Vol. 105, No. 1, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 15, 1982 Pages 231-235

# IDENTITY OF PEROXY RADICALS PRODUCED FROM VITAMIN K IN OXYGENATED SOLUTIONS AS STUDIED BY PULSE RADIOLYSIS TECHNIQUE

Parinam S. Rao* and Robert E. Olson

Department of Medicine and Biochemistry
St. Louis University School of Medicine
St. Louis, Missouri 63104

Received November 30, 1981

SUMMARY: The transient optical absorption spectra of the peroxy radicals of vitamin K were observed in aqueous solutions using the fast reaction technique of pulse radiolysis and kinetic absorption spectrophotometry. From the change in absorbance of the transient spectra with pH, the ionization constants (pK) for these radicals were determined. The pK values for the hydroxyl and peroxy radical adducts were found to be 5.7 and 5.5, respectively. The efficiency and rate of electron transfer from these peroxy radicals to p-benzoquinone (BQ) were determined. Electron transfer from the peroxy radical anion to BQ is very efficient (60%) leading to the formation of $\cdot BQ^-$. The importance of these radicals in oxidation and biochemical reactions are discussed.

The vitamin K dependent carboxylation of glutamic acid to γ-carboxy glutamate in prothrombin precursors requires $O_2$, $CO_2$ and NADPH. A free radical mechanism involving the oxygen intermediates (1) and hydroperoxide of vitamin K (2) are postulated to be the species involved. Direct study of this process which probably involves peroxy radicals of vitamin K is in its infancy due to experimental difficulties in observing and identifying them. The electron spin resonance spectra of the peroxy radicals have no hyperfine structure except that due to $^{17}O$. The optical absorption spectra on the other hand are very similar to $\cdot HO_2$ and $\dot{O}_2$ (3). Pulse radiolysis provides a convenient method for the production of peroxy radicals in oxygenated aqueous solutions without the necessary intermediate formation of an associated excited state. Recently (4), the absorption characteristics, pK's and their rate of reaction with $O_2$ for the semiquinone radicals of vitamin K have been determined. We now report on the existence, identity, and acid-base properties of the vitamin K peroxy radical adduct in aqueous solution.

*Present address: Department of Medicine--Division of Cardiology, Montefiore Hosp., 111 East 210th St., NY 10467

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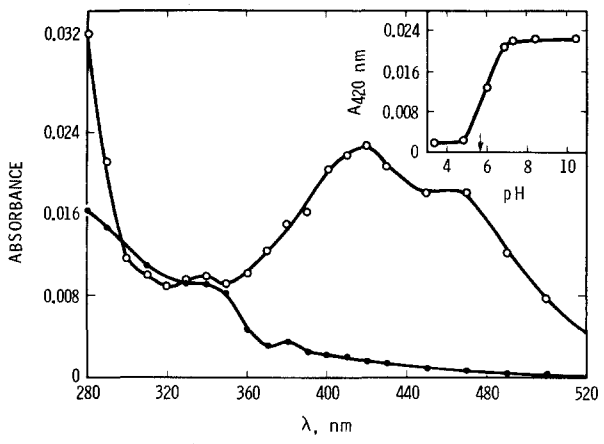



Fig. 1 Transient optical absorption spectra produced from the reaction of OH radicals with vit K ($2.10^{-5}$ M in the presence of 1 atm $N_2O$) at pH 10.4 (●) and pH 3.4 (o). Insert: absorbance at 420 nm versus pH.

## MATERIALS AND METHODS

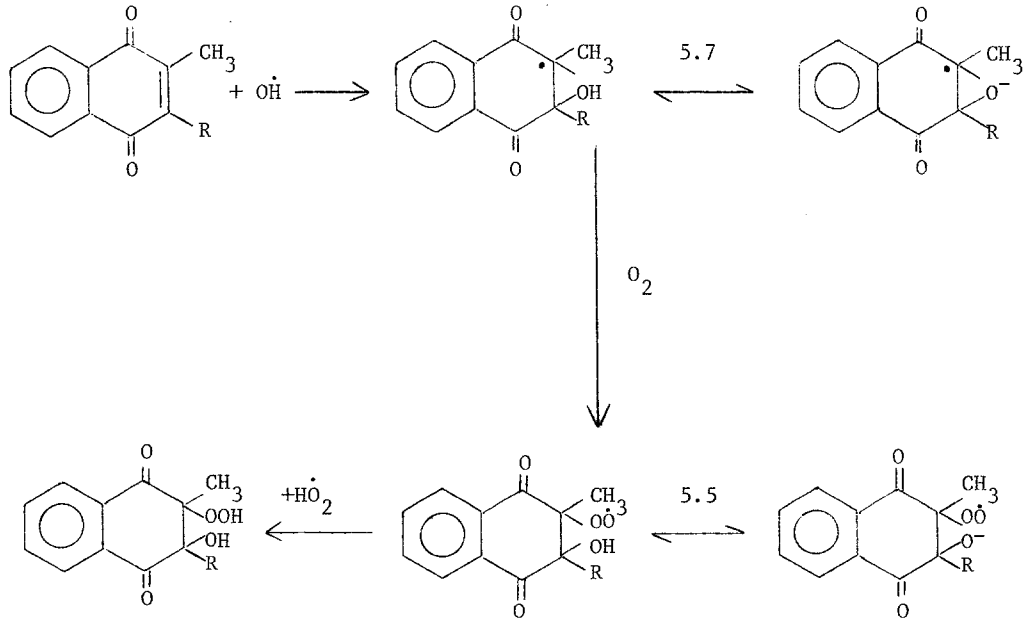
The technique of pulse radiolysis and fast kinetic absorption spectroscopy as described earlier was used (5). Electron pulses ranging from 100-800 ns duration from a 3-4 MeV electron linear accelerator were used. The concentration of transients produced was of the order of $10^{-6}$ to $10^{-7}$ M. Transient absorptions (250-520 nm) were determined using an RCA 7200 photomultiplier as detector and a Bausch and Lomb grating monochromator type 33-86-25 (band width 3 nm) with appropriate corning filters. The radical formation and decay kinetics data were automatically printed out by a computerized Hewlett Packard 8010 system. The vitamin K-hydroxyl radical adduct was produced by reaction of OH radicals with vitamin K ($2 \times 10^{-5}$ M) in aqueous solution in presence of 26 mM $N_2O$ (to convert $e^-_{aq}$ to OH). The peroxy radical adduct was produced using 13 mM $N_2O$ and 0.7 mM $O_2$. Total dose used was 7 krads/pulse for determinations of transient absorption spectra, pK's of the radical adducts, and ~1.0 krads/pulse when studying the electron transfer properties of these radicals to p-BQ. Dosimetry was carried out using aerated thiocyanate solutions ($1.10^{-2}$ M) taking G ($CNS^-$) = 2.9 and $\varepsilon (CNS)^-_2$ = 7600 $M^{-1}$ $cm^{-1}$ at 500 nm. Vitamin K was obtained from Sigma Chemical Co. and its concentration determined using $\varepsilon_{270}$ of 19,100 in alcohol. Perchloric acid, KOH, phosphates and tetraborate (1 mM) were used as buffers. The solutions were saturated with appropriate gases in dark using the syringe technique (6) and transferred into 2 cm quartz reaction cells (with high purity silica windows) under argon.

## RESULTS AND DISCUSSION

The nature and absorption spectra of vitamin K hydroxyl radical adducts are obtained from $N_2O$ saturated solutions of vitamin K by monitoring the radical spectra in the range of 250-520 nm (cf. Fig. 1). The spectrum produced by reaction of OH radicals with vitamin K has a rather similar transient absorption to that produced from menaquinone (7), except that the extinction coefficients are smaller and with more intense shoulders. The radical is designated vitamin K-OH. Similar data were reported earlier (7) by one of the authors using vitamin

$K_3$. The formation of vitamin K-hydroxyl radical adduct at other positions on the ring has been ruled out due to the absence of the characteristic cyclohexadienyl radical absorbalnce peak at 540 nm. The radical formation by side chain of vitamin K has also been ruled out as the spectra obtained is similar to that of menaquinone which has no side chain (cf. Ref. 7). On monitoring the change in absorbance with pH at a fixed wavelength (420 nm), a titration type curve is obtained (inset Fig. 1) from which a pK value of 5.7 0.1 is derived.

Formation and Ionization of Peroxy Radical Adduct: The transient optical spectra of the intermediates produced in the presence of $O_2$ are distinctly different from those in its absence. Fig. 2 shows the spectrum obtained on pulse radiolysis of aqueous solution of $2.10^{-5}$ M, vitamin K at pH 10.4 and 3.4 in 13 mM $N_2O$ and 0.7 mM $O_2$. The radical anion at pH 10.4 has a maximum at 275 nm ($\varepsilon_{275}$ = 1200 $cm^{-1}$). In contrast, without $O_2$ the vitamin $\dot{K}$-OH adduct produced at this pH has a maximum well below 280 nm with another peak in the visible ($\varepsilon_{420}$ = 280). At pH 3.4 in the presence of $O_2$ the absorbance is small ($\varepsilon_{255}$ = 442 $m^{-1}$ $sec^{-1}$). The pK of the peroxy radical anion - radical adduct is determined to be 5.5 ±0.1 (inset, Fig. 2).




On pulse radiolysis of 2 x $10^{-5}$ M vitamin K in the presence of 13 mM $N_2O$, 0.7 mM $O_2$ and 10 μM BQ, all the OH radicals produced react with vitamin K, all the $e^-_{aq}$ with $N_2O$ and essentially none with $O_2$ or BQ. At pH 3.4 when all the $\dot{O}_2$

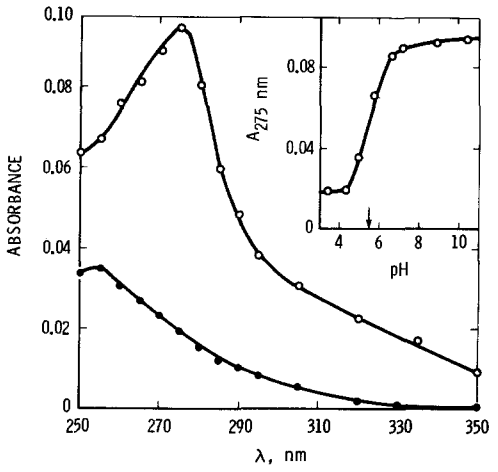



Fig. 2 Transient absorption spectra of the peroxy radical of $O_2$ vit K-OH at pH 10.4 (•) and 3.4 (o) produced on pulse radiolysis of aqueous solution of $2.10^{-5}$ M vit K in the presence of 13 mM $N_2O$ and 0.7 mM $O_2$. Insert: absorbance at 275 nm versus pH.

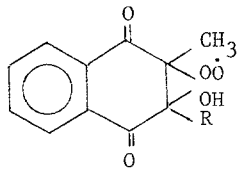
vitamin K-OH is present in its acidic form, very little (10%) formation of the semiquinone radical of BQ ($BQ^{\cdot-} - H^+$) is observed at 400 nm. On the other hand, at pH 10.4, the ionized form of the peroxy radical reacts with BQ and the characteristic absorption at 430 nm due to the semiquinone radical anion ($\dot{B}Q^-$) is observed (60% transfer). The electron transfer from Na formate ($\dot{C}O_2^- + BQ \rightarrow \dot{B}Q^-$) at 430 nm is taken as 100% (Table 1).

TABLE 1

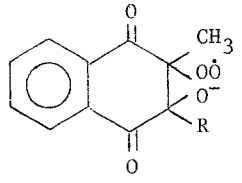
Extinction, Ionization Constants of Radicals Produced from Vitamin K and Rate-Efficiency of Electron Transfer to BQ in Aqueous Solutions

| Substrate | pH | Radical | ε/nm | pK | 2k | Electron Transfer to BQ* | |
|---|---|---|---|---|---|---|---|
| | | | | | | Efficiency | Rate k |
| | | | | | $m^{-1}s^{-1}$ | % | $m^{-1}s^{-1}$ |
| $2.10^{-5}$ M Vit K | 10.4 | ·Vit K-O$^-$ | 280(420) | 5.7 | $1.1x10^{-9}$ | 90 | $4x10^9$ |
| 26 mM $N_2O$ | 3.4 | ·Vit K-OH | 430(280) | | $8.1x10^9$ | 70 | $8x10^9$ |
| $2.10^{-5}$ M Vit K | 10.4 | ·$O_2$ Vit K-O$^-$ | 1200(275) | 5.5 | $1.0x10^8$ | 60 | $2x10^9$ |
| 13 mM $N_2O$ | 3.4 | ·$O_2$ Vit K-OH | 442(255) | | $8.0x10^8$ | 10 | — |
| 0.7 mM $O_2$ | | | | | | | |

*$2.10^{-5}$ M Vit K, 13 mM $N_2O$, 0.7 mM $O_2$ and 1 x $10^{-5}$ M BQ




+ BQ ⟶‖ $BQ\text{-}H^+$ + R + $O_2$




+ BQ ⟶ $\dot{B}Q^-$ + R + $O_2$

This presents a direct evidence for the existence of $\dot{O}_2$ vitamin K-OH radical. Its spectrum is sufficiently different from that of $\dot{O}_2^-/(\dot{H}O_2)$ to rule out electron transfer to $O_2$.

The ability of ionized peroxy radicals to undergo electron transfer reactions to various acceptors having higher redox potentials, whereas unionized peroxy radicals do not, can be considered very significant. Particularly this is so since it would seem that most peroxy radicals have pK's in the physiological pH range of 5-7.

Thanks are due to Dr. Robert E. Olson for his encouragement and suggestions.

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