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L-methionine induced inhibition of powdery mildew and its reversal by folic acid

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Abstract

L-methionine inhibits development of powdery mildew on cucumber leaf discs, floating on a 8×10^{-4} M solution of this compound. All other natural amino acids are ineffective in this way. This effect was reversed by 10^{-4} M folic acid. The possible interference of L-methionine with folic acid metabolism, and its importance for powdery mildew development are discussed.

Introduction

Several amino acids have been reported to be systematically active against certain fungal plant diseases. Often, only those amino acids that are not normally involved in the nitrogen metabolism of plants showed this effect. An exception is L-methionine. Papavizas and Davey (1963) showed that this amino acid suppresses *Aphanomyces* root rot of peas, and Dekker (cited by van Andel, 1966) found that it inhibits development of powdery mildew on cucumber. The effect of L-methionine on powdery mildew is dealt with in this communication.

Materials and methods

The amino acids were purchased from S.A.F. Hoffman-La Roche & Co. Ltd., Basel, Switzerland. To screen them for chemotherapeutic effect on powdery mildew, cucumber leaf discs 12 mm in diameter were floated on an aqueous solution of the compound to be tested in open Petri dishes. Inoculation was carried out by dusting conidia of *Sphaerotheca fuliginea* on the upper surface of the discs and evaluation was made after 8 days of incubation in the greenhouse; per treatment usually seven discs were used.

Results

Effect on powdery mildew

The following amino acids were tested against powdery mildew on cucumber leaf dscs at concentrations of 8×10^{-4}M, 4×10^{-4}M, and 2×10^{-4}M: glycine, DL-α-alanine, DL-β-alanine, DL-valine, DL-leucine, DL-isoleucine, DL-phenylalanine, DL-tyrosine, DL-tryptophane, DL-serine, DL-threonine, DL-aspartic acid, DL-glutamic acid, DL-lysine, DL-arginine, DL-histidine, DL-cystine, DL-cysteine, DL-proline, DL-hydroxyproline, DL-ornithine, DL-citrulline, DL-bromoserine, DL-threo-β-phenylserine,

Table 1. Activity of L-, D- and DL-methionine against cucumber powdery mildew. Leaf disc test: 0 = no powdery mildew, 5 = discs covered with powdery mildew.

		8×10^{-4} M	4×10^{-4} M	2×10^{-4} M
L-methionine		0.0	0.1	3.3
D-methionine		2.6	4.3	5.0
DL-methionine		0.2	2.3	4.0
control (water)	5.0			

Tabel 1. Werking van L-, D- en DL-methionine tegen komkommermeeldauw. Bladschijffestoets: 0 = geen meeldauw, 5 = schijffes bedekt met meeldauw.

DL-ethionine and DL-methionine. Of these amino acids only DL-methionine and DL-ethionine proved active. Ethionine, which is an antimetabolite of methionine, is known to be a fungitoxic compound. Methionine, however, does not show fungitoxicity in vitro against various non-obligate parasites. In this report primary attention has been focused upon methionine.

When testing D- and L-methionine separately, the latter proved to be far more active than the former (Table 1).

In the leaf disc test, L-methionine, at 8×10^{-4}M, appeared also active against the other powdery mildews tested, viz. on wheat, peas, beets and lupins. When examining the development of powdery mildew microscopically on the epidermis of cucumber leaf discs, stripped at certain periods after inoculation, germination was normal on L-methionine treated discs, but growth of the fungus stopped during penetration or shortly after formation of the first haustoria.

Administration of L-methionine to the roots of cucumber plants did not reduce powdery mildew at 8×10^{-4}M and only slightly at 32×10^{-4}M; application as a spray was even less effective. The following derivatives of methionine were inactive against powdery mildew on cucumber leaf discs: DL-methionine hydroxamate, DL-methionine methyl sulfonium chloride, DL-methionine sulfone and DL-methionine sulfoxide.

Specificity of action of L-methionine

For the testing of L-methionine against fungi which do not germinate on a dry leaf surface, a somewhat modified leaf disc test had to be used. The leaf discs were first floated for 2 days on a solution of the amino acid, then placed on a wet filter paper impregnated with the same solution and inoculated with a spore suspension. In order to ensure a moist environment during infection, the Petri dishes were kept closed for 24 h. When tested in this way, L-methionine at 8×10^{-4}M appeared inactive against cucumber scab, caused by *Cladosporium cucumerinum*, cucumber anthracnose, caused by *Colletotrichum lagenarium*, and chocolate spot on broad beans, caused by *Botrytis fabae*.

In vitro, germination of the conidia of *C. cucumerinum*, *C. lagenarium* and *B. fabae* was not inhibited at 8×10^{-4}M, nor was mycelial growth of these fungi and of *Pythium debaryanum*, *Stereum purpureum*, *Thanathephorus cucumeris* and a number of other non-obligate parasites.

Table 2. Reversal of the effect of L-methionine against cucumber powdery mildew by folic acid. Leaf disc test.

water (control	5.0
folic acid, 1×10^{-4} M (control)	5.0
L-methionine, 8×10^{-4} M	0.0
L-methionine + folic acid	3.0

Tabel 2. Antagonering van het effect van L-methionine tegen komkommermeeldauw door folinezuur. Bladschijffes methode.

In contrast to the diseases caused by non-obligate parasites, development of rust on French beans, caused by *Uromyces appendiculatus*, was suppressed by 8×10^{-4}M L-methionine in the leaf disc test. In this case the discs were inoculated with a dry mixture of uredospores and talc, 1 : 9, and floated on the amino acid solution in Petri-dishes, which were kept closed during 24 h after inoculation. The number of rust pustules per leaf disc was reduced from 38 in the control to 2 in the L-methionine treated discs. Germination of the uredospores in vitro was not reduced by 8×10^{-4}M L-methionine.

Reversal by folic acid

The effect of L-methionine against cucumber powdery mildew could be reversed by addition of folic acid to the amino acid solution (Table 2).

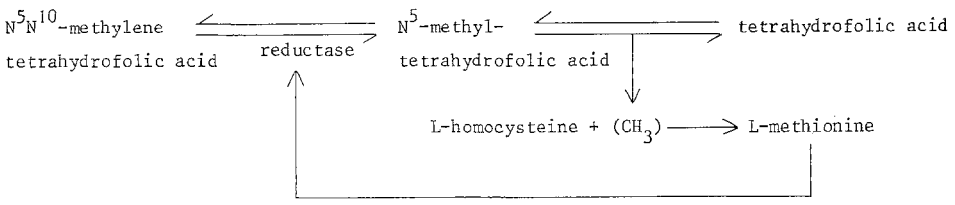
No reversion was obtained by adding 10^{-4}M p-aminobenzoic acid or L (+) p-aminobenzoyl glutamate to the medium. The effect of L-methionine against bean rust was not reversed by folic acid.

Discussion

It has been shown that L-methionine may suppress certain plant diseases. Since this natural amino acid is not a fungicide in vitro, it possibly acts specifically on the powdery mildew fungus or interferes with the metabolism in the plant in such a way that it becomes unsuitable as a host for the fungus.

The reversal of the effect of L-methionine against powdery mildew by low concentrations of folic acid might throw some light on the possible mode of action of this amino acid. Data from the literature indicate that inhibition of the functioning of folic acid may lead to a suppression of powdery mildew. Crowdy et al. (1958) obtained control of powdery mildew on wheat and cucumber by administration of sulfanilamide to the roots. The latter compound is known to be a competitive antagonist of para-aminobenzoic acid, a part of the folic acid molecule. Moreover, the effect of sulfanilamide against the powdery mildew diseases was reversed by folic acid.

Taylor et al. (1966), working with *Escherichia coli* showed that L-methionine caused end-product repression of the enzyme N^5N^{10}-methylene tetrahydrofolate reductase, which catalyzes the conversion of N^5N^{10}-methylene tetrahydrofolic acid into N^5-methyltetrahydrofolic acid. The latter compound acts as a donator of methyl groups for the formation of L-methionine from L-homocysteine:



This repression disturbs the balance of the different forms of folate compounds in the cell. Important is the level of tetrahydrofolate, as regulated by its rate of formation by the transmethylase reaction, which determines the extent of other folate-dependent reactions. Taylor et al. (1966) did not investigate whether the effect of L-methionine upon *E. coli* was reversed by folic acid. It is known that this compound may be reduced to tetrahydrofolic acid in living cells. Furthermore Sakami and Ukstins (1961) obtained evidence for the formation of N^5-methyltetrahydrofolic acid in a system containing tetrahydrofolic acid-^{14}C, NADP, Mg and pig liver enzyme.
It seems worthwhile to investigate whether the inhibition of powdery mildew development by L-methionine is due to repression of the enzyme N^5N^{10} methylene tetrahydrofolate reductase, and whether the reversion of this effect by folic acid could be explained by a restoration of the balance of the different folate compounds in the cell.

Samenvatting

Onderdrukking van meeldauw door L-methionine, en de antagonering van dit effect door folinezuur

L-methionine verhindert de ontwikkeling van meeldauw op schijfjes komkommerblad, die op een 8×10^{-4}M oplossing van deze verbinding drijven. Alle andere natuurlijke aminozuren zijn in dit opzicht onwerkzaam. Een antagonering van dit effect werd verkregen door 10^{-4}M folinezuur aan de oplossing toe te voegen. De mogelijke invloed van L-methionine op de folinezuurstofwisseling en de gevolgen hiervan voor de ontwikkeling van meeldauw worden besproken.

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