Mechanism of oxygenation of vitamin K hydroquinone

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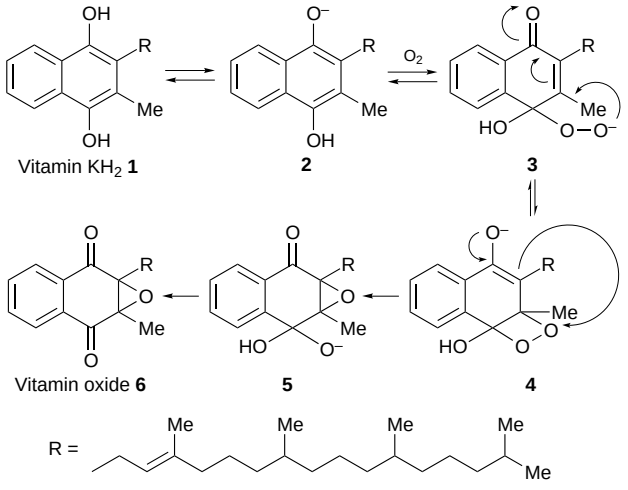
When the monoanion of vitamin K hydroquinone is treated with $^{18}O_2$ in THF, the product, vitamin K oxide, carries a full atom of ^{18}O at the epoxide oxygen and partial incorporation (34%) of a second atom of ^{18}O at the carbonyl oxygen, which is most reasonably ascribed to the dioxetane mechanism.

Vitamin K in its hydroquinone form, vitamin KH_2, is required as a cofactor for a microsomal enzyme that converts N-terminal glutamates in proteins of the blood clotting cascade to γ-carboxyglutamates.[1] Vitamin KH_2 is transformed into vitamin K oxide concurrent with the abstraction of the γ-hydrogen of glutamate, leading to γ-carboxyglutamate.[2] In studies of the mechanism of action of vitamin K, the role of molecular oxygen in the formation of vitamin K oxide was explored.[3,4] Key features of this mechanism are the rearrangement to a strong alkoxide base **5** *via* the dioxetane intermediate **4** (Scheme 1), proposed on the basis of results of a parallel oxygen-16 and oxygen-18 experiment,[3] and the corresponding oxidation of a model system.[4]

The nonenzymic model approach that supports the chemical principle used 2,4-dimethylnaphthol in place of vitamin KH_2.[4] However, the most important difference between the model and the vitamin K dependent carboxylase resides in the structure of vitamin K. Vitamin K is a 1,4-dioxygenated naphthalene, whereas the model carries only one oxygen. Since molecular oxygen can add to the 2-position of vitamin KH_2 **1** yielding a hydroperoxy anion **7** which might produce vitamin K oxide **6**,[5] it was interesting to compared the *in vivo* oxygenation reaction of vitamin K hydroquinone itself, in the absence of the enzyme.

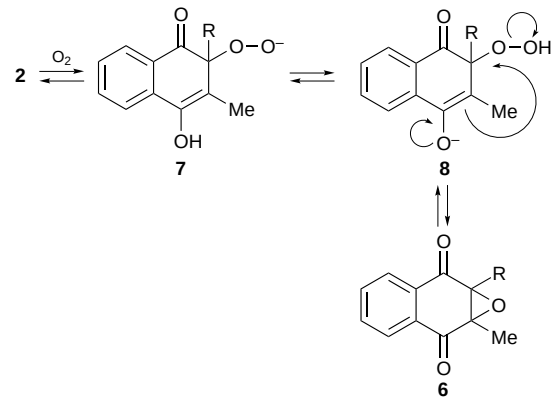
It is well-established that hydroquinones are unstable in air and rapidly oxidize to yield quinone. Indeed, when a chloroform solution of vitamin K hydroquinone **1** was stirred under an oxygen atmosphere, vitamin K was formed within 30 min. By contrast, when the monoanion of vitamin KH_2 **2** was stirred under an oxygen atmosphere, vitamin K oxide **6** was isolated in 88% yield (Scheme 2). These spontaneous oxidations in the chemical model system are clearly important for the mechanism of action of vitamin KH_2 in biological systems. Therefore, experiments employing oxygen-18 were next used to examine the involvement of the dioxetane intermediate, as shown in Scheme 1, because 2-hydroperoxide **8** could be differentiated from the dioxetane mechanism by ^{18}O labelling experiments.



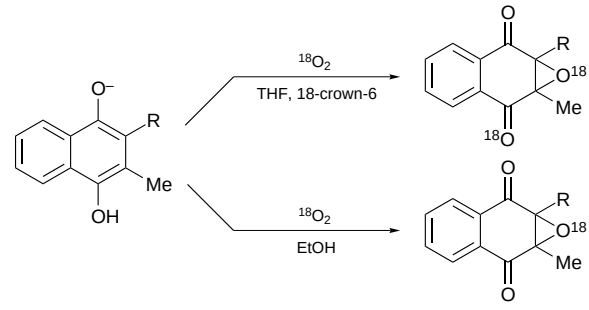
Scheme 1

Typical experimental conditions consisted of stirring a THF solution of a monoanion of vitamin KH_2 **2** at room temperature with 18-crown-6 and delivering $^{18}O_2$ from a sealed tube to yield vitamin K oxide **6**.† Examination of the mass spectrum reveals that an atom of ^{18}O has been incorporated into the vitamin K oxide **6**, since the molecular ion peak moves from *m/z* 466 to 468. The ^{18}O label could, in principle, be located at any of the three oxygen positions, but the mass spectrum is most consistent with location of the ^{18}O at the epoxide position. Thus, the *m/z* 423 peak is unchanged when comparing the unlabelled with the labelled spectrum; the $M^+ - 43$ peak at *m/z* 423 arises from cleavage of the epoxide and loss of a MeCO fragment.[3] Moreover, the intensity of the peak at *m/z* 470 was 41% of the parent peak at *m/z* 468, which is much larger than would be expected on the basis of natural abundance ^{13}C.[6] After correcting for natural abundance ^{13}C, the presence of 36% of a second atom of ^{18}O in the vitamin K oxide from the labelling experiment is indicated, and this is most reasonably ascribed to the dioxetane mechanism (Scheme 3).

In EtOH, the product of oxygenation of vitamin KH^- **2** under $^{18}O_2$ resulted in a shift of the *m/z* 466 peak to 468, with no increase in the intensity of the $M^+ + 4$ peak. The path leading to



Scheme 2



Scheme 3

the formation of vitamin K oxide in an aprotic solvent could be different from that in protic solvent. In the oxygenation of the vitamin K anion **2** in EtOH, the 2-peroxy anion intermediate **7** might predominate and might be protonated by EtOH, yielding the hydroperoxide **8**. If the phenolic hydroxy group of peroxide **7** is deprotonated by the resulting ethoxide, and with the assistance of a hydrogen bonding of solvent, the hydroperoxide could yield vitamin K oxide.

This solvent-dependent regioselectivity observed in the oxygenation of the vitamin K anion **2** might be rationalized in terms of the stability of the peroxy anion intermediates **3** and **7**. Since it is known that the potassium ion associates with crown ether more strongly than with protic solvents,[7] the peroxy anion intermediate would be naked. The resulting free peroxy anion **7** at the 2-position might experience electronic repulsion by the carbonyl group, whereas the peroxy anion **3** at the 4-position is stabilized by hydrogen bonding with the *gem*-hydroxy group.[8] therefore, the addition of oxygen using THF with 18-crown-6 might be preferred in the 4-position (Fig. 1).

The peroxy anion intermediate in EtOH seems to undergo stabilization by association with the counter cation K^+ and partly also by solvation through hydrogen bonding.[7] In this case, the stabilization of the peroxy anion **7** at the 2-position appears to be due to chelation with the carbonyl group of the intermediate.

The biological transformation of vitamin K oxide **6** appears to be shielded from water by the enzyme or membrane environment.[9] Therefore the chemical formation of vitamin K oxide **6** has parallels in the biological formation. However, another possibility for incorporation of ^{18}O into vitamin K oxide **6** would involve label exchange between ^{18}O labelled hydroxide and the product in THF, even though formation of hydroxide anion might be unfavourable under aprotic conditions. This path cannot be ruled out at present, and the appropriate control reactions remain to be conducted.

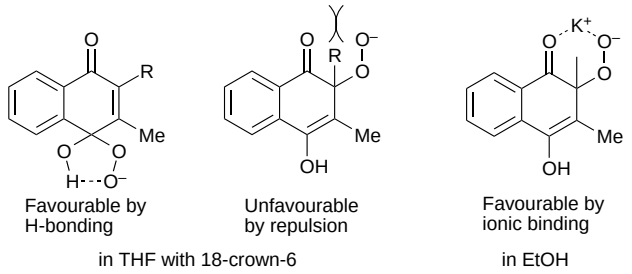


Fig. 1 Proposed solvent-dependent regioselectivity of peroxide in the oxygenation of the vitamin K hydroquinone monoanion

This research was generously supported by a grant from the Ministry of Education (BSRI-96-3416). The authors also acknowledge partial support from Chung-Ang University.

Footnotes

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† Typical experiment: oxygenation of **2** with $^{18}O_2$. A solution of 142.8 mg (0.316 mmol) of vitamin K hydroquinone **1** in 4 ml of dry THF was added to a suspension of 12.6 mg (0.315 mmol) of potassium hydride and 1 ml of dry THF *via* a double tipped needle and stirred for 10 min under an atmosphere of argon at room temperature. 18-Crown-6 (60.0 mg, 0.227 mmol) was then added. $^{18}O_2$ [96% enriched in 18-oxygen (ICON), 100 ml] was added through a break seal. After stirring for 20 min at room temperature, the reaction mixture was quenched with 5 ml of water and extracted with diethyl ether (3 × 10 ml). The combined diethyl ether layers were washed with saturated aqueous potassium chloride, dried over $MgSO_4$ and concentrated to yield 101.8 mg of the crude product as a yellow oil, which was checked by GC–MS (Hewlett-Packard 5890 Series II gas chromatograph and a Hewlett-Packard 5970 mass selective detector).

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Received in Cambridge, UK, 17th February 1997; Com. 7/01091E