Catalytic effect of monovalent cations on the amine oxidation by cofactor TTQ of quinoprotein amine dehydrogenases

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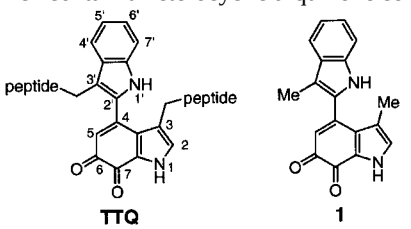
Received (in Cambridge, UK) 9th November 1999, Accepted 19th January 2000

Oxidation of benzylamine by a model compound of cofactor TTQ (tryptophan tryptophylquinone) of quinoprotein amine dehydrogenases is made possible by coordination of a monovalent cation such as Li^+ in anhydrous MeCN.

Bacterial methylamine dehydrogenase (MADH) and aromatic amine dehydrogenase (AADH) comprise a new class of enzymes which contain a heterocyclic *o*-quinone cofactor, TTQ



(tryptophan tryptophylquinone), at their active sites in the light subunits.[1–3]

Monovalent cations have recently been shown to influence not only the UV–VIS spectrum of the enzyme but also the redox reactivity of TTQ in the amine oxidation and the subsequent electron transfer to biological electron acceptor proteins such as amicyanin.[4–9] The enzymes have two different cation binding sites, one of which is located near the quinone carbonyl oxygen O(6) of the cofactor.[4] Thus, the cationic species incorporated into this binding pocket have been suggested to interact with the quinone moiety directly, affecting the electronic structure of the cofactor as well as the reactivity both in the amine oxidation reaction and the subsequent electron transfer process.[5–9] However, little is known about the binding model of the cationic species toward TTQ or how it affects the electronic structure as well as the reactivity of the TTQ cofactor. In this study, we have investigated the interaction of a TTQ model compound[10] with monovalent cations to demonstrate that an alkaline metal ion such as Li^+ binds to the quinone, leading to a significant enhancement of the amine oxidation reaction in anhydrous organic media.

Addition of a certain amount of $LiClO_4$ to an anhydrous MeCN solution containing TTQ model compound **1** (5.0×10^{-5} mol dm^{-3}) and benzylamine (1.0×10^{-2} mol dm^{-3}) caused a rapid spectral change in the UV–VIS region. Fig. 1 shows the spectral change of the titration of **1** with $LiClO_4$ (0–0.15 mol dm^{-3}) in the presence of benzylamine (1.0×10^{-2} mol dm^{-3}).[11] The characteristic absorption band at 408 nm due to the quinone itself decreases, accompanied by an increase in a new absorption band at 461 nm, with increasing the concentration of added $LiClO_4$. The binding constant K_{ML} for 1:1 complex formation between the quinone (Q) and the metal ion (M^+) can be expressed by eqn. (1), where A_0 and A_∞ are the initial and final absorptions of the titration, and $[M^+]_0$ and $[Q]_0$ denote the concentration of the added metal ion and the initial quinone concentration, respectively. The plot of $(A - A_0)/(A_\infty - A)$ *vs.* $([M^+]_0 - \alpha[Q]_0)$ [$\alpha = (A - A_0)/(A_\infty - A_0)$] gives a straight line passing through the origin, as shown in the inset in Fig. 1, from which a K_{ML} value of 15.6 dm^3 mol^{-1} was obtained from the slope. A similar spectral change was obtained in the titration of **1** with $NaClO_4$ although the binding constant was smaller (K_{ML} = 5.5 dm^3 mol^{-1}, λ_{max} = 454 nm).[12,13] It should be noted that the spectral change obtained in this system is fairly close to that observed in the titration of MADH with monovalent cations.[4,8] This result suggests that the monovalent cation binds to the quinone moiety of **1** at a similar position to that proposed in the enzymatic systems.[14]

$$\frac{A - A_0}{A_\infty - A} = K_{ML}([M^+]_0 - \frac{A - A_0}{A_\infty - A_0}[Q]_0) \quad (1)$$

Although *no* reaction takes place between benzylamine and quinone **1** in an aprotic solvent such as anhydrous MeCN, **1** was converted into the reduced form of TTQ in the presence of Li^+. Thus, the reaction of quinone **1** (5.0×10^{-5} mol dm^{-3}) and benzylamine (1.5×10^{-2} mol dm^{-3}) in the presence of $LiClO_4$ (0.15 mol dm^{-3}) in anhydrous MeCN at 25 °C under anaerobic conditions resulted in a drastic spectral change where the absorption band at 461 nm due to the Li^+ complex of the quinone decreases, accompanied by an increase in a new band at 323 nm due to the reduced TTQ in the aminophenol form (Fig. 2).[15] As reported in the previous model reactions between the TTQ model compound and benzylamine in MeOH,[16] the reaction consists of three distinct steps, where the first one [Fig. 2(*a*)] corresponds to addition of the amine to the quinone (k_1), the second one [Fig. 2(*b*)] is due to the spontaneous (k_2) and the amine-catalyzed (k_2') rearrangement from the substrate imine to the product imine intermediates, and the third one [Fig. 2(*c*)] is ascribed to the imine exchange reaction (k_3) to generate the aminophenol and *N*-benzylidenebenzylamine ($PhCH_2N{=}CHPh$), as summarized in Scheme 1.[15] Each process has its own isosbestic point, at 418, 383 and 372 nm, respectively, demonstrating the accuracy of the stepwise mechanism shown in Scheme 1. Since the difference in the rate

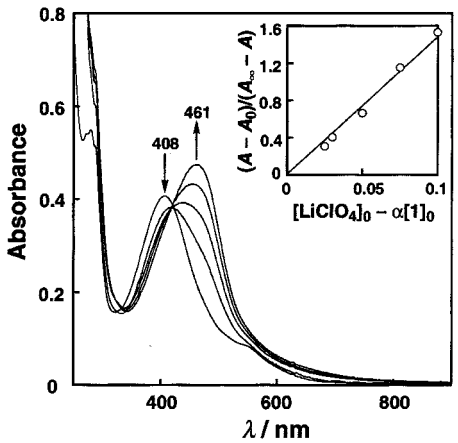


Fig. 1 Spectral change observed upon addition of $LiClO_4$ (0–0.15 mol dm^{-3}) to an MeCN solution of **1** (5.0×10^{-5} mol dm^{-3}) in the presence of benzylamine (1.0×10^{-2} mol dm^{-3}) at 25 °C. Inset: plot of $(A - A_0)/(A_\infty - A)$ *vs.* $[LiClO_4]_0 - \alpha[\mathbf{1}]_0$ $\{\alpha = (A - A_0)/(A_\infty - A_0)\}$.

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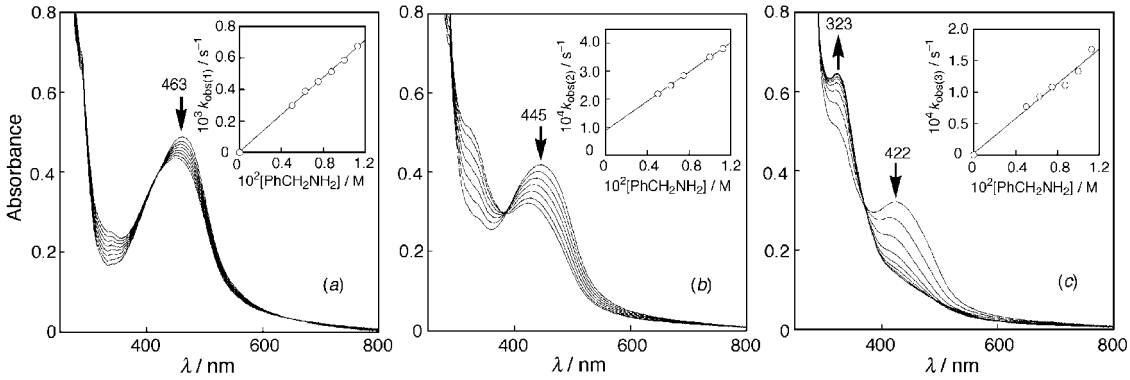
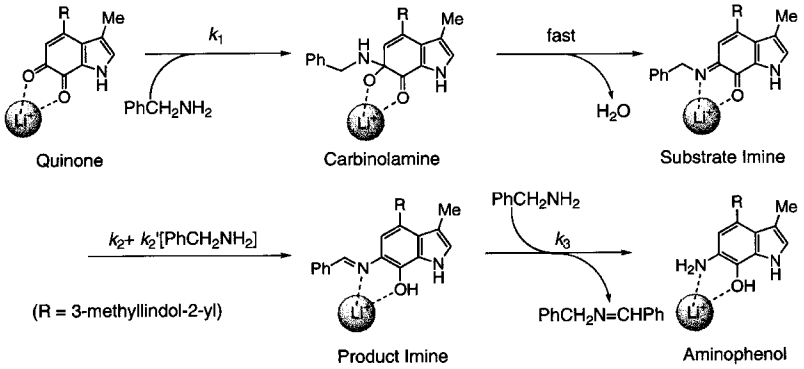


Fig. 2 Spectral change for the reaction of **1** (5.0×10^{-5} mol dm^{-3}) with benzylamine (1.5×10^{-2} mol dm^{-3}) in the presence of $LiClO_4$ (0.15 mol dm^{-3}) in anhydrous MeCN at 25 °C under anaerobic conditions. (*a*) The first stage (0–7200 s), 800 s interval. Inset: plot of $k_{obs(1)}$ *vs.* [$PhCH_2NH_2$]. (*b*) The second stage (9600–24000 s), 2400 s interval. Inset: plot of $k_{obs(2)}$ *vs.* [$PhCH_2NH_2$]. (*c*) The third stage (24000–49600 s), 3200 s interval. Inset: plot of $k_{obs(3)}$ *vs.* [$PhCH_2NH_2$].



Scheme 1

of each step was not large enough to determine the rate independently, the pseudo-first order rate constants for the three steps ($k_{obs(1)}$, $k_{obs(2)}$ and $k_{obs(3)}$) were determined simultaneously by computer simulation of the time course of the absorption change using a non-linear curve-fitting program (Mac curve fit) as reported in the previous study.[16] From the dependence of k_{obs} in each step on the amine concentration the rate constants were determined as $k_1 = 5.9 \times 10^{-2}$ M^{-1} s^{-1}, $k_2 = 8.7 \times 10^{-5}$ s^{-1}, $k_2' = 2.6 \times 10^{-2}$ M^{-1} s^{-1}, and $k_3 = 1.4 \times 10^{-2}$ M^{-1} s^{-1}, as shown in the insets in Fig. 2. It should also be emphasized that the oxidation of benzylamine by the Li^+ complex of **1** proceeds catalytically, and molecular oxygen is used as an electron acceptor to regenerate the iminoquinone form from the reduced TTQ. Thus, benzylamine (0.10 mol dm^{-3}) was converted into *N*-benzylidenebenzylamine quantitatively when it was treated with a catalytic amount of **1** (1.0×10^{-3} mol dm^{-3}; 1 mol %) in the presence of $LiClO_4$ (1.0 mol dm^{-3}) under aerobic conditions for 24 h.

In summary, a monovalent cation such as Li^+ has been demonstrated for the first time to bind to TTQ at its quinone moiety and makes it possible for the catalytic amine oxidation to occur efficiently in anhydrous MeCN. The Li^+ binding may accelerate the addition step of the amine (k_1) and enhances the stability of the intermediates, leading to the efficient catalytic oxidation of benzylamine. These results gave us an important insight into the catalytic mechanism of the monovalent cations in MADH- and AADH-catalysed reactions.

The present study was financially supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Culture, and Sports of Japan.

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11 Each spectrum in Fig. 1 was taken just after the addition of $LiClO_4$, since the redox reaction between **1** and benzylamine gradually proceeded over a prolonged reaction time.
12 Because of the low solubility of NH_4ClO_4, $KClO_4$, $CsClO_4$ and CsOTf in anhydrous MeCN, interaction between the quinone and those monovalent cations could not be examined accurately.
13 In the absence of benzylamine, the spectral changes (bathochromic shifts) and the K_{ML} values are much smaller than those measured in the presence of the amine: for Li^+, λ_{max} = 430 nm; K_{ML} = 3.6 dm^3 mol^{-1}; for Na^+, λ_{max} = 425 nm; K_{ML} = 2.3 dm^3 mol^{-1}. Benzylamine may abstract the pyrrole proton of **1** to enhance the Li^+ binding.
14 In spite of our great efforts, a single crystal of the Li^+ complex of **1** suitable for X-ray analysis has yet to be obtained. The ^{1}H NMR spectrum of **1** (1×10^{-3} mol dm^{-3}) in CD_3CN in the presence of $LiClO_4$ (1 mol dm^{-3}) showed downfield shifts for 1-H, 2-H and 5-H ($\Delta\delta$ = 0.39, 0.16 and 0.05 ppm, respectively), suggesting the coordination of Li^+ at the quinone moiety of **1**.
15 Although the reaction intermediates (substrate imine and product imine) and the aminophenol product of TTQ have been successfully isolated in the previous reaction in MeOH (ref. 16), isolation and detailed characterization of those species in the present system were hampered by Li^+ existing in large excess. The spectral change of the present reaction is, however, fairly close to that of the reaction of **1** and benzylamine in MeOH, (ref. 16), strongly supporting the idea that the intermediates and reduced product are the same in both systems.
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Communication a908914d