapid quenching of the gas phase conformational equilibrium of BAPH on a cold surface we have found by IR matrix isolation spectroscopy that the abundance of the *trans*-conformer does not exceed 15%.

(12) MCPH was prepared by sodium dithionite reduction of the corresponding cycloheptapyridinium iodide prepared from 6,7,8,9-tetrahydro-5*H*-cyclohepta[*b*]pyridin-5-one, which was in turn synthesized according to the known procedures. Epsztajn, J.; Bieniek, A.; Brzeziński, J. Z. *Pol. J. Chem.* **1980**, *54*, 341. Caprathe, B. W.; Jaen, J. C.; Wise, L. D.; Heffner, T. G.; Pugsley, T. A.; Meltzer, L. T.; Parvez, M. *J. Med. Chem.* **1991**, *34*, 2736.

(13) The feasibility of such a reaction is presently being tested in our laboratories.

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(15) (a) Wu, Y.-D.; Houk, K. N. *J. Org.Chem.* **1993**, *58*, 2043. (b) Brewster, M. E.; Pop, E.; Huang, M.-J.; Bodor, N. *Heterocycles* **1994**, *37*, 1373.