This student paper was written as an assignment in the graduate course

Free Radicals in Biology and Medicine

(77:222, Spring 2003)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2003 Term

Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

The Fine Print:

Because this is a paper written by a beginning student as an assignment, there are no guarantees that everything is absolutely correct and accurate.

In view of the possibility of human error or changes in our knowledge due to continued research, neither the author nor The University of Iowa nor any other party who has been involved in the preparation or publication of this work warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for the results obtained from the use of such information. Readers are encouraged to confirm the information contained herein with other sources.

Vitamin K

Antioxidant versus Prooxidant

By
Gang Niu

Free Radical and Radiation Biology Program
B-180 Medical Laboratories
The University of Iowa
Iowa City, IA 52242-1181, USA

For 077:222 Spring 2003
17th, February 2003
Paper II

Abbreviations

GST	Glutathione *S*-transferase
IUPAC	International Union of Pure & Applied Chemistry
KH_2	Vitamin K hydroquinone
mEH	Microsomal epoxide hydrolase
PT	Prothrombin
VKD	Vitamin K–dependent
VKOR	Vitamin K epoxide reductase

Table of Contents

Abstract

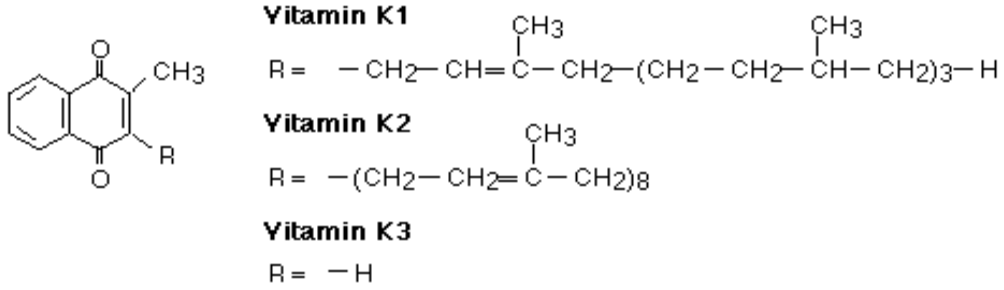
Vitamin K is a group name for several related compounds that have in common a methylated naphthoquinone ring structure and that vary in the aliphatic side chain attached at the 3-positions. They all exhibit ultraviolet, infrared and nuclear magnetic resonance absorption spectra that are characteristic of the naphthoquinone nucleus. The function of vitamin K in higher organisms is as a cofactor for an enzymatic carboxylation reaction, which is necessary for vitamin K–dependent (VKD) proteins. The vitamin K metabolite during the reaction, vitamin K_1 2,3-epoxide, is reduced by liver enzyme(s) back to the hydroquinone cofactor form of the vitamin, vitamin K_1H_2 to form Vitamin K cycle. Vitamin K_1H_2 may act as a potent antioxidant and radical scavenger in lipid peroxidation. However, in their one electron reduction process, semiquinone radicals are generated and ready to react with molecular oxygen resulting in generation of ROS.

Introduction

In 1935 Dam et al proposed that the antihemorrhagic vitamin of the chick was a new fat-soluble vitamin, which he called “vitamin K” [1]. Not only was K the first letter of the alphabet which was not used to describe an existing or a postulated vitamin activity at that time, but it was also the first letter of the German word *Koagulation*. The methylated naphthoquinone ring structure of vitamins make them potent antioxidant, but the mechanism still need be clarified.

Structure and Nomenclatrue

Vitamin K is a group name for several related compounds that have in common a methylated naphthoquinone ring structure and that vary in the aliphatic side chain attached at the 3-position



(Figure 1) [2].

Figure 1. Structure of three forms of Vitamin K

The nomenclature of these compounds was summarized in Table 1 [3]. The menaquinones can be subdivided further with respect to their aliphatic side-chain length into menaquinone-4 (MK-4, containing four isoprenoid residues) and the long-chain menaquinones (MK-n, containing n isoprenoid residues). It is generally accepted that the naphthoquinone is the

functional group, so the mechanism of action is similar for all K vitamins. Substantial differences may be expected, however, in intestinal absorption, transport, tissue distribution, and bio-availability. These differences are caused by the different lipophilicities of the various side chains and by the different food matrices in which they occur [4].

Table 1. The Nomenclature of Vitamin K

Chemical name	Old	IUPAC
2-Methyl-1,4-naphthoquinone(I)	K_3	Menadione
2-Methyl-3-phytyl-1,4-naphthoquinone(II)	K_1	Phylloquinone(K)
2-Methyl-3-multiprenyl-1,4-naphthoquinone(I)(class)	$K_{2(n)}$	Menaquinone-n(MK-n)
2-Methyl-3-farnesylgeranyl-geranyl-1,4-aphthoquinone(III)	$K_{2(35)}$	Menaquinone-7(Mk-7)

Physical Charaterization and Detection

The various forms of the vitamin can readily be characterized by a number of physical methods. They exhibit an ultraviolet spectrum which is characteristic of the naphthoquinone nucleus with four distinct peaks between 240 and 280 nm, and a less sharp absorption at around 320-330 nm. These compounds also exhibit characteristic infrared and nuclear magnetic resonance absorption spectra. Again, the characteristic features of the spectra are largely those of the naphthoquinone ring. Nuclear magnetic resonance analysis of phylloquinone has been used to firmly establish that natural phylloquinone is the trans isomer. Mass spectroscopy has been useful in determining the length of side chain and the degree of saturation of vitamins of the menaquinone series isolated from natural sources [5]. The ultraviolet, infrared, nuclear magnetic resonance, and mass fragmentation spectra of phylloquinone are shown in Figure 2 [3]. The spectra of vitamins of the menaquinone series are similar.

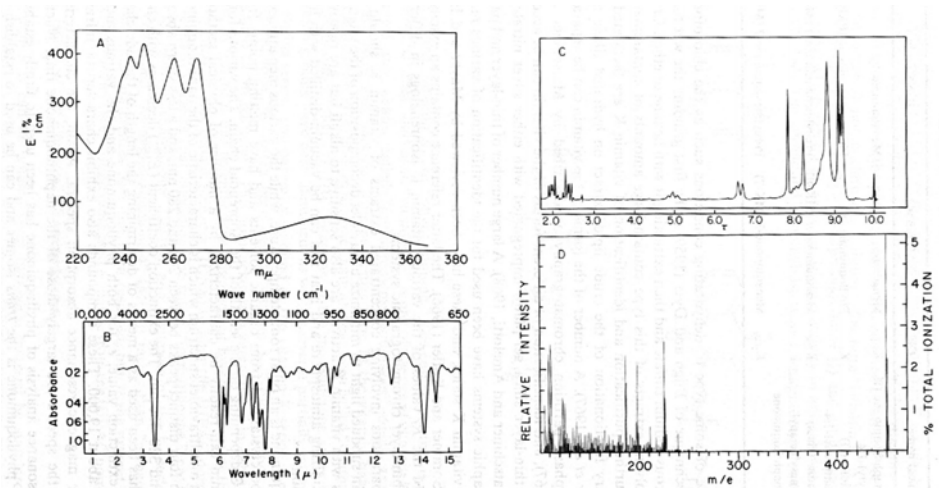


Figure 2. A: Ultraviolet absorption spectrum of phylloquinone in petroleum ether. B: Infrared absorption spectrum of phylloquinone. C: Nuclear magnetic resonance spectrum of phylloquinone in $CDCl_3$ at 60 Mc. D: Mass fragmentation spectrum of phylloquinone, the parent molecular ion, at m/e 450.

Physiological Function and Vitamin K Cycle

The only recognized function for vitamin K in higher organisms is as a cofactor for an enzymatic reaction that converts glutamyl residues (glu's) to γ-carboxylated glutamyl residues (gla's) in a class of proteins referred to as vitamin K–dependent (VKD) proteins. These proteins are modified by the VKD- or γ-carboxylase as they are secreted through the endoplasmic reticulum (ER). A model has been proposed in which a weak base (cys) generates a strong base (e.g., vit K alkoxide) that is sufficient for the stereospecific abstraction of a hydrogen ion from the γ-glutamyl position [6] . Subsequent addition of CO_2 to the carbanion intermediate produces the gla residue. Each cycle of glu to gla conversion results in the oxidation of vitamin K hydroquinone (KH_2) to vitamin K epoxide, and the carboxylase is also an epoxidase.

Carboxylation, which is required for the biological activity of VKD proteins, occurs at multiple residues within a region called the gla domain. Carboxylation results in Ca^{++}-binding and a conformational change in the gla domain, with consequent insertion of hydrophobic residues within this domain into phospholipid bilayers in which VKD proteins exert their biological effects [7].

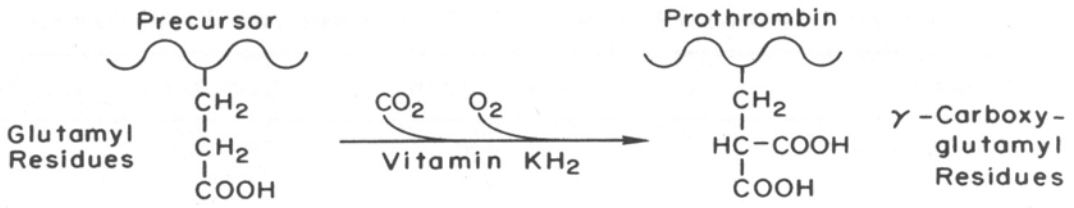


Figure 3. The vitamin K dependent carboxylation [3].

The VKD proteins presently comprise a family of ~12 proteins. The first such proteins identified were ones involved in hemostasis, including prothrombin (PT), factor IX (fIX), factor VII (fVII), factor X (fX), protein C (PC) and protein S (PS)[8]. Subsequent identification of other VKD proteins has shown that carboxylation is important to a broader range of biological functions, including bone morphogenesis [bone gla protein (BGP) and matrix gla protein (MGP)] and growth control [(gas)6] [9]. VKD proteins with potential functions in signal transduction [proline-rich gla protein (PRGP)-1 and PRGP-2] [10] and a VKD protein of unknown function (protein Z) have also been discovered. Finally, the carboxylase itself has been shown to be a VKD protein [11]. This observation was a surprise because the carboxylase does not share any homology with the other VKD proteins. It is very likely that other VKD proteins remain to be

discovered. Tissue distribution of the carboxylase is widespread, thus accommodating such a possibility.

γ-Carboxylation of one Glu residue is coupled stoichiometrically to formation of 1 molecule of the vitamin K metabolite, vitamin K_1 2,3-epoxide. The epoxide is reduced by liver enzyme(s) back to the hydroquinone cofactor form of the vitamin, vitamin K_1H_2, and this cyclic conversion establishes a redox cycle for vitamin K in liver, known as the vitamin K cycle.

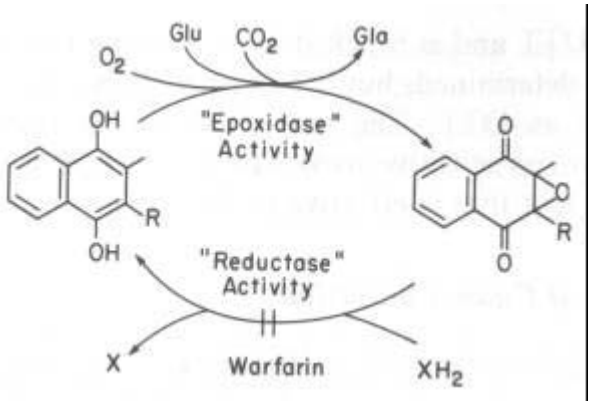


Figure 4. Vitamin K cycle [3].

In 1985, Wallin *et al.* [12] found that the human liver contained a warfarin-sensitive enzyme- vitamin K epoxide reductase (VKOR) whose function was to reduce K_1 epoxide to K_1H_2. This enzyme is inhibited by 4-OH coumarin analogs (e.g., warfarin), leading to the rapid depletion of the KH_2 supply.

Antioxidant Versus Prooxidant

The 1,4-naphthoquinone structure of the K vitamins resembles the benzoquinone structure, and thus these vitamins may contain antioxidative properties as well. Menadione is a very potent inhibitor (IC50<<5μM) of microsomal lipid peroxidation. Its activity is believed to result mainly from its capacity to divert electrons from membrane lipids. Active vitamin K differs, however,

from menadione by a poly-isoprenoid(menaquinoese or vitamin K2) or a phytyl(phylloquinone or vitamin K1) chain at the 3 position. Talcott et al. showed that 3-substitution weakened the activity of menadione as an inhibitor of lipid peroxidation [13]. Furthermore, the water insolubility of the vitamin Ks may prevent them from catching electrons. Indeed, studies from several groups showed only weak activity (IC50>>100μM) of K vitamins as inhibitors of lipid peroxidation in various test system. On the other hand, the hydroquinone of vitamin K1 may act as a potent antioxidant. Mukai et al. found the hydroquinone to be 10 times as potent as α-tocopherol in the reaction with phenoxyradicals and 100 times as potent as ubiqunol in regenerating vitamin E from its radical [14].

Regeneration of the Vitamin KH2 is warranted by vitamin K epoxide reductase, which reduces the epoxide to the quinone and thereafter to the KH2. The notion that this vitamin K cycling may accommodate potent antioxidant activity is supported by the fact that 1) epoxidation can be dissociated from carboxylation and 2) the microsomal vitamin K epoxide reductase is present in reserve.

The bioreductive activation step of vitamin K consists of one-electron and two-electron reductions. The two-electron reduction pathway has generally been considered to predominate in the vitamin K cycle. Coumarin anticoagulants block the vitamin K cycle by inhibition of vitamin K epoxide reductase and the two-electron reduction and thus leads to anticoagulation. However, the anticoagulation can be overcome by administration of large doses of vitamin K. The antidote effect of the vitamin is due to the formation of vitamin K hydroquinone via the one-electron reduction pathway which is unaffected by coumarins. The one-electron reduction generates the semiquinone radical which can readily react with molecular oxygen to produce active oxygen

species and hence undergoes a futile redox cycling [15]. The net result of this redox cycling is oxidative stress such as hepatotoxicity resulting from the generation of ROS. The toxicity of quinones is enhanced by the addition of coumarin, a potent inhibitor of the enzyme for two-electron reduction [16].

Summary

The core structure of Vitamin K is a methylated naphthoquinone ring which confers characteristic ultraviolet, infrared and nuclear magnetic resonance absorption spectra. Vitamin K is necessary for vitamin K–dependent (VKD) proteins' carboxylaiton. The bioreductive activation step of vitamin K consists of one-electron and two-electron reductions. The two-electron reduction pathway has generally been considered to predominate in the vitamin K cycle. Vitamin K_1H_2 may act as a potent antioxidant while semiquinone radicals generated in their one electron reduction pathway are ready to react with molecular oxygen to give active oxygen species.

References

1. Dam H. (1935) The antihemorrhagic vitamin of the chick: Occurrence and chemical nature. *Nature.* **135:**652-653.
2. Vermeer C, Schurgers LJ. (2000) A comprehensive review of vitamin K and vitamin K antagonists. *Hematol Oncol Clin North Am*. **14:**339-353
3. DeLuca HF. (1978) *Handbook of lipid research: the fat-soluble vitamins*. New York: Plenum Press.
4. Groenen-van Dooren MM, Ronden JE, Soute BA, Vermeer C. (1995) Bioavailability of phylloquinone and menaquinones after oral and colorectal administration in the vitamin K-deficient rat. *Biochem Pharmacol.* **50:**797-801
5. Sommer P, Kofler M. (1966) Physicochemical properties and methods of analysis of phylloquinones, menaquinones, ubiquinones, plastoquinones, menadione, and related compounds. *Vitam Horm.* **24:**349-399
6. Dowd P, Hershline R, Ham SW, Naganathan S. (1995) Vitamin K and energy transduction: a base strength amplification mechanism. *Science.* **269:**1684-1691
7. Sunnerhagen M., Drakenberg T., Forsen S., Stenflo J. (1996) Effect of Ca^{2+} on the structure of vitamin K-dependent coagulation factors. *Haemostasis.* **26:**45-53
8. Furie B, Furie BC. (1988) The molecular basis of blood coagulation. *Cell.* **53:**505-518
9. Price P A. (1988) Role of vitamin-K-dependent proteins in bone metabolism. *Annu Rev Nutr.* **8:**565-583
10. Kulman JD, Harris JE, Haldeman BA, Davie EW. (1997) Primary structure and tissue distribution of two novel proline-rich γ-carboxyglutamic acid proteins. *Proc Natl Acad Sci USA.* **94:**9058-9062
11. Berkner KL, Pudota BN. (1998) Vitamin K-dependent carboxylation of the carboxylase. *Proc Natl Acad Sci USA.* **95:**466-471
12. Wallin R, Martin LF. (1985) Vitamin K-dependent carboxylation and vitamin K metabolism in liver. Effects of warfarin. *J Clin Invest.* **76:**1879-1884
13. Talcott RE, Smith MT, Giannini DD.(1985) Inhibition of microsomal lipid peroxidation by naphthoquinones: structure-activity relationships and possible mechanisms of action. *Arch Biochem Biophys.* **241:**88-94
14. Mukai K, Morimoto H, Kikuchi S, Nagaoka S. (1993) Kinetic study of free-radical-scavenging action of biological hydroquinones (reduced forms of ubiquinone, vitamin K and tocopherol quinone) in solution. *Biochim Biophys Acta.* **1157:**313-317
15. Smith MT. (1985) Quinones as mutagens, carcinogens, and anticancer agents: introduction and overview. *J Toxicol Environ Health.* **16:**665-672
16. Thor H, Smith MT, Hartzell P, Bellomo G, Jewell SA, Orrenius S. (1982) The metabolism of menadione (2-methyl-1,4-naphthoquinone) by isolated hepatocytes: a study of the implications of oxidative stress in intact cells. *J. Biol. Chem.* **257:**12419–12425.