2682 © 1990 The Chemical Society of Japan Bull. Chem. Soc. Jpn., 63, 2682—2686 (1990) [Vol. 63, No. 9

NAD(P)+-NAD(P)H Models. 72. Isotope Effects to Prove the Multi-Step Mechanism in the Reduction with an NAD(P)H Analog

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(Received May 28, 1990)

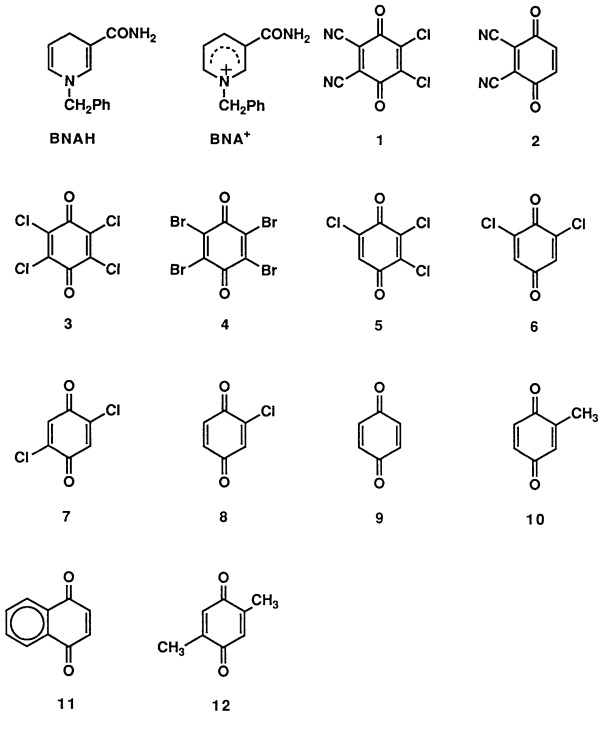
Reduction of 1,4-benzoquinone and its various derivatives with 1-benzyl-1,4-dihydronicotinamide (BNAH) and its 4-deuteriated derivative was studied. The kinetic deuterium primary isotope effect (k^H/k^D) for this reaction appears always larger than the isotopic ratio in the product, BNA^+(Y^H/Y^D; product isotope effect). In the absence of magnesium ion, Y^H/Y^D increases as the substrate becomes less electron-demanding. In the presence of magnesium ion which accelerates the reaction, Y^H/Y^D stays small whereas k^H/k^D is not affected. The results reveal that the reaction proceeds with a multi-step mechanism in which the product-determining and rate-determining steps differ each other.

About a decade ago, we reported that the kinetic primary deuterium isotope effect (k^H/k^D) in the reduction of $\alpha,\alpha,\alpha,$-trifluoroacetophenone with an NAD(P)H analog is smaller than the ratio in the yield of protiated and deuteriated products (Y^H/Y^D; product isotope effect).[1] The result was interpreted in terms of a multi-step mechanism; since the initial electron-transfer process has the largest activation energy among those of the processes involved, this is the rate-determining step and k^H/k^D stays small reflecting small movement of hydrogen nucleus during this process, whereas the deuterium-content in the product is determinied by the following proton (or hydrogen atom) transter process which is associated by a large kinetic isotope effect. As the substrate becomes more electron-demanding, the kinetic importance of the initial electron-transfer process becomes less and k^H/k^D increases asymptotically to the upper limit of Y^H/Y^D.

The interpretation on the discrepancy between the kinetic and product isotope effects was claimed by Powell and Bruice based on their finding of a by-product as well as the isotopic scrambling during the reaction.[2] It was, however, confirmed that the claim cannot be applied to our system.[3] Later on, Kreevoy et al. reported that the rates of a wide range of redox reactions between a dihydropyridine derivatives and their corresponding onium salts can excellently be reproduced by the Marcus equation, and the observation was considered to be a good supprt for a one-step reaction meachanism.[4,5] However, it has been known that a charge-transfer complex is formed between these reduced and oxidized species.[6,7] The reaction starting from the charge-transfer complex may follow the Marcus equation without contradicting the observation and theory by Kreevoy et al.

We now wish to report that the reduction of 1,4-benzoquinone and its derivatives with 1-benzyl-1,4-dihydronicotinamide (BNAH) exerts larger k^H/k^D than Y^H/Y^D because the activation energy for the initial electron transfer process in the present reaction is reasonablly small and the proton (or hydrogen atom) transfer process remains to be rate-determining. Whereas the deuterium content in the product is defined by the structure of the intermediate complex (relative position of the transferring hydrogen (deuterium) nucleus against the substrate), or the initial electron transfer process is the product-determining step.

It should be emphasized that the kinetic isotope effect for the present reaction has been demonstrated to follow the Marcus relationship[8] and the k^H/k^D should not exceed the Y^H/Y^D if Powell and Bruice's prediction is valid. It is also noteworthy that the stereochemistry of the reduction of a series of substituted and unsubstituted 1,4-benzoquinones with a chiral NAD(P)H analog is linearly correlated with the oxidation potential of the substrate.[9,10]



Scheme 1.

Results and Discussion

Kinetic and product isotope effects were observed at 298 K in acetonitrile in the presence and absence of magnesium ion. The results are listed in Table 1 and logarithms of the isotope effects are plotted in Fig. 1 against the redox potential of the substrate.[8)]

The product isotope effect was measured by observing the relative intensity of the ^{1}H NMR signal from the proton at the C_4-position in the produced 1-benzyl-3-carbamoylpyridinium ion (BNA$^+$). The present technique is different from the previous ones[1,2)] in that Y^H/Y^D is the direct measure for the ratio of hydrogen and deuterium dissociated from BNAH-4-*d*. In other words, the value of Y^H/Y^D is free from the discrimination of isotopes amoug the (by)-products, abortive adducts, or different intermediates, if any, as pointed out previously.[2)] Since we confirmed that no by-product could be detected in the reaction mixture within the limit of ^{1}H NMR spectroscopy, Y^H/Y^D can safely be regarded as the ratio of isotopes employed for the reaction we are now interested in.

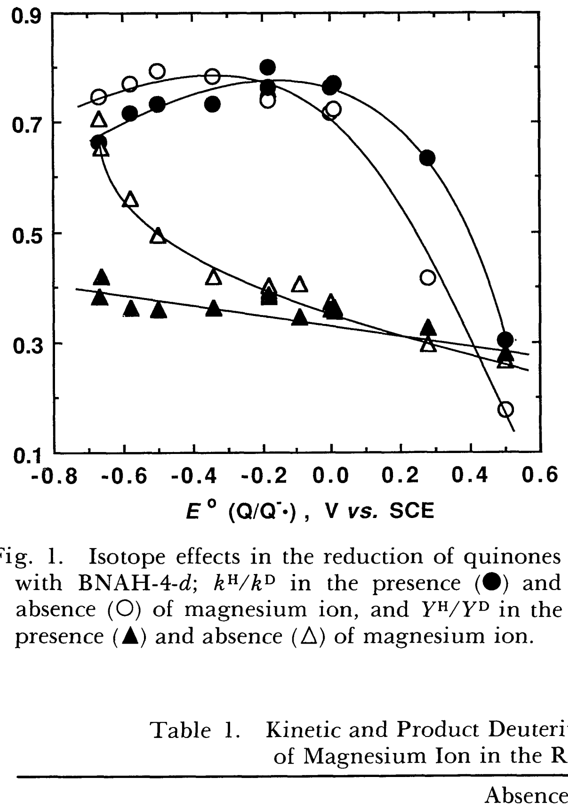


Fig. 1. Isotope effects in the reduction of quinones with BNAH-4-*d*; k^H/k^D in the presence (●) and absence (○) of magnesium ion, and Y^H/Y^D in the presence (▲) and absence (△) of magnesium ion.

As described above, the quadratic relationships between the kinetic isotope effect and the redox potential in the presence and absence of magnesium ion are nicely reproduced by the Marcus equation,[8)] which, according to Kreevoy's proposal, should be a proof for a one-step mechanism for the present reaction. However, it is clearly seen that the value of Y^H/Y^D deviates largely from the value of the corresponding k^H/k^D, and the discrepancy in the value of Y^H/Y^D observed in the reactions in the presence and absence of magnesium ion becomes important as the substrate becomes less electron-demanding.

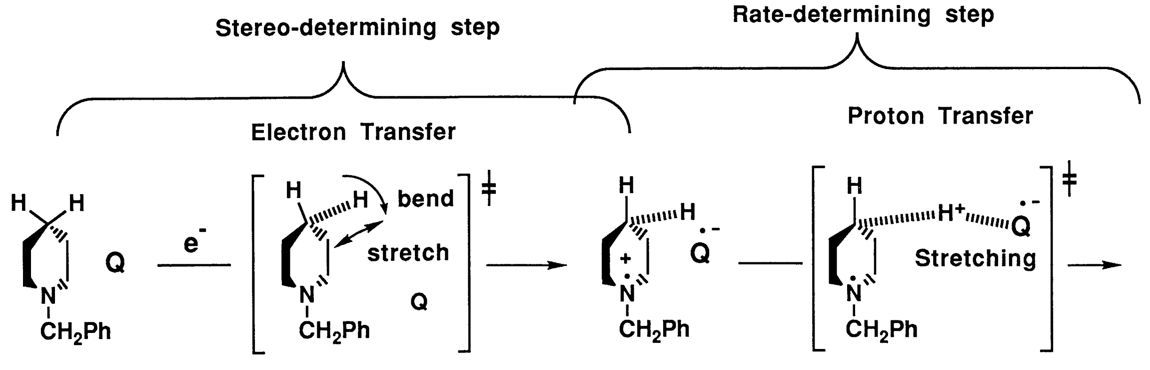
The observation cannot be rationalized by assuming a one-step mechanism. Instead, we believe that the reaction involves separated product- and rate-determining steps, and, at least for a majority of the substrates of redox potential between −0.5—+0.2, the product-determining step is associated by a small kinetic isotope effect whereas the rate-determining step is accompanied by a large effect.[11,12)] In addition, in order to keep the Y^H/Y^D smaller than the k^H/k^D, the rate-determining step has to come later than the product-determining step.

To fulfill this requirement, it will be the most reasonable idea to assume a process in which the bending mode of the H-C_4-H(D) moiety contributes largely with little contribution from the C_4-H(D) stretching mode for the product-determining step, whereas the succeeding rate-determining step is mostly composed of the C_4-H(D) stretching vibration. Thus,

Table 1. Kinetic and Product Deuterium Isotope Effects in the Presence and Absence of Magnesium Ion in the Reduction of Quinones with BNAH[a)]

Quinone	$E°(Q/Q^{\cdot-})$[b)]	Absence of Mg^{2+}			Presence of Mg^{2+}		
		Yield/%	Y^H/Y^D	k^H/k^D	Yield/%	Y^H/Y^D	k^H/k^D
1	+0.50		1.84	1.5	20	1.90	2.0
2	+0.28	10	1.97	2.6	4	2.11	4.3
3	+0.01		2.30	5.3	43	2.27	5.9
4	0.00	100	2.36	5.2	48	2.29	5.8
5	−0.09[c)]	100	2.55		40	2.22	
6	−0.18		2.47	5.6	11	2.41	5.8
7	−0.18		2.53	5.5	11	2.43	6.3
8	−0.34		2.62	6.1	100	2.31	5.4
9	−0.50	30	3.13	6.2	100	2.29	5.4
10	−0.58	65	3.65	5.9	100	2.30	5.2
11	−0.66[d)]	37	4.5		100	2.62	
12	−0.67[e)]	15	5.1	5.6[e)]	100	2.42	4.6[e)]

a) The values for kinetic isotope effects and redox potentials are taken from Ref. 8. b) In V vs. SCE. c) Average of tetrachloro- and dichloro-1,4-benzoquinones. d) Corrected from the values obtained under different experimental conditions: cf. K. B. Patel and R. L. Willson, *J. Chem. Soc. Faraday Trans. 1,* **1973**, 814. e) Values for 2,6-dimethyl-1,4-benzoquinone.



Scheme 2.

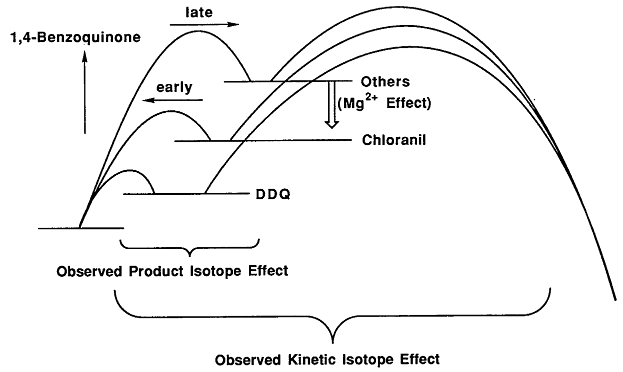


Fig. 2. Schematic energy diagram for the reduction of quinones with BNAH.

the interpretation of the results inevitably leads us to the proposal of the formation of an intermediate, probably of an electron transfer type, which is the product-determining step. The product-determining step is then followed by the rate-determining transfer of a hydrogen (deuterium) nucleus (See Scheme 2).

The energy diagram shown in Fig. 2 explains every observation so far we have.

1. For a long time we have proposed that a bivalent ion in the present reaction system catalyzes initial electron tranfer process.[13,14] In the presence of magnesium ion, therefore, the activation energy for the initial step becomes smaller or the transition state comes earlier, regardless of the reactivity of the substrate. Since the movement of nuclei in the H-C$_4$-H(D) group is small in an early transition state, the isotope effect exerted by the process of this kind is small. Since there is no chance to select hydrogen and deuterium after the hydrogen (deuterium) face of BNAH-4-*d* was chosen by the substrate, this initial electron transfer process to form the intermediate is the product-determining step in the total reaction.

Since the succeeding proton (or hydrogen atom) transfer process is rate-determining, the kinetic discrimination between the isotopes is large and the $k^{\mathrm{H}}/k^{\mathrm{D}}$ appears large. However, since this process is little affected by the magnesium ion, its presence or absence does not change the value of $k^{\mathrm{H}}/k^{\mathrm{D}}$ significantly.

2. In the absence of magnesium ion, the electron transfer becomes more difficult than its presence. This is particularly true for the substrates of very little electron-demanding. In order to undergo the reaction with the substrate of this kind, the electron transfer has to be assisted by a large movement of the nuclei in the H-C$_4$-H(D) moiety, which, in turn, means that the electron transfer process is largely associated not only by bending but also by the stretching elongation of the C$_4$-H(D) bond and the $Y^{\mathrm{H}}/Y^{\mathrm{D}}$ becomes large as the result of large movement of the hydrogen (deuterium) in the process of the electron transfer. When the transition state for the electron transfer process becomes higher in energy than that for the proton (or hydrogen atom) transfer process, the product-determining step coincides with the rate-determining step. In this case, a large discrimination of the isotopes can be seen in both electron and proton (or hydrogen atom) transfer processes. Therefore, the isotopic distribution in the product ($Y^{\mathrm{H}}/Y^{\mathrm{D}}$) becomes larger than the kinetic isotope effect ($k^{\mathrm{H}}/k^{\mathrm{D}}$).[15] This is the case for the reductions of α,α,α-trifluoroacetophenone and its derivatives or ethyl benzoylformate.[1] In the present reaction, 2,5-dimethyl-1,4-benzoquinone, which is the least electron-demanding substrate among those studied, exerts similar values for $k^{\mathrm{H}}/k^{\mathrm{D}}$ and $Y^{\mathrm{H}}/Y^{\mathrm{D}}$. Thus, $Y^{\mathrm{H}}/Y^{\mathrm{D}}$ depends largely on the reactivity (redox potential) of the substrate in the reaction without magnesium catalysis.

3. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) is a special substrate as was already pointed out.[10,16] Since this substrate is sufficiently electron-defficient, electron-transfer to this substrate takes place very quickly with little association of the nuclei movement, which means that the electron transfer intermediate is very much reactant-like. Following the discussion described above, the values of 1.5—2.0 exerted by this substrate for the $k^{\mathrm{H}}/k^{\mathrm{D}}$ seems reasonable as the kinetic isotope effect origined from the difference in the zero-

point stretching energy of C–H and C–D bonds with somewhat non-linear transition-state (vide infra).[2,17,18]

4. The large discrepancy observed for k^H/k^D and Y^H/Y^D with the substrates of redox potential between −0.5—+0.2 is ascribed to the quantum mechanical tunneling effect.[4,19–21]

As mentioned above, the electron transfer process we propose is not the Frank–Condon-type.[22] Instead, it has to be assisted by the nuclei movement. For a majority of the substrates studied, the assistance is so large that the electron transfer intermediates resemble the corresponding products in structure, where the quantum mechanical tunneling can occur quite easily. On the other hand, DDQ forms a reactant-like intermediate and the proton (or hydrogen atom) transfer from the intermediate requires a large movement of the hydrogen nucleus, or tunneling effect is hardly expected here. Judging from the value of k^H/k^D the quantum mechanical border for the tunneling seems to exist between DDQ and chloranil (tetrachloro-1,4-benzoquinone).

We have emphasized that our multi-step mechanism for the (net) hydride-transfer reaction does not necessarily mean that the process for the transfer of hydrogen nucleus is completely separated from that for the transfer of electron, which is the Frank–Condon-type process.[14,23] Instead, the energy barrier for the initial electron transfer process may be overlapped by that for the proton (or hydrogen atom) transfer process. The extent of the overlapping depends on the reactivity (redox potential) of the substrate. By introducing the concept of the quantum mechanical tunneling effect, the idea has become much clearer; that is, a substrate which is less electron-demanding forms a product-like electron transfer complex as the intermediate and the possibility of the tunneling becomes larger for the succeeding proton (or hydrogen atom) transfer process. On the other hand, the probability of quantum mechanical tunneling is much lower from the reactant-like intermediate because the separation of potential wells for the reactant[24] and product systems are large.[25]

When the overlap of barriers for the electron and proton (or hydrogen atom) transfers is sufficiently large, one may say that the reaction proceeds with the one-step mechanism. However, we prefer to keep the idea of the multi-step mechanism in order to explain all the reactions with a variety of substrates; say, from DDQ[10] or metal ions[26] to various non-DDQ quinones or ethyl benzoylformate, with a united concept.

Experimental

Instruments. ^{1}H NMR spectra were recorded at 200 and 400 MHz on a Varian VXR200 and a JEOL GX400 FT-NMR spectrometers, respectively, in D_2O or $CDCl_3$ with TSP-d_4 or TMS as an internal standard, respectively.

Materials. BNAH and BNAH-4-*d* were prepared according to the literature method.[27] Quinones were purchased from commercial sources and purified by a standard method[28,29] except for chloro-,[30] trichloro-,[31] and 2,3-dicyano-1,4-benzoquinones,[32] which were synthesized after the respective literature procedures. Magnesium perchlorate was dried at 160 °C in vacuo for 6 h and used immediately. Acetonitrile was distilled over calcium hydride before use.

Observation of Product Isotope Effect. A 1,4-benzoquinone (0.2—2 mmol depending on the reactivity of the quinone) was added to a solution of BNAH-4-*d* (4.0 mg, 0.019 mmol) in acetonitrile (4 ml) at 25 °C. Magnesium perchlorate (8.3 mg, 0.038 mmol) was also added to this solution, if necessary. After the stirring for a period equivalent to 10 half-lives, 4 ml of 1 M HCl (1 M=1 mol dm^{-3}) was added to the reaction mixture and the aqueous phase was washed three times with ethyl acetate.[33] The solvent was evaporated to dryness. The residue was dissolved into D_2O (0.5 ml) and 1.0 μl of methanol was added to this aqueous solution, and the solution was subjected to ^{1}H NMR spectroscopy to elucidate the isotopic ratio and the chemical yield of the product, BNA$^+$. The product isotope effect, Y^H/Y^D, is equal to [BNA$^+$-4-*d*]/[BNA$^+$]. ^{1}H NMR (D_2O; δ from TSP-d_4): 5.92 (CH_2, s, 2H). 7.52 (Ph, s, 5H). 8.20 (5-H, dd and d, 1H), 8.92 (4-H, d, *x*H), 9.08 (6-H, d, 1H), and 9.37 (2-H, s, 1H), where x=[BNA$^+$]/([BNA$^+$-4-*d*]+[BNA$^+$]).

It was confirmed by ^{1}H NMR spectroscopy that neither BNAH nor BNAH-4,4-d_2 was formed from BNAH-4-*d* (and BNA$^+$) under the reaction condition.

The authors wish to thank Prof. Schowen of the University of Kansas for his valuable advice. This work was financially supported by the Japanese Minister of Education, Science and Culture under the Grant-in Aid, No. 1470022.

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