THE ENZYMATIC REDUCTION OF K-VITAMINS INCORPORATED IN THE MEMBRANE OF LIPOSOMES

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1. Introduction

20 years ago one of us had described [1,2] a flavin enzyme which can be reduced by NADH or NADPH and reoxydized by a number of quinones, especially by methylnaphthoquinone. Because this reaction is strongly inhibited by low concentrations of dicumarol or other anticoagulants, we believed the biological function of this enzyme to be to reduce the quinone form of the K-vitamins and called it vitamin K-reductase. This name, however, was not generally accepted because it was not possible to demonstrate a reaction of the reduced form of the enzyme with vitamin K_1 or other water-insoluble K-vitamins which had been brought in solution with the aid of solubilizers like Tween. These apparently protect the naphthoquinone from being attacked by the flavin enzyme. Now we are able to show that these vitamins easily react with the vitamin K-reductase if they are incorporated into the membranes of liposomes and thus can transport hydrogen from outside into the interior of such vesicles.

2. Materials and experimental procedure

The liposomes were prepared in the usual manner from a mixture of 95% egglecithin (grade 1. Lipid Products, South Nutfield, England) and 5% dicetylphosphate (Sigma) using a Branson sonifier Mod.S-110 with microtip (step 3.6 A output). The aqueous medium contained Tris buffer pH 7.7 (≈ 60 mM) and $K_3[Fe(CN_6)]$ (0.2 M). The sonification was carried out at 20°C under nitrogen during 35–45 min and gave liposomes of a diameter between 150 and 250 Å which were separated by gel filtration with Sephadex G-50. The vitamin K-reductase used in this work was prepared from rat liver following the somewhat simplified procedure of Märki and Martius [2] and was about 12% pure. The K vitamins were a gift of Hoffmann-LaRoche AG. (Basel). If such vitamin K containing liposomes are incubated with a solution containing NADH and vitamin K-reductase, the following sequence of reactions takes place:

outer space: NADH + FAD (reductase + H^+ → NAD + $FADH_2$ (reductase) $FADH_2$ + vit.K (membrane bound) → FAD + vit.K.H_2 (membr. bd.)

inner space: vit.K.H_2 (membrane bound) + $Fe(CN)_6^{3-}$ → vit.K (membrane bound) + 2 $Fe(CN)_6^{4-}$ + $2H^+$

The rate of this oxidation-reduction reaction can be measured spectrophotometrically at 436 nm. (ϵ = 760 for $(Fe\ CN_6)^{3-}$ under these experimental conditions). The velocity of the reaction is dependent upon the concentration of the enzyme (fig.1) and on the concentration and structure of the K-vitamin (fig.2). The K-antimetabolite 2-chloro-3-phytyl-1,4-naphthoquinone [3] reacts only to a very low degree and if incorporated together with a K-vitamin in the liposomes acts as an inhibitor (50% inhibition at a molar ratio phylloquinone/2-chloro analog = 10).

* This paper is based partially on undergraduate research work by R.G and A.V.

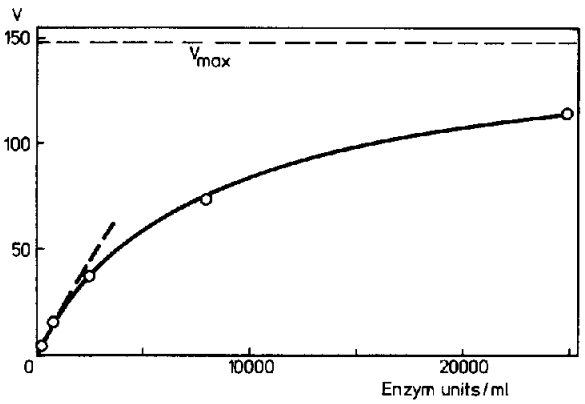

Fig.1. Influence of enzyme concentration. Reaction mixture: 0.7 ml suspension of liposomes containing 3.2 mol % vitamin K_1; 50 µl NADH 0.6%; total vol: 1 ml. T=25°C; Tris–HCl buffer pH 7,7.

3. Discussion

The result of our experiments clearly show that even the most water insoluble K-vitamins can react enzymatically with the reduced form of a flavin enzyme ('vitamin K-reductase') if they are incorporated in the membrane of liposomes. This is in accordance with our conception that it is the biological function of this enzyme to reduce the vitamin K bound by cell membranes. Strong support is given to this idea by the observation that the vitamin K antimetabolic action of the water insoluble 2-chloro-3-phytyl-1,4-naphthoquinone can also be demonstrated in vitro. We would like, however, to emphazize that in all probability the sequence of reactions in vivo is more complex than the simple artificial system described in this paper.

References

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[2] Märki, F. (1959) Thesis ETHZ Nr. 2908; Märki, F. and Martius, C. (1960) Biochem. Z. 333, 111; Märki, F. and Martius, C. (1961) Biochem. Z. 334, 293.
[3] Lowenthal, J., McFarlane, J. A. and McDonald, K. M. (1960) Experientia 16, 428.

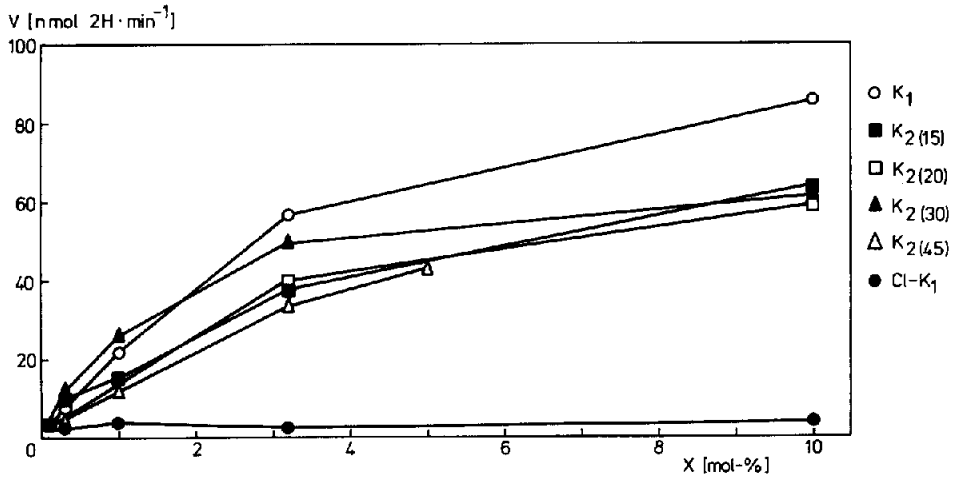

Fig.2. Variation of the vitamin K concentration in the liposomal Membrane: (mol. wt of the phosphatide mixture ≃ 760) 0,1; 0.32; 1.0; 3,2; 10 mol%. Reaction mixture: same as in fig.1; 6000 enzyme units/ml. Cl-K_1 = 2-chloro-3-phytyl-1,4-naphthoquinone.