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Effect of Vitamin K_3 on plasma membrane-bound H^+-ATPase and reductase activities in plants

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Abstract

Vitamin K-like compounds are widely diffused in plants, but their role and function are still partially unknown. Vitamin K_1 – phylloquinone – is largely present in tylacoid membranes as electron carrier inside the PSI redox chain. More recently, it has been found that Vitamins K_1 and K_3 may also affect the plasmalemma-bound H^+-ATPase and reductase activities. In this article we report the effects of Vitamin K_3 treatment on the above activities in three different conditions: in the presence of Vitamin K_3; after the addition of Vitamin K_3 to the hydroponic solution; after sonication of plasma membrane in the presence of Vitamin K_3 in the solution. Results showed that Vitamin K_3 was able to improve both plasmalemma H^+-ATPase and reductase activities at a concentration that is very well tolerated by the whole plant. Our findings support the hypothesis that Vitamin K is involved in plasmalemma activities of both mono and dicotyledonous plants.

Keywords: H^+-ATPase; Menadione sodium bisulfite; Phylloquinone; Reductase activity; Vitamin K

1. Introduction

Naphthoquinonic compounds are widely diffused in plant tissues but the physiological function of most of them still remains questionable [1]. Interestingly, among this miscellaneous group of substances there is one that has been studied and investigated for several decades since its isolation in alfalfa hay [2]. The interest came mainly from its nutritional and physiological consequences on animals fed with it rather than for the role played in plants; this substance was Vitamin K. The generic name of Vitamin K refers to a pool of compounds having the basic structure of 2-methyl-1,4-naphthoquinone (Menadione or Vitamin K_3) substituted in position 3 with a lateral polyisoprenoid chain of variable length (Fig. 1). In plants the most common moiety is Vitamin K_1 or phylloquinone, which presents an isophytol as lateral chain, but also Vitamin K_3 has been isolated in plant tissues [3].

In animals Menadione does not exert a direct Vitamin K activity, being a pro-vitamin which must be further metabolised in the liver into the active form (Vitamin K_2) through the addition of the side chain in position 3. Vitamin K is a lipophilic molecule whose physiological functions are likely to be related to the redox properties provided by the double quinonic radical. In animals Vitamin K is an essential cofactor needed to carry out the γ-carboxylation of glutamic acid residues of some proteins (Fig. 2). The most important proteins that undergo a Vitamin K-dependent post-translational modification are those involved in blood clotting (namely prothrombin and clotting factors VII, IX and X), even if an increasing number of Vitamin K-dependent proteins not related to blood coagulation have been discovered in animal tissues since the mid 1970s [4,5]. For a general review on Vitamin K see Suttie [6].

In plants Vitamin K is synthesized from corismic acid, an intermediate produced in the shikimate pathways, and its function is not as clear as in animals. Vitamin K_1 is involved in the photosynthetic electron transport chain, where it constitutes the second step of photosystem I (PSI) redox chain [7]. Its precise localization inside the tylacoid membrane has been investigated [8].

A direct role for Vitamin K as a constituent of the plasmalemma redox chain has been first hypothesized by Barr et al. [9,10], working on carrot cell cultures, and by Böttger and

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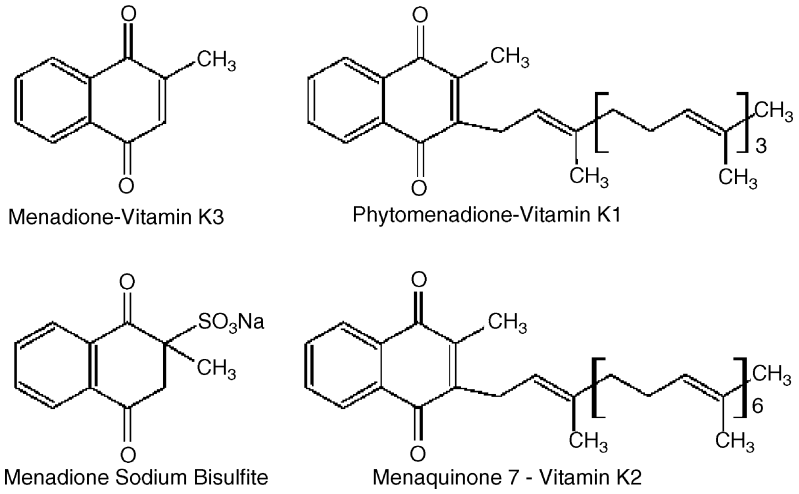

Fig. 1. Vitamin K forms.

co-workers, who were able to measure in maize roots an increase in the rate of reduction of external electron acceptors as a consequence of a pre-treatment with Vitamin K_3 [11,12]. On the basis of these and other observations [13], it has been postulated that Vitamin K could be part of a plasma membrane redox chain, whose real sequence has still to be assessed [14–16]. Some data indicate that Vitamin K-like substances may also be connected to the enzymatic pool of the nitrate reductase [17,18].

Other studies on phospholipidic model membranes show a strong antioxidant effect of Vitamin K, which is crucial to protect lipids from free radical damage [19–21] and indeed this activity has also been detected in biological membranes [22].

In this paper we report the results of three different sets of experiments on the effect of Vitamin K_3 in the form of the water soluble derivative, Menadione sodium bisulfite (MSB), on the reductase and also on the H^+-ATPase activities in plasma-lemma-enriched fractions obtained from roots of mono-(*Zea mays* L.) and dicotyledonous (*Cucumis sativus* L., *Lycopersicon esculentum* L.) plants. In the first set of experiments we compared the reductase and H^+-ATPase activities of purified plasmalemma fractions in the presence or in the absence of Vitamin K_3. In the second set, we compared the membrane activities of purified plasmalemma fractions isolated from plants grown in the presence or in the absence of Vitamin K. In the third set of experiments, the reductase and the H^+-ATPase activities of purified plasmalemma fractions were compared after a sonication treatment of the membrane in the presence or not of Vitamin K_3.

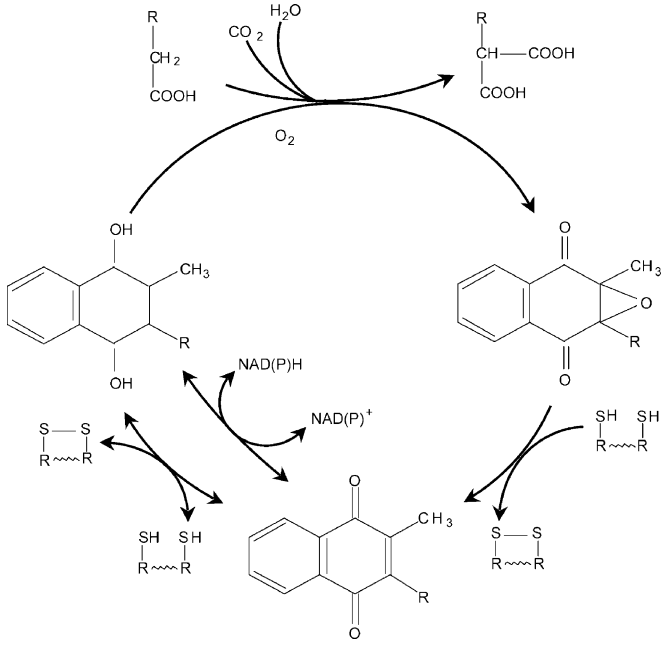

Fig. 2. Vitamin K related activities. Modified from Suttie [6].

2. Materials and methods

2.1. Plant material and growth conditions

Seeds (*Z. mays* L., *C. sativus* L., *L. esculentum* L.) were allowed to germinate in agriperlite moistened with 0.5 mM $CaCl_2$. After germination seedlings were transferred for hydroponic culture to a nutrient solution with the following composition: 2 mM $Ca(NO_3)_2$; 0.75 mM K_2SO_4; 0.65 mM $MgSO_4$; 0.5 mM KH_2PO_4; 0.1 mM $Fe^{(III)}EDTA$; 10^{-2} mM H_3BO_3; 10^{-3} mM $MnSO_4$; 5×10^{-4} mM $CuSO_4$; 5×10^{-4} mM $ZnSO_4$; 5×10^{-5} mM $(NH_4)_6Mo_7O_{24}$; the pH was brought to 6.2 with NaOH. The solution was weekly renewed and constantly aerated. Vitamin K_3 (Menadione sodium bisulfite – Vanetta S.p.A. Milano), a water soluble derivative, was added at the desired concentration to the fresh hydroponic solution at the beginning of each week. Plants were grown in a growth chamber with a day/night regime of 16/8 h and a PPDF of 200 μmol m^{-2} s^{-1}; temperature was 26 °C in the light and 18 °C in the dark. All tests were performed on plants grown for 20 days.

2.2. Preparation of cell membrane

Roots of 20-day-old plants were excised, rinsed in distilled water and homogenised in a mortar at 2–4 °C with four volumes