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CoQ Semiquinone Radical

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Abstract

CoQ is a vital component of cellular respiration. It is a very hydrophobic molecule that can be found in the lipid bilayers of the mitochondrial membrane. It consists of a quinone ring structure and an isopreniod tail [1]. CoQ transfers electrons from complex II and I to complex III in the electron transport chain. When CoQ accepts a single electron, a CoQ semiquinone radical is formed. This radical species carries a negative charge and an unpaired electron that can react with a variety of molecules. The semiquinone radical is a relatively stable free radical and it can be directly measured by EPR [1]. The semiquinone radical also reacts directly with molecular oxygen to produce superoxide [2].

Introduction

CoQ is an essential biological molecule that is found in lipid bilayer of membranes. It is most notably found in the mitochondrial membrane where it is an electron carrier in the cellular respiration. CoQ has many other functions in the cell. It is an efficient antioxidant and protector against lipid peroxidation. CoQ is also involved in free radical production and even has a hand in vitamin E regeneration. Many of the functions of CoQ involve oxidation and reduction reactions. These reactions can create a very interesting species of CoQ, the semiquinone radical. This free radical species is relatively stable as well as reactive. This paper will provide an overview and insight into the CoQ semiquinone radical including its structure, location, and cellular reactions.

Structure of CoQ

CoQ is also known as ubiquinone or quinone. It is composed of a 2,3-dimethoxy-5-methyl-6-polyprenylbenzoquinone ring structure and an isopreniod tail. This tail can vary in length from 1 to 12 subunits with the ten-subunit form being the most prevalent. This paper will focus on the 10-subunit form of CoQ. All data and figures presented will pertain to the 10-subunit homolog unless otherwise noted. The long tail gives the molecule a strong hydrophobic characteristic. CoQ has structural features that are related to fat-soluble vitamins. The melting point of CoQ is 49^{o}C and a molecular weight of 862 g. The pKa of CoQ in water is 13.3 at 23^{o}C [1].

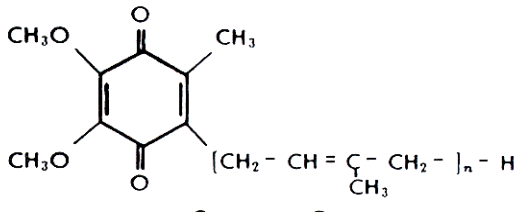
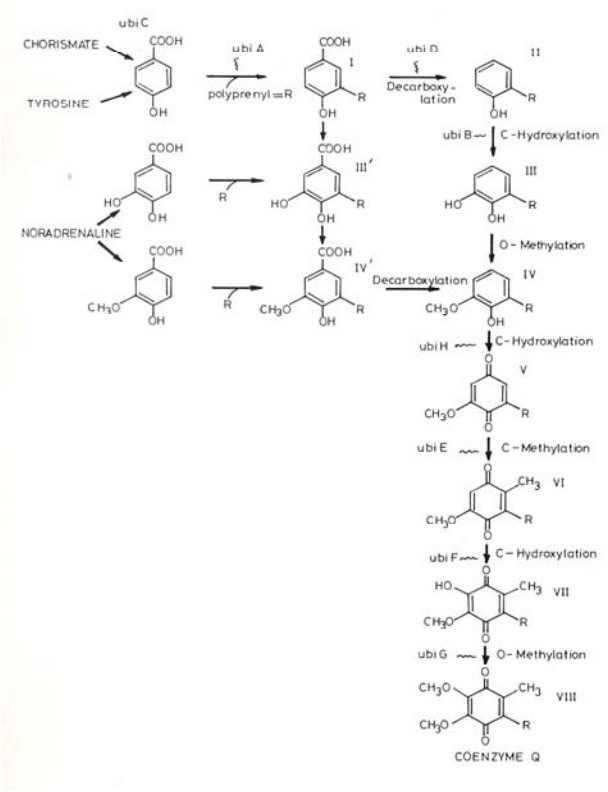


Figure 1. The structure of CoQ. Isopreniod tail can exist from n=0 through n=10 [3].

The structure of CoQ can exist in two conformation forms, folded and extended. The reduced form of CoQ exists in the folded form, while the oxidized form is found in the extended conformation. The folded confirmation is a lower energy, more stable conformation. The length of the molecule is approximately 21 angstroms. [4]

Biosynthesis of CoQ

The biosynthesis of CoQ requires the formation of the ring structure and isopreniod tail side chain. The formation of the ring requires eight reactions – 3 C-hydroxylations, 2 O-methylations, 1 C-methylation, 1 decarboxylation, and 1 C-alkylation by a polyprenyl group. The formation and length of the side chain is dependent on the polymerization of isopentenyl pyrophosphate. Scheme 1 shows a detailed description of the reactions involved in the ring formation of CoQ [1].



Scheme 1. Biosynthesis pathway for the formation of the ring structure of CoQ (coenzyme Q). "R" represents the location of the isopreniod tail side chain [1].

CoQ Localization

CoQ is a very hydrophobic molecule that is naturally found in lipid bilayers. CoQ exists unbound in the lipid bilayer and is free to move and disperse throughout the membrane. CoQ transfers electrons from the NADH-CoQ reductase complex (complex I) and the Succinate-CoQ reductase complex (complex II) to the $CoQH_2$-Cytochrome c reductase complex (complex III) in the mitochondrial membrane. While CoQ binding proteins do exist in the lipid bilayer, the majority of CoQ is unbound and free to move about the lipid bilayer. CoQ that is used in the electron transport chain comes from a Q-pool that is deplenished and replenished by the oxidation/ reduction reactions of CoQ.

As CoQ accepts and donates electrons semiquinone radicals are formed and dismantled. During this process protons drive a process known as the 'Q-cycle' which recycles the population of oxidized and reduced CoQ [5].

CoQ Semiquinone Radical Formation

When CoQ is reduced by a single electron, the CoQ semiquinone radical species is formed: $CoQ + e^- \longrightarrow CoQ^{\bullet -}$.

The semiquione radical is relatively stable when compared with other free radical species. Because of this stability this radical can go on to react in a variety of different pathways. It, like its parent molecule CoQ, is most notably involved in the electron transport chain. The CoQ semiquinone radical has a pK_a of approximately 4.0 in water, while CoQ has a pK_a of 13.3 in water [1] Since CoQ is a two-electron acceptor, the semiquionone radical also plays the role as an intermediate as CoQ is reduced and oxidized [5].

$$CoQ + e^- \longleftrightarrow CoQ^{\bullet -}$$

$$CoQ^{\bullet -} + e^- + 2H^+ \longleftrightarrow CoQH_2$$

These reactions most notably take place in the mitochondria where CoQ is accepts and transports electrons.

CoQ Semiquinone Radical Structure

The structure of the semiquinone radical is slightly altered by the addition of an electron. The two double-bonded oxygen are now single bonded. One oxygen carries a negative charge, while the other carries one free unpaired electron. The ring structure of

the semiquinone radical contains three double bonds, one more than CoQ, as shown in figure 2 [1].

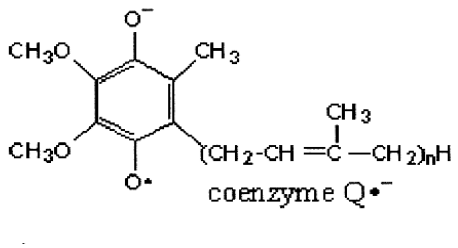


Figure 2. The structure of the CoQ semiquinone radical (coenzyme $Q^{\bullet -}$ = CoQ semiquinone radical) [adapted from 3].

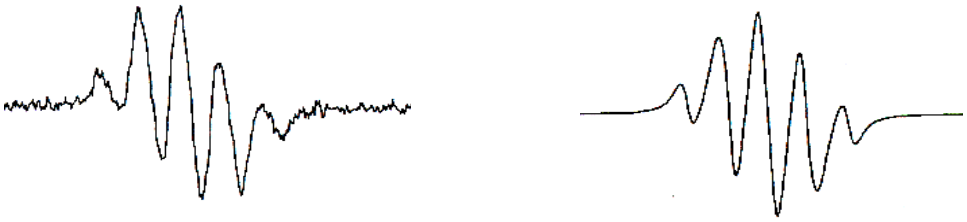
CoQ/ Semiquinone Radical Detection

CoQ was originally isolated by saponification of beef heart mitochondria with alkali in the presence of pyrogallol and extracted with petroleum ether. CoQ can also be isolated by lipid extraction from wet tissues in various solvents and from dry tissues with hydrocarbon solvents. Separation and purification of CoQ can be accomplished by chromatography. HPLC is the most sensitive, detects CoQ down to 2.5 ng, and is the fastest method, but others methods such as column, thin-layer, and gas chromatography can be used. [1]

The existence of semiquinone radicals is best measured by electron paramagnetic resonance (EPR). The measurement of the majority of free radicals is limited because they are highly reactive and therefore have a short lifetime. The CoQ semiquinone radical is a relatively stable free radical species. Its stability and longer lifespan makes it a good candidate to be measured directly by EPR spectroscopy. EPR is one of the best methods for identifying semiquinone radicals because of its detection characteristics: it

can directly identify the semiquinone radical, it is technically simple, and it does not require the isolation or chemical characterization of organic oxidation products [6].

To determine if a given sample contains the CoQ semiquinone radical its EPR spectrum is compared with EPR spectrum of established standards for the CoQ semiquinone radical. EPR determination involves points of comparison other than just the spectrum. One very important point of comparison is the g- value. The g-value is a measurement of the local magnetic field experienced by the electron. A free electron has a g- value of 2.0023. The g-value of CoQ semiquinone radical is very similar, as is many other free radicals [7].



A. B.

Figure X. EPR spectrum of a semiquinone radical. A.) is an example of experimental data. B.) is a computer simulated characteristic of a semiquinone radical. The Experimental reaction of 1.2 M human HbO2 and 0.5 mM CoQ was carried out in borate buffer (pH 9.0)(g- values not provided in the literature.) [8].

CoQ can also be accurately identified by UV spectroscopy. The special features of the CoQ UV spectrum are due to its benzoquinone ring. CoQ displays a main absorbance in the 270-280 nm (exact absorbance dependent on solvent; H_2O= 282nm, petroleum ether= 270nm) due to electronic transitions of the benzoquinone ring. CoQ semiquinone radicals show complex spectra with a major band at 445 nm [1].

CoQ Semiquninone Radical Reaction with O_2

The CoQ semiquinone radical can react directly with molecular oxygen. In this reaction an electron is passed from the semiquinone radical to molecular oxygen. This reaction produces a molecule of superoxide and CoQ [2].

$$CoQ^{\bullet-} + O_2 \longleftrightarrow CoQ + O_2^{\bullet-}$$

The rate constant for the forward reaction is $k \cong (1\text{-}58) \times 10^7$ and the rate constant for the reverse reaction is $k \cong (5\text{-}20) \times 10^7$ [2]. Thus, the kinetics of this reaction allows the equilibrium to be shifted from one direction to the other, even with only small changes in reactant concentration. The reaction direction is largely dependent on reactant concentration. Like the reaction kinetics the redox potentials for CoQ/ $CoQ^{\bullet-}$ and O_2/ $O_2^{\bullet-}$ are also similar. The redox potential of CoQ/ $CoQ^{\bullet-}$ is $E_0 \cong -(0.11\text{-}0.15)$ V and the redox potential of O_2/ $O_2^{\bullet-}$ is $E_0 \cong -(0.11\text{-}0.16)$ V [9]. The balance of this reaction allows the products to be easily altered with either addition or subtraction of reactants. Cellular processes that can increase or decrease the concentration of the reactants can regulate the products of this reaction.

Summary

When CoQ accepts a single electron, it forms a stable free radical species. This semiquinone radical is found in the lipid bilayers. It plays a vital role in the electron transport chain and other biological reactions. It can react directly with molecular oxygen to produce superoxide, which was described in this paper. CoQ and its semiquinone radical are involved in many vital biological pathways. As more research,

such as my own, focuses on the electron transport chain and its role in cancer and aging, it may be discovered that CoQ plays a vital role in cause or treatment.

References

1. Lenaz, G., ed. (1985) *Coenzyme Q*. John Wiley and Sons publications.
2. Quinn, Peter. (1999) *Expansion of antioxidant function of vitamin E by coenzyme Q*. In: Lenaz, ed. *Biofactors 9*. IOS Press; pp 14
3. Beyer. An analysis of the role of coenzyme Q in free radical generation and as an antioxidant. (1991) *Biochem. Cell. Biol*. **70**: 390-403.
4. Lenaz, Giorgio. (1999) *Localization and mobility of coenzyme Q in lipid bilayers and membranes*. In: Lenaz, ed. *Biofactors 9*. IOS Press; pp87-93.
5. Brandt, Ulrich. (1996) Bifurcated ubihydroquinone oxidation in the cytochrome bc complex by proton-gated charge transfer. *FEBS Letters*. **387**: 1-6.
6. Barbehenn, R.V. (2003) Semiquinone and ascorbyl radicals in the gut fluids of caterpillars measured with EPR spectrometry. *Insect Biochemistry and Molecular Biology*. **33**: 125-130.
7. Campbell, I.D. and Dwek R.A., ed. (1984) *Biological spectroscopy*. Benjamin/Cummings Publishing Comp; pp179-215.
8. Guo, Quiong. Electron spin resonance investigation of semiquinone radicals formed from the reaction of ubiquinone 0 with human oxyhemglobin. *The Journal of Biological Chemistry*. **277**(2): 6104-6110.
9. Stoyanovsky, Detcho A. Ubiquinone-dependent recycling of vitamin E radicals by superoxide. *Archives of Biochemistry and Biophysics*. **323**: pp 343-351.