Heteroatom Chemistry
Volume 7, Number 5, 1996

Rapid Communication

Model Systems for Cofactor Activity. Biomimetic Reduction of Vitamin K by 1,3-Propanedithiol

Jason Imbriglio, Paresh Patel, and Vincent M. Rotello*

Department of Chemistry, University of Massachusetts, Amherst, MA 01003

Received 25 April 1996

ABSTRACT

Vitamin K is reduced to vitamin KH_2 by lipoate in biological systems. To provide a model system for this reaction, we have studied the in vitro reduction of vitamin K by 1,3-propanedithiol. This reaction occurs rapidly in dimethyl sulfoxide (DMSO) at 23°C, demonstrating the validity of this model system.

Vitamin K (**1**) is an essential cofactor in blood clotting [1]. In its reduced form, vitamin KH_2, it is an essential cofactor for the enzyme that is responsible for the carboxylation of at least seven proteins of the blood-clotting cascade (prothrombin, clotting factors VII, IX, and X, and proteins C, S, and Z). In the initial step of this process, enzymatic reduction of vitamin K by lipoate (**4a**) provides the vitamin KH_2 hydroquinone (**2**) with concomitant formation of disulfide **5** (Scheme 1). Reaction of hydroquinone **2** with dioxygen in the enzyme in the presence of clotting factors and carbon dioxide results in the carboxylation of the N-terminal glutamates of the proteins, with concomitant formation of diketoepoxide **3** [2]. Epoxide **3** is then reduced by lipoate (**4a**) to regenerate vitamin K (**1**) [3].

In the course of our recent research, we have used model systems to explore the role of noncovalent interactions in the redox chemistry of biological cofactors [4]. These models allow us to examine important biological processes in simplified systems. This allows us to isolate and quantify the enzyme-cofactor interactions responsible for biological activity. The first step in the design of a viable model system is the creation of an appropriate metaphor for the enzymatic environment. The suitability of the model chosen can then be assessed on a functional basis. For reactions occurring in the generally anhydrous environment of the enzyme active site, aprotic media provide the most logical choice for model systems. We report here the initial results of our model studies of the crucial dithiol-mediated reduction of vitamin K to the active vitamin KH_2 hydroquinone.

To gain a better understanding of the enzymatic reduction of vitamin K (**1**) to vitamin KH_2 (**2**), we have studied the reaction of **1** with dithiols. Addition of excess 1,3-propanethiol (**4b**) (Scheme 1) to degassed solutions of vitamin K in dimethyl sulfoxide (DMSO) at ambient temperatures resulted in a rapid decolorization of the yellow mixture that could be followed by UV-Vis spectroscopy [5]. This reaction was reversible: upon exposure to air, the solution rapidly regained its original yellow color and UV spectroscopic absorptions. The positive identifica-

Dedicated to Professor Louis D. Quin, a scientist, colleague, and gentleman, on the occasion of his retirement from the University of Massachusetts at Amherst.

*To whom correspondence should be addressed.

1042-7163/96/050293-02

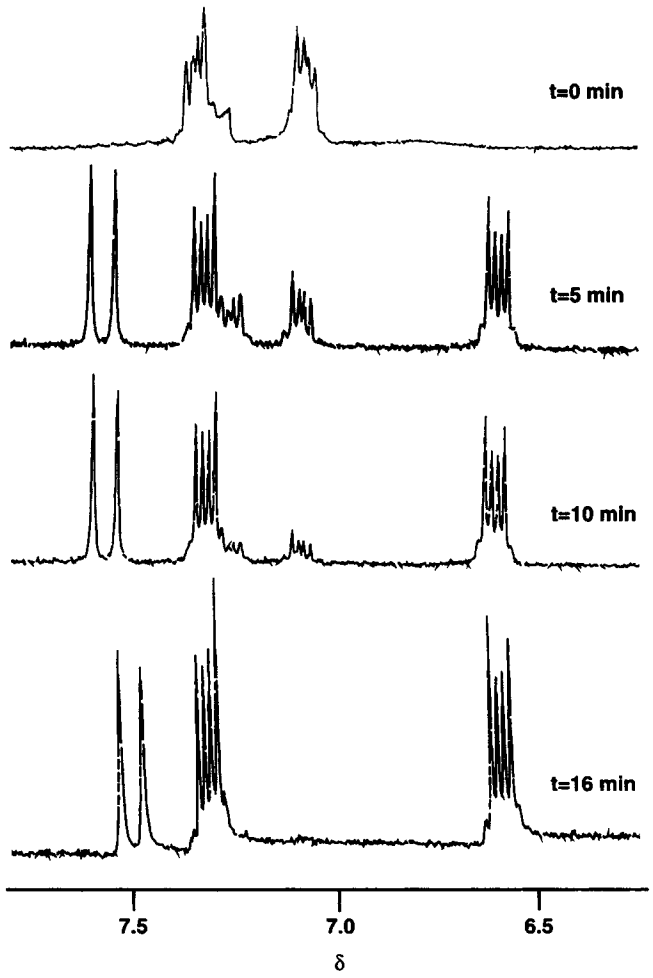
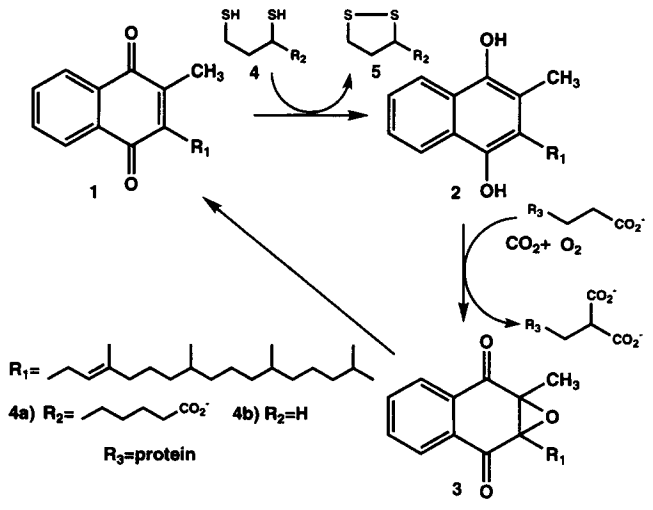


FIGURE 1 Reduction of vitamin K to KH_2 in DMSO-d_6 [7].



SCHEME 1

tion of the colorless product was complicated by its instability toward aerobic oxidation. As a result, infrared and mass spectroscopic studies were inconclusive. To determine whether the product formed in this reaction was the hydroquinone **2**, or a quinone-thiol adduct [6], we followed the reaction in situ by ^{1}H NMR spectroscopy. As shown in Figure 1, treatment of vitamin K (**1**) with **4b** in DMSO-d_6 resulted in dramatic changes in the aromatic region of the ^{1}H NMR spectrum of (**1**) [7]. These spectral changes coincide fully with those previously observed during the hydrosulfite reduction of quinone **1** to provide the hydroquinone vitamin KH_2 (**2**) (Scheme 1).

In summary, we have established that 1,3-dithiols effect the biomimetic reduction of vitamin K in an aprotic medium. Experiments designed to explore the effects of noncovalent forces on this process are currently underway and will be reported in due course.

ACKNOWLEDGMENT

This research was supported by the National Science Foundation (CHE-9528099) and the Petroleum Research Fund of The American Chemical Society (30199-G4).

REFERENCES

[1] J. W. Suttie, *Biofactors, 1,* 1988, 55; R. Olson, *Annu. Rev. Nutr., 4,* 1984, 281.

[2] For model studies of the transformation of **2** to **3**, see: (a) P. Dowd, S. Ham, Geib, *J. Am. Chem. Soc., 113,* 1991, 7734; (b) R. Flowers, S. Naganathan, P. Dowd, E. Arnett, S. Ham, *J. Am. Chem. Soc., 115,* 1991, 9409.

[3] For studies on the reduction of epoxide **3** to vitamin K, see: R. Silverman, *J. Am. Chem. Soc., 103,* 1981, 5939; P. Preusch, J. Suttie, *J. Org. Chem., 48,* 1983, 3301.

[4] E. Breinlinger, A. Niemz, V. M. Rotello, *J. Am. Chem. Soc., 117,* 1995, 5379; E. Lambert, E. Breinlinger, V. Rotello, *J. Org. Chem., 60,* 1995, 2646; A. Niemz, V. M. Rotello, *J. Mol. Rec.,* in press.

[5] Vitamin K is unreactive toward thiols in protic media such as ethanol/water mixtures: K. Takamura, M. Sakamoto, Y. Hayakawa, *Anal. Chim. Acta, 106,* 1979, 261.

[6] For an example of adducts formed by reaction of thiols with vitamin K analogs, see: Y. Hayakawa, K. Takamura, *Yakagaku Zasshi, 95,* 1975, 1173.

[7] A solution of vitamin K (**1**) (Aldrich, 98%) (18 mg, 40 μmol) in DMSO (2.0 mL) was degassed by three cycles of freeze–pump–thawing. To this mixture was added 1,3-propanethiol (**4a**) (20 μL, 22 mg, 200 μmol). NMR spectra (200 MHz) were then accumulated at 23°C until the reaction was complete.