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A quantum chemical study of the mechanism of action of Vitamin K epoxide reductase (VKOR) II. Transition states ☆

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Abstract

A reaction path including transition states is generated for the Silverman mechanism [R.B. Silverman, Chemical model studies for the mechanism of Vitamin K epoxide reductase, J. Am. Chem. Soc. 103 (1981) 5939–5941] of action for Vitamin K epoxide reductase (VKOR) using quantum mechanical methods (B3LYP/6-311G**). VKOR, an essential enzyme in mammalian systems, acts to convert Vitamin K epoxide, formed by Vitamin K carboxylase, to its (initial) quinone form for cellular reuse. This study elaborates on a prior work that focused on the thermodynamics of VKOR [D.W. Deerfield II, C.H. Davis, T. Wymore, D.W. Stafford, L.G. Pedersen, Int. J. Quant. Chem. 106 (2006) 2944–2952]. The geometries of proposed model intermediates and transition states in the mechanism are energy optimized. We find that once a key disulfide bond is broken, the reaction proceeds largely downhill. An important step in the conversion of the epoxide back to the quinone form involves initial protonation of the epoxide oxygen. We find that the source of this proton is likely a free mercapto group rather than a water molecule. The results are consistent with the current view that the widely used drug Warfarin likely acts by blocking binding of Vitamin K at the VKOR active site and thereby effectively blocking the initiating step. These results will be useful for designing more complete QM/MM studies of the enzymatic pathway once three-dimensional structural data is determined and available for VKOR.

Keywords: Vitamin K; VKOR; Reductase; Mechanisms; Transition states

1. Introduction

When the human vascular system is compromised, an exquisite sequence of reactions is catalyzed in response to the assault, with the immediate goal being the formation of a blood clot to re-seal the damaged system. A number of the enzymes that participate in the response system depend on the presence of Vitamin K for the post-translational modifications that render them active [1].

The main role of Vitamin K, an essential component of human physiology, is to provide for the conversion of glutamic acid (Glu) to gamma-carboxyglutamic acid (Gla). This post-translational modification occurs for some Glu residues that reside in the membrane-binding domain of several critical blood coagulation proteins. It is currently believed that the three forms of Vitamin K – Vitamin K, Vitamin K epoxide and Vitamin K hydroquinone – are involved in a catalytic cycle (Fig. 1).

Although Vitamin K was discovered in 1934 [2], it was only in the mid 1980s when the general details of the mechanism began to be unraveled [3]. Recently, the two key enzymes in the

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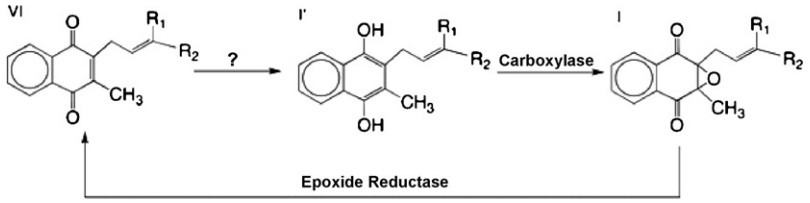

Fig. 1. The Vitamin K catalytic cycle. The result of the carboxylase activity is to convert Glu to Gla in a number of sites in Vitamin K-dependent proteins. The enzyme for the initial reduction (? in the figure) is not known. Structure I is Vitamin K epoxide, structure I′ is Vitamin K hydroquinone and structure VI is Vitamin K.

catalytic cycle, the Vitamin K carboxylase [4] and the Vitamin K epoxide reductase [5,6] (VKOR) have been identified, sequenced and cloned. The identity of the reducing enzymatic species for (VI → I′, Fig. 1) is still an open question. A pertinent News and Views column in Nature [7] summarizes our knowledge to the present.

The drug Warfarin is the most studied inhibitor of the reductase, VKOR. The design of this agent was based on an isolate from fermented sweet clover, a deadly poison to livestock [8,9]. Over 7 million prescriptions of this drug were written for US patients in the year 2003 [10]. One of the interesting outcomes of the human genome project has been the quantitative details necessary to understand why all patients treated with drugs such as Warfarin do not respond the same way, i.e., there is considerable variability in the human phenotype. This situation leads to the danger of over/under prescribing Warfarin for any given individual. For instance, it has been shown recently that there are a significant number of single nucleotide polymorphisms for VKOR [11], which is the target of Warfarin, and at least one significant polymorphism for the cytochrome P450 gene 2C9 that metabolizes Warfarin [12]. The desire to ultimately be able to treat patients with knowledge-based enzyme reactivity along with genetic profile information drives the current work.

In this work, we will focus on the epoxide reductase. The significant studies of Dowd et al. [13,14], Silverman [15,16], Goodstadt and Ponting [17] and others, have made it possible to establish a (pseudo-enzymatic) mechanism by which the essential epoxide reductase activity may be modeled. The sequence of reactions proposed by Silverman [15] has been modified by practical consideration:

$$\mathrm{Ii} + \mathrm{H_2O} + \mathrm{HSCH_3} + {}^{-}\mathrm{SCH_3} \rightarrow \mathrm{Ia}$$

$$\mathrm{Ia} \rightarrow \mathrm{TS1} \rightarrow \mathrm{Ib}$$

$$\mathrm{Ib} \rightarrow \mathrm{IVi} + {}^{-}\mathrm{SCH_3} + \mathrm{H_2O}$$

$$\mathrm{IVi} + \mathrm{H^+} \rightarrow \mathrm{Vi}$$

$$\mathrm{Vi} + {}^{-}\mathrm{SCH_3} + \mathrm{H_2O} \rightarrow \mathrm{Va}$$

$$\mathrm{Va} \rightarrow \mathrm{TS2} \rightarrow \mathrm{Vb}$$

$$\mathrm{Vb} \rightarrow \mathrm{VI} + \mathrm{CH_3SSCH_3} + 2\mathrm{H_2O}$$

$$\text{Net}: \mathrm{Ii} + \mathrm{HSCH_3} + {}^{-}\mathrm{SCH_3} + \mathrm{H^+} \rightarrow \mathrm{VI} + \mathrm{CH_3SSCH_3} + \mathrm{H_2O}$$

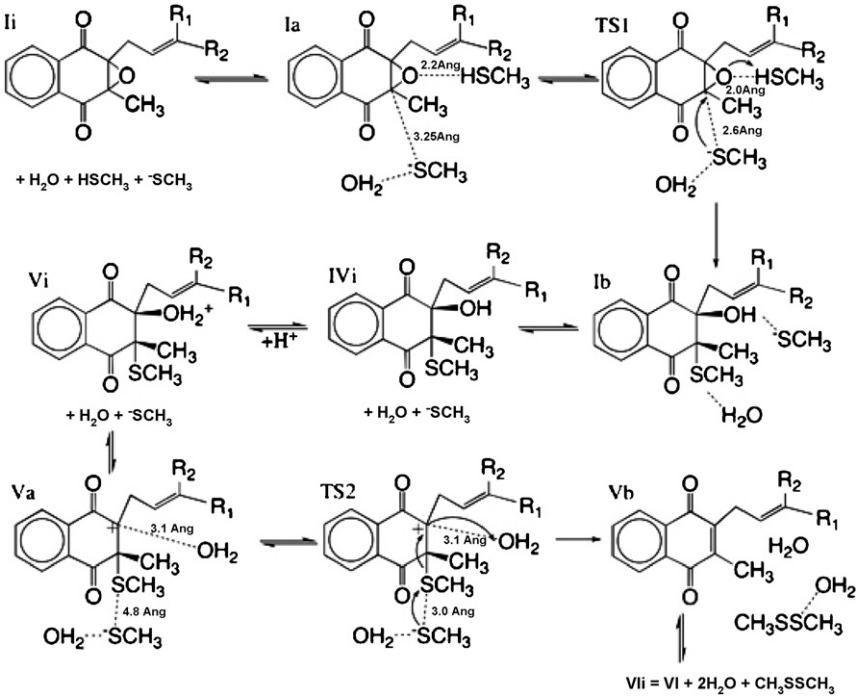

Fig. 2. A thiol/disulfide-based mechanism to reduce the Vitamin K epoxide to the quinone. This is a modification of the Silverman mechanism [15].