Rapid Enzyme-Catalyzed Heterolytic C−H Bond Cleavage by a Base Strength Amplification Mechanism: A Theoretical Examination of the Mechanism of Oxidation of Vitamin K

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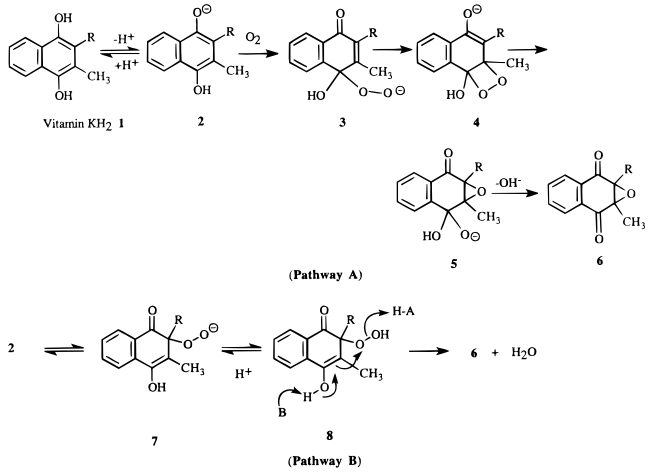
Received September 8, 1997

Vitamin K is the essential cofactor for vitamin K-dependent γ-glutamyl carboxylase, which catalyzes the conversion of N-terminal glutamates (Glu) in proteins of the blood clotting cascade to corresponding γ-carboxyglutamates (Gla).[1] The carboxylation catalyzed by γ-glutamyl carboxylase is accompanied by concomitant oxidation of the hydroquinone form of vitamin K to its quinone 2,3-epoxide. Recent studies have demonstrated that the catalytic carboxylation occurs via formation of a carbanion intermediate.[1,2] The C−H bond which is ionized is α to the terminal carboxyl group of the involved Glu with a pK_a of around 22.[3] Thus, it is neither thermodynamically nor kinetically favorable for any protein base to abstract the methylene hydrogen adjacent to the γ-carboxylate group unless the enolate anion intermediates can be effectively stabilized.[4] According to the reports of Paul Dowd and co-workers,[5−8] the oxidation of vitamin K is supposed to provide the needed strong base for this difficult proton abstraction. The process for the generation of this base has been termed base strength amplification.[8] Questions still remain as to exactly what is the strong base involved in the proton abstraction. There are suggestions that the base is **5** or HO^- generated by dissociation of **5** to **6** (Scheme 1). Here we report a theoretical investigation[9] of the possible intermediates involved in the oxidation of vitamin K. On the basis of the present study, an alternative pathway is suggested which is more consistent with recent experimental observations.

Scheme 1 displays two possible pathways for the formation of vitamin K 2,3-epoxide from the KH^- anion. Pathway A was proposed by Dowd and co-workers on the basis of studies on the model compound 2,4-dimethylnaphthol.[5−8] Pathway B is not feasible with 2,4-dimethylnaphthol but is a reasonable pathway for the oxidation of KH^-. Figure 1 shows the calculated geometry for each species involved in the oxidation of the reduced vitamin K.

Scheme 1



Addition of O_2 to compound **2** could occur at either C-2 or C-4 positions, forming the corresponding peroxides **3** and **7**. According to our calculations, peroxide **3** is favored in a vacuum since the negatively charged oxygen in **3** can be stabilized through intramolecular hydrogen bonding. Peroxide **3** is about 21.1 kcal/mol lower in energy than **7**. The initially formed peroxide intermediate **3** is not stable, and it rearranges to a dioxetane intermediate **4**, which then forms a much more stable epoxide alkoxide intermediate **5**. The dioxetane intermediate **4** is less stable than **3** by 10.4 kcal/mol. Intermediate **4** is separated from the alkoxide intermediate **5** by a small barrier of 3.9 kcal/mol. The alkoxide intermediate **5** is about 54.0 kcal/mol more stable than intermediate **4**. At the HF/6-31+G(d) level, intermediate **7** is about 30.5 kcal/mol lower in energy than the transition state (**9**) for the interconversion of **4** to **5**; however, at the B3LYP/6-31+G(d) level, it is higher in energy than **9** by 6.7 kcal/mol. Clearly, electron correlation has a significant effect on the calculated energetics. Intermediate **7** is probably still accessible even in low dielectric environment. The protonated species of **3** and **7** (**3H** and **7H)** are essentially isoenergetic. Therefore, both species should be accessible in a polar environment.

In the peroxide intermediate **3** (Figure 1**)**, there is an intramolecular hydrogen bond between the hydroxyl hydrogen and the oxyanion with a hydrogen bonding distance of 1.732 Å; the peroxide O−O distance is 1.450 Å and the C−O(−O) distance is 1.384 Å. Although the peroxide O−O distance in **7** remains about the same as in **3** (1.453 vs 1.450 Å), the C−O(−O) distance becomes shorter in **7** by 0.02 Å. In the transition state (**9**) for the interconversion of **4** and **5**, the breaking O- - -O distance is 1.829 Å and the forming O- - -C distance is 2.100 Å. In **5H** (the neutral form of **5**), the two C−O(−H) distances are 1.399 and 1.384 Å for the cis and trans hydroxyl groups, respectively. Structural information regarding these reactive intermediates are difficult to obtain experimentally. However, X-ray crystal structures of compounds analogous to **3H** and **5H** are known.[5] Indeed, our calculated structures for **3H** and **5H** are very similar to the X-ray crystal structures for analogous compounds investigated previously by Dowd and co-workers.[6]

The calculated gas-phase protonation energies for anions **2**−**5** and **7** are given in Table 1. Interestingly, peroxide intermediate **7** is more basic than **3** owing to the intramolecular hydrogen bonding in **3**. According to the present calculations, the gas-

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S0002-7863(97)03140-5 CCC: $15.00
Published on Web 02/04/1998

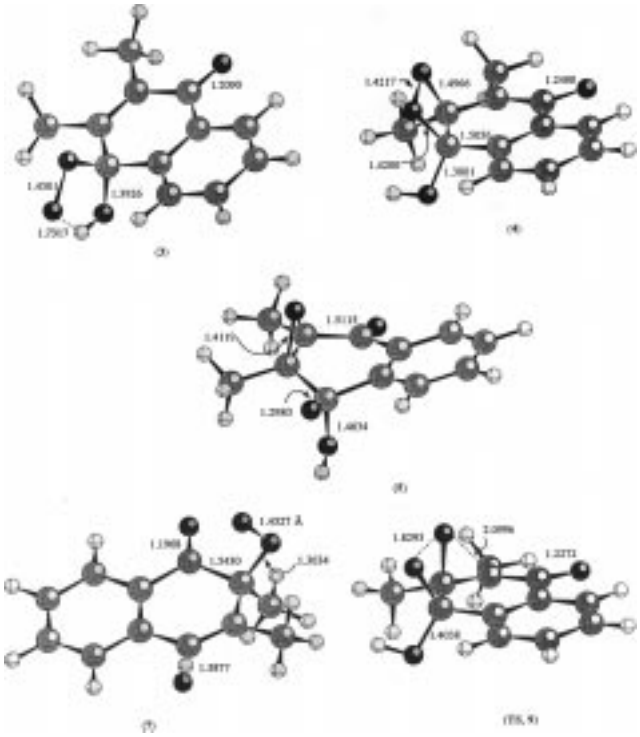

Figure 1. HF/6-31+G(d)-optimized structures for compounds **3**−**7** and **9**.

Table 1. Calculated Protonation Energies for **2**−**5** and **7** at the HF/6-31+G(d) and B3LYP/6-31+G(d) Levels of Theory

compd	HF/6-31+G(d)	B3LYP/6-31+G(d)
2	353.4	343.8
3	352.3	342.7
4	350.2	339.0
5	356.0	345.4
7	372.2	364.5

phase basicity follows the following order: **7** > **5** > **2** > **3** > **4**. As expected, the alkoxide intermediate **5** is more basic than any other species on pathway A.

One way to differentiate pathway A from pathway B is to carry out oxidation in the presence of $^{18}O_2$. The vitamin K oxide formed from pathway A should contain two ^{18}O atoms, while the vitamin K oxide from pathway B should have only one ^{18}O atom. It has recently been demonstrated[10] that oxidation of potassium 18-crown-6 salt of reduced vitamin K by O_2 (room temperature in a THF solution) provides about 34% product containing two ^{18}O atoms. This indicates that pathway A is operative. However, it is unclear why only 34% of the product contains two ^{18}O atoms. One possible explanation is that the proton scrambles between the two oxygen atoms (oxyanion and the hydroxyl) in **5** and the hydroxide cis to the epoxide oxygen is cleaved preferably. Indeed, according to our calculation, in **5H**, bonding of the hydroxyl group cis to the epoxide is weaker, having a longer C−O bond than the trans hydroxyl group (see Figure 2).

Alternatively, one may argue that the less than 100% double ^{18}O incorporation is an indication of the involvement of pathway B. The possible involvement of this latter mechanism in the epoxidation reaction was considered as long ago as 1978.[11] If pathway B is operative in the nonenzymatic oxidation of KH^-, only one ^{18}O will be incorporated into the vitamin K oxide. If both pathway A and B are involved, it could explain why only 34% of the vitamin K oxide product contains two ^{18}O atoms.

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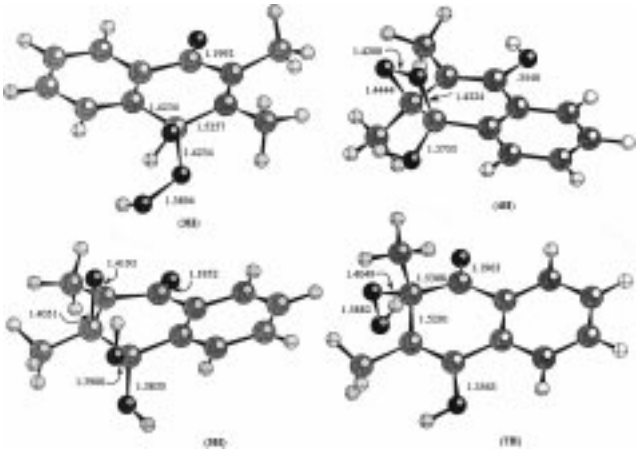
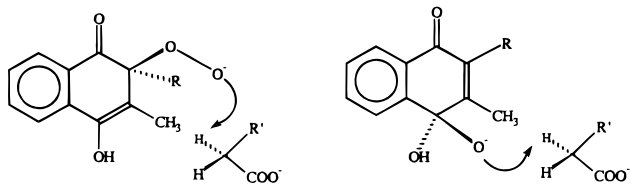


Figure 2. HF/6-31+G(d)-optimized structures for compounds **3H**−**5H** and **7H**.

Scheme 2



10 **11**

Surprisingly, when $^{18}O_2$ oxidation of KH^- was carried out in protic solvent such as ethanol, no second ^{18}O incorporation was detected in the vitamin K oxide product.[10] This latter experimental observation seems to suggest that, in polar environment such as in protic solvent, pathway B is indeed operative. This is also in agreement with our calculations that **3H** and **7H** are equally stable. Thus, it is very likely that in THF both pathways are operative, with pathway A being the dominant one. Apparently, in protic solvent such as ethanol, pathway B is favored since the peroxide **7** can then be stabilized through intermolecular hydrogen bonding.

Since an isotope incorporation study with carboxylase has shown that about 18% of vitamin K epoxide contains two ^{18}O atoms, pathway A must be involved in the enzymatic reaction.[8] As to why only 18% of the vitamin K oxide contains the second ^{18}O atom, there are two possible explanations as in the nonenzymatic oxidation reaction discussed previously. The first possible explanation is that the enzyme somehow favors the preferable cleavage of the ^{18}OH of **5** as seen in the coenzyme B_{12}-dependent dioldehydratase.[6,12] The involvement of pathway B provides a simple alternative explanation. It is conceivable that the two bases **5** and **7** compete for the carbon acid hydrogen. If so, the stereochemistry of the intermediates would have to be as shown in structures **10** and **11** (Scheme 2). The involvement of pathway B in the enzymatic reaction would be more consistent with recent experimental observation[13] that, in the absence of propeptide or glutamate-containing substrate, the epoxidation of vitamin K does not occur, indicating that the enzyme plays a role in the mechanism of vitamin K epoxidation. Epoxidation via pathway A does not require the participation of the enzyme while the involvement of enzyme is necessary for pathway B.

Acknowledgment. This work was supported by the Petroleum Research Fund of the American Chemical Society. The support by National Center for Supercomputing Applications is also appreciated.[14]

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