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Allicin, a naturally occurring antibiotic from garlic, specifically inhibits acetyl-CoA synthetase

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Allicin is shown to be a specific inhibitor of the acetyl-CoA synthetases from plants, yeast and mammals. The bacterial acetyl-CoA-forming system, consisting of acetate kinase and phosphotransacetylase, was inhibited too. Non-specific interaction with sulfhydryl-groups could be excluded in experiments with dithioerythritol and *p*-hydroxymercuribenzoate. Binding of allicin to the enzyme is non-covalent and reversible. [^{14}C]-Acetate incorporation into fatty acids of isolated plastids was inhibited by allicin with an I_{50}-value lower than 10 μM. Other enzymes of the fatty acid synthesis sequence were not affected, as was shown using precursors other than acetate.

Acetate kinase; Acetyl-CoA synthetase; Allicin; Phosphotransacetylase; (Garlic)

1. INTRODUCTION

Allicin, the major flavour product of garlic (*Allium sativum* L.), is a well-known antimicrobial agent [1,2] which originates from alliin [3]. In *Allium* plants the enzyme, alliinase, and the corresponding cysteine sulfoxide, alliin, are thought to be located in different compartments [4]. When the cell is damaged, alliinase cleaves alliin to allicin, pyruvate, and ammonium (fig.1). There are several physiological processes in microorganisms which are affected by allicin such as lipid biosynthesis [5,6], RNA synthesis [7] or, in mammals, lowering of lipids [8] and aggregation of platelets [9]. Hitherto, no specific enzyme target was known, although some non-specific effects on some enzymes were observed [10], but in these cases the inhibitory potency of allicin could be overcome by sulfhydryl reagents such as cysteine or dithioerythritol. In our studies on the mode of action of allicin and in search of its target enzyme we investigated its effect on de novo fatty acid biosynthesis in plants. Subcellular organelles (plastids) and enzyme preparations capable of fatty acid biosynthesis from different radioactive precursors were applied. After detection of the target enzyme, the investigations were extended to systems from yeast, mammals and bacteria.

2. MATERIALS AND METHODS

Allicin was purchased from Roth (Karlsruhe, FRG) as a 3.5% trituration in silicic acid. Acetate kinase (EC 2.7.2.1), phosphotransacetylase (EC 2.3.1.8), and acetyl-CoA synthetase (EC 6.2.1.1) from yeast were obtained from Boehringer (Mannheim, FRG). Radiochemicals were from Amersham (UK). All other reagents were analytical grade. Fresh bovine heart was from the local slaughterhouse and the bovine acetyl-CoA synthetase was assayed by preparing mitochondria as outlined in [11]. Plastids were isolated and tested for de novo fatty acid biosynthesis as described [12]. Fatty acid biosynthesis from different radioactive precursors was performed with an enzyme fraction from barley [13]. This fraction was also used for the plant acetyl-CoA synthetase assay. Measuring acetyl-CoA synthetase activity is based on the non-enzymic acylation of dithioerythritol [14] and worked out to an enzyme assay [15] with some modification: 0.1 M tricine, pH 8; 5 mM $MgCl_2$; 0.25 mM acetate (= 0.15μCi); 0.5 mM CoA; 2 mM ATP and 20 mM dithioerythritol in a final volume of 50 μl. The reaction was stopped after 10 min with an equal volume of 1 M NaCl and [^{3}H]acetyl-CoA as an internal standard and acylation proceeded for 1 h at 30°C. Thereafter the resulting product was extracted twice with diethylether. Radioactivity was measured in a liquid scintillation counter. The values in the tables and figures represent the mean of at least 3 determinations. Maximum deviations were between 5 and 10%.

3. RESULTS

In our laboratory we established different test systems to screen inhibitors (herbicides, antibiotics) of

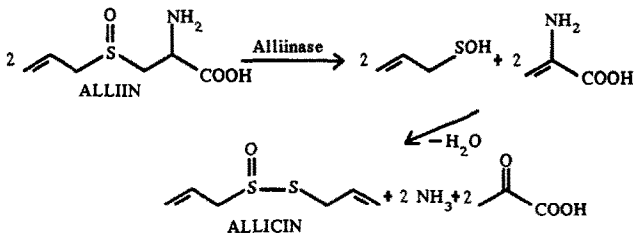

Fig.1. Formation of allicin from alliin by the action of alliinase.

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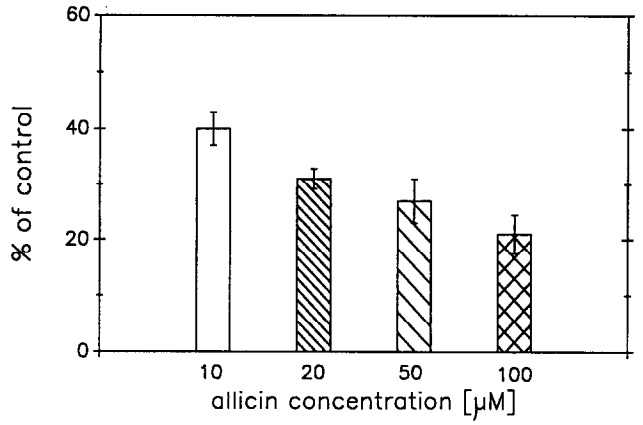

Fig.2. Inhibition of acetate incorporation by allicin into fatty acids in isolated radish etioplasts.

de novo fatty acid biosynthesis (e.g. [12,16]). By using isolated etioplasts from radish (*Raphanus sativus*) we could show that allicin inhibits the incorporation of acetate into the fatty acid fraction in a dose-dependent manner with an I_{50}-value lower than 10 μM (fig.2). Oat chloroplasts were inhibited to a similar extent with an inhibition rate of 85% at a concentration of 100 μM allicin. To identify the target enzyme of allicin, we applied an enzyme system capable of incorporating different radioactive precursors into fatty acids. Allicin inhibits the incorporation of acetate, but not that of acetyl-CoA or malonate, into fatty acids (fig.3). This indicates that within the fatty acid biosynthesis sequence only acetyl-CoA synthetase (EC 6.2.1.1) is inhibited by allicin. Acetyl-CoA synthetase forms acetyl-CoA from acetate, ATP and CoA by releasing acetyl-CoA, AMP and pyrophosphate. In higher plants this enzyme is believed to be exclusively located in the plastid compartment [17]. Our results also indicate that the malonate thiokinase, which appears to be not or little affected, possesses a reaction mechanism which is different to that of the inhibited acetate thiokinase (acetyl-CoA synthetase). The slight reduction of malonate incorporation by allicin (fig.3) may be due to

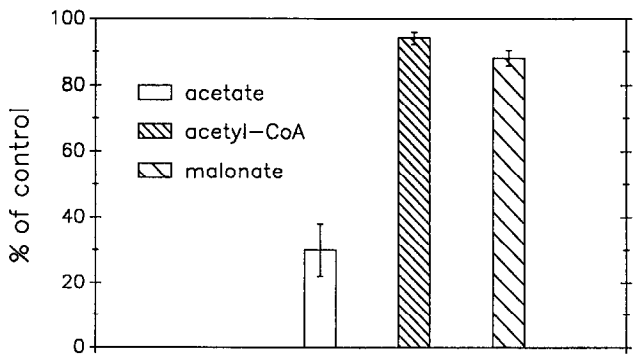

Fig.3. Effect of allicin (100 μM) on the incorporation of different radioactive precursors into total fatty acids by an enzyme system from barley.

Table 1

Comparison of the percentage inhibition of different acetyl-CoA-forming enzyme systems by different allicin concentrations

	100 μM	75 μM	50 μM
Barley acetyl-CoA synthetase	76	65	53
Yeast acetyl-CoA synthetase	77	–	49
Bovine acetyl-CoA synthetase	66	45	20
Bacterial acetate kinase/phospho-transacetylase	52	51	39

impurities of acetate present in the commercially available malonate preparation [18]. Incorporation of labelled pyruvate into fatty acids, as assayed in oat chloroplasts, was not inhibited by allicin.

Since allicin affects a broad spectrum of organisms [1,2], we decided to test the acetyl-CoA synthetases from other phylogenetic groups. By using the commercially available yeast enzyme and a crude enzyme fraction from bovine heart, we were also able to detect an inhibition of these acetyl-CoA synthetases by allicin (table 1). All enzymes were inhibited in a dose-dependent manner. Slight differences were found depending on the investigated species. By including the bacterial systems, which possess another enzymic organization, we were able to demonstrate that all enzyme systems so far investigated, which form acetyl-CoA from acetate, are blocked by allicin (table 1). The bacterial system consisting of acetate kinase and phosphotransacetylase first generates acetylphosphate (from acetate and ATP releasing ADP) and in a second step transfers the activated acetate group to CoA.

In order to insure that the block of the acetyl-CoA formation is a specific, non-sulfhydryl-group effect, we compared the influence of allicin on the yeast enzyme with that of *p*-hydroxymercuribenzoate, a well-known thiol-group blocker of acetyl-CoA synthetase [11]. The inhibition of the enzyme with *p*-hydroxymercuribenzoate could be antagonized with the thiol-group reagent, dithioerythritol, whereas the inhibition rate of

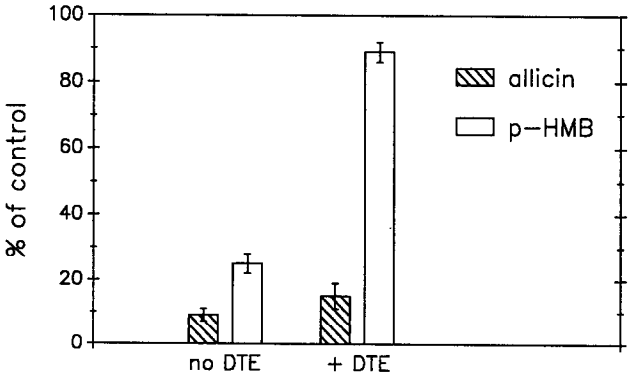

Fig.4. Comparison of the sulfhydryl effect of dithioerythritol (DTE; 20 mM) on the inhibition of yeast acetyl-CoA synthetase by 100 μM concentrations of allicin and *p*-hydroxymercuribenzoate (p-HMB).

allicin was unaffected (fig.4). Another experiment was performed to determine the binding mechanism of allicin. By passing a yeast enzyme/allicin mixture (100 μM allicin, inhibition rate 85%) through a gel filtration column (PD10, Pharmacia) we were able to restore the original enzyme activity without allicin. This result demonstrates that allicin is a reversible, non-covalent inhibitor of acetyl-CoA synthetase.

4. DISCUSSION

Many reports have shown the in vivo activity of allicin, but hitherto no target enzyme could be identified. In view of the broad spectrum of effects and sensitive organisms, one would expect that a central point of metabolism must be affected by allicin. Inhibition of lipid and fatty acid biosynthesis causes large changes in the viability of cells. It has been pointed out before that lipid metabolism (yeast, mammals) may be affected by allicin [5–8]. By measuring the acetate incorporation in plastids we could show here that the lipid biosynthesis of plants is also affected by allicin and that the target enzyme is the acetyl-CoA synthetase. The correct I_{50}-values (of allicin) are certainly lower than 10 μM, since it is difficult to fully extract allicin from silica gel and due to the fact that allicin is relatively unstable in solution. In view of this, allicin, as a natural antibiotic, exhibits a good potency as compared to the antibiotics, cerulenin and thiolactomycin, two other inhibitors of plant fatty acid biosynthesis [19]. Further support of the specificity of the allicin inhibition is that all investigated acetyl-CoA synthetases from plants, yeast and mammals and also the bacterial acetate kinase/phosphotransacetylase system are affected. The non-reversibility of the allicin inhibition by thiol reagents and the specific block of acetate incorporation into fatty acids by allicin, whereby incorporation of pyruvate or malonate is unaffected, are additional evidence for the specificity of the mode of action of allicin. The inhibition of fatty acid and lipid formation by allicin may shed new light on the understanding of the application in folk medicine of allicin and garlic to cardiovascular diseases.

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