Mevinolin: A Highly Specific Inhibitor of Microsomal 3-Hydroxy-3-Methylglutaryl-Coenzyme A Reductase of Radish Plants

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The fungal metabolite mevinolin, known to be a potent hypocholesterolemic agent, excerts *in vitro* a strong inhibitory effect on microsomal HMG-CoA reductase from etiolated radish seedlings at a concentration of about three magnitudes lower than the K_m towards the natural substrate (S)-HMG-CoA ($I_{50} = 2.5 \times 10^{-9}$ M). Beside this, mevinolin significantly inhibits the root elongation of radish as well as of wheat seedlings already at low concentrations of 10 to 100 ppb ($= 2.5 \times 10^{-8}$ to 2.5×10^{-7} M).

Introduction

During the last years, a series of hypocholesterolemic agents was detected in different strains of ascomycetes. Compactin was first isolated from *Penicillium brevicompactum* as an antifungal metabolite [1]. Later on, the isolation of compactin (ML-236 B) together with two related compounds, ML-236 A and ML-236 C, from *Penicillium citrinum* was reported [2]. Mevinolin, obtained from *Aspergillus terreus* [3], is identical with a compound isolated independently from *Monascus ruber* designated as monacolin K [4]. Very recently, the isolation of dihydromevinolin from *A. terreus* [5] and of dihydrocompactin from *P. citrinum* [6] was also achieved. All of these structurally related entities (Fig. 1) are potent competitive inhibitors of animal HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis [2−8]. Mevinolin proved to be the most effective inhibitor. Compactin (ML-236 B) has been found to be an useful research tool in studies on the regulation of vertebrate isoprenoid synthesis [8, 9]. Similar to animal tissue (for reviews see [10−12]) there is an evidence for a key-regulating role of HMG-CoA reductase in the plant isoprenoid synthesis [12−14]. Therefore, it was of interest to study the direct *in vitro*-effect of the most inhibitory metabolite, mevinolin, on plant HMG-CoA reductase. The present communication describes its effect on the microsomal HMG-CoA reductase of radish seedlings and its effect on the growth of intact radish and wheat roots.

Methods

Mevinolin was converted to the Na-salt as described [15]. Seedlings (*Raphanus sativus* var. "Saxa Treib" and *Triticum aestivum* var. "Kolibri") were grown in the dark on water and on water supplied with mevinolin. The concentrations (range 1−4000 ppb) are given in the figures. The maximum amount of ethanol needed for solubilization of mevinolin (stock solution: 4 mg mevinolin/ml) was finally less than 0.1‰. These low ethanol amounts had no inhibition effect on plant growth (controls).

The homogenization of the etiolated radish seedlings (dim green light) for the isolation of membrane pellets and the radioactive assay of the HMG-CoA reductase activity has been described in detail [12, 14]. The stock solution of mevinolin was diluted with water to yield the final concentrations in the assay sytem immediately before use.

Results and Discussion

As shown in Fig. 3 mevinolin applied in its water-soluble sodium salt form causes the *in vitro*-inhibition of the microsomal HMG-CoA reductase from etiolated

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a) ML-236 A : $R_1 = -H$, $R_2 = -OH$

b) ML-236 B : $R_1 = -H$, $R_2 = \ldots$
   (=Compactin)

c) ML-236 C : $R_1 = -H$, $R_2 = -H$

d) Mevinolin : $R_1 = -CH_3$, $R_2 = \ldots$
   (=Monacolin K)

e) 4a,5-Dihydrocompactin ⎤ are the reduced ana-

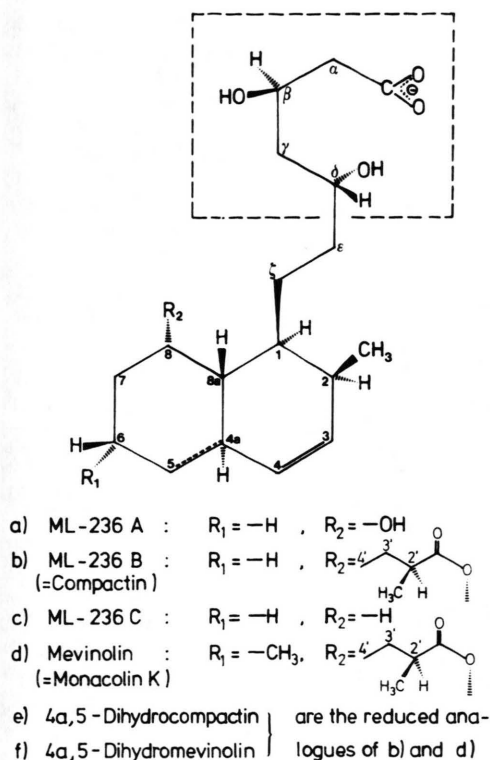f) 4a,5-Dihydromevinolin ⎦ logues of b) and d)

Fig. 1. Chemical structures of the hydroxy-carboxylate forms of mevinolin and of related entities. Part of these compounds include a portion (encircled) that resembles the HMG moiety of HMG-CoA.

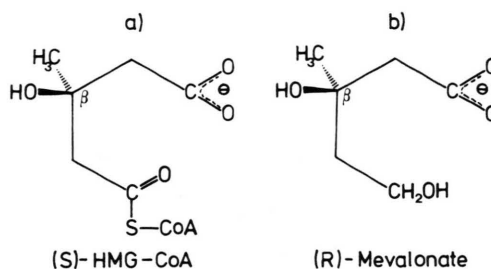(S)-HMG-CoA   (R)-Mevalonate

Fig. 2. Structures of a) (S)-HMG-CoA and b) (R)-mevalonate as the natural substrate and product of the HMG-CoA reductase catalyzed reaction.

From the occurrence and localization of the different ascomycetes strains in nature, which are producing similar inhibitory metabolites (Fig. 1) an effect of mevinolin not only on animal tissue but also on plants and the plant HMG-CoA reductase could be expected. A specific inhibition of the isoprenoid biosynthetic pathway of plants and soil organisms of the rhizosphere could be the possible natural function of the mevinolin-type metabolites. In order to ascertain an *in vivo*-effect of mevinolin on plant growth, preliminary studies were carried out using developing seedlings. The influence of different concentrations of mevinolin on the root elongation of the dicotyledonous radish and of the monocotyledonous wheat seedlings was investigated. Mevinolin inhibits the root elongation growth of both darkgrown species, which is paralleled by a reduced fresh weight. The threshold values for significant inhibition are reached in the range of 10

radish seedlings. The concentration for a 50% inhibition of the enzyme (I_{50} value) is 2.5×10^{-9} M; this is about three magnitudes lower than the K_m-value towards the natural substrate (S)-HMG-CoA [12]. Other inhibitory compounds known for this plant enzyme like $NADP^+$ or HS-CoA excert comparable kinetic effects only at concentrations higher than 10^{-4} M [12]. The concentration range for this highly specific inhibition of the plant HMG-CoA reductase by mevinolin is similar to that obtained with an enzyme preparation from rat liver [3, 4]. In the case of animal tissue the high affinity of mevinolin (Fig. 1) to HMG-CoA reductase is thought to be a function of a) the 2-methylbutyrate portion (R_2) and b) the methylgroup (R_1) at the hexahydronaphthalene moiety of the molecule [3, 4, 8]. This assumption is based on the observation that the structurally related ML-236 C that lacks such substituents R_1 and R_2 is far less inhibitory on animal sterol synthesis [8] than mevinolin [3].
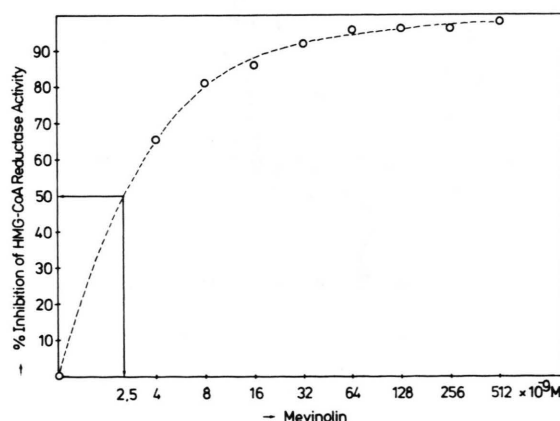

Fig. 3. Inhibition of microsomal HMG-CoA reductase from etiolated radish seedlings by increasing concentrations of mevinolin (Na-salt). Mean of three samples.
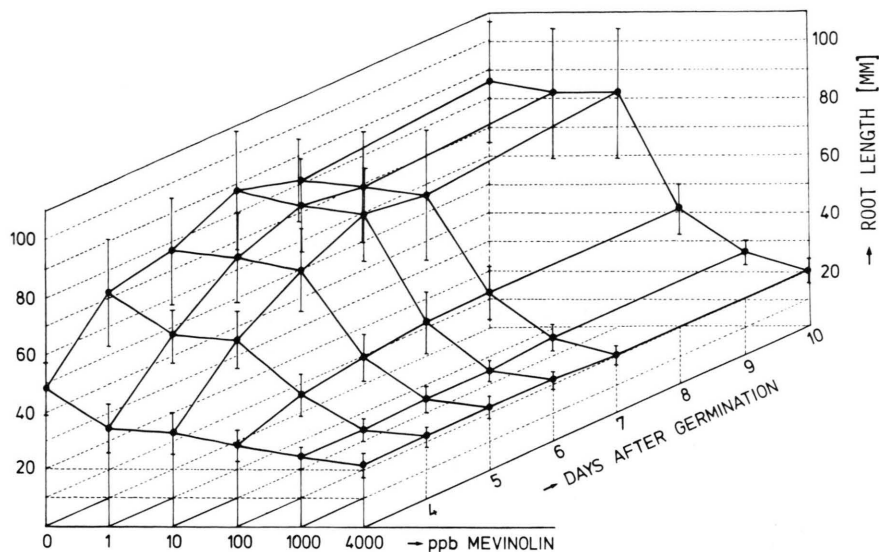
Fig. 4. Mevinolin-induced inhibition of the main root of radish seedlings. A significant drop in root elongation is obtained between 10 and 100 ppb ($= 2.5 \times 10^{-8}$ and 2.5×10^{-7} M). Mean values from 50 plants per each condition with standard deviations.

to 100 ppb (2.5×10^{-8} to 2.5×10^{-7} M) for both plants (Fig. 4 and 5 a, b). A concentration of more than 1000 ppb shows apparently no additional effect. The formation of roots cannot be completely blocked by the application of mevinolin. A minimum root length of about 2 cm is obtained in 3 day old radish seedlings also at a high mevinolin concentration far above the threshold level (Fig. 4). The stock of endogenous sterols needed for membrane synthesis may be sufficient for a certain primary root growth. In other Brassicaceae plants (*e. g. Brassica napus, B.*

campestris, B. juncea, B. nigra, Sinapis alba, and *S. arvensis*) good amounts of sterols have been found in the seeds [16], which may also be the case for radish seeds. The inhibition of the *de novo* sterol synthesis by mevinolin would then block further root development. In the 3 day old radish seedlings the root tips are wilting, turn brown and growth ceases completely at higher mevinolin concentrations (above 10^{-7} M).

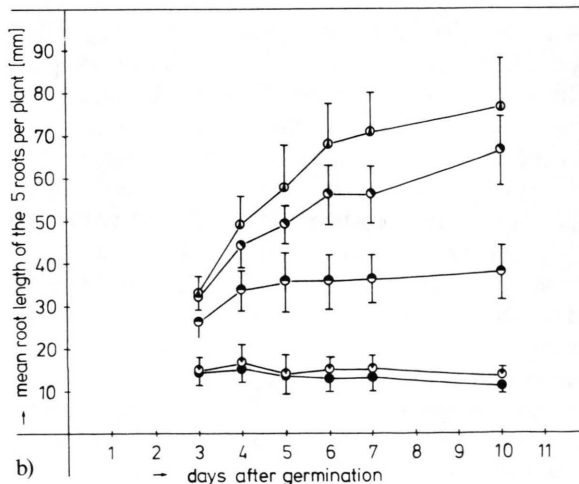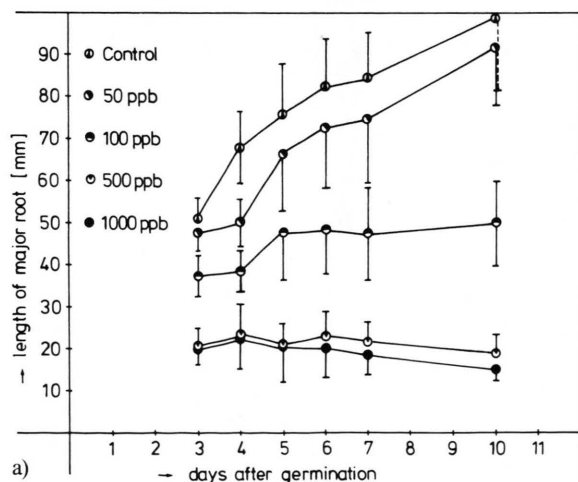Whether the plant growth retarding ability of mevinolin is solely due to the inhibition of sterol

Fig. 5. Effect of mevinolin (Na-salt) application on the elongation of a) the most developed wheat root and b) the mean root length, calculated from measuring all 5 to 6 roots of the seedlings. Mean values were obtained by comparing the roots of 40 plants per each growth condition.

synthesis or also to a change *e. g.* in the levels of the isoprenoid hormones gibberellic or abscisic acid cannot be judged from this first investigation. A decrease of the gibberellic acid content is postulated for the inhibitory potency of sterol-lowering agents like Amo 1618 or others [17, 18], and of the fungicides triadimefon [19, 20] and triadimenol [20]. The latter compounds are, however, effective usually at much higher concentrations than the mevinolin levels used in this investigation. The opposite effect, the acceleration of plant growth *e. g.* after treatment of seeds with the biologically active steroid constituent of brassins is similarly reached only at high brassin concentrations [21]. Very recently it was reported that ML-236 B at a concentration of 500 ppb could inhibit to 98% the incorporation of radioactive acetate into sterols of cell cultures from *Acer pseudoplantanus* [22]. An influence of ML-236 B on HMG-CoA reductase was, however, not established. The results reported here show that the fungal metabolite acts in plants as a herbicide with a clear structure-function relationship by inhibition of the mevalonate synthesizing HMG-CoA reductase.

It has been shown by several authors that light causes a sharp decrease in microsomal HMG-CoA reductase activity of etiolated seedlings via active phytochrome [12, 14, 23, 24], which is paralleled by the reduced growth rate of radish seedlings (roots, hypocotyls, fresh weight) [12]. From this and the mevinolin experiments described here, we conclude that there exists an interrelationship between HMG-CoA reductase activity and stem or root elongation growth of plants.

In contrast to animal tissues [cf. 8−11, 15] there is little information on the regulation of terpenoid and prenyllipid synthesis in the different plant compartments via the intracellular supply with the specific precursor mevalonate. The application of mevinolin in further experiments will help to demonstrate, whether a "multivalent feedback regulation" of HMG-CoA reductase, as shown in vertebrate tissue [9], also occurs in plants. In our attempt to define the possible autonomy of plant organelles in isoprenoid synthesis [25] mevinolin may also prove a suitable tool.

Acknowledgements

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