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NOTES

Allium sativum (Garlic) Inhibits Lipid Synthesis by *Candida albicans*

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The effect of aqueous garlic extract on the macromolecular synthesis of *Candida albicans* was studied. Protein and nucleic acid syntheses were inhibited to the same extent as growth, but lipid synthesis was completely arrested. Blockage of lipid synthesis is likely an important component of the anticandidal activity of garlic.

Numerous reports indicate that garlic extract has broad-spectrum antimicrobial activity (1); however, the mechanism of this growth inhibition is not known. In the absence of reports on this topic, we undertook to study the effect of aqueous garlic extract on the biosyntheses of macromolecules by *Candida albicans* and found that lipid synthesis was completely inhibited. We suggest, therefore, that blockage of lipid production by garlic extract is an important physiological mechanism for growth inhibition.

The major growth inhibitory component in garlic extract is believed to be allicin (diallyl thiosulfinate) (5, 7, 8, 10, 15, 17). However, because this substance is unstable and not commercially available, nearly all reported studies have used freshly prepared, unfractionated garlic extracts designated as allicin or presumed to contain allicin (3, 6, 11, 13, 14, 16, 17). In this study, dehydrated garlic powder (McCormick and Co., Gilroy, Calif.) was used. It was prepared from fresh garlic bulbs by low-temperature dehydration and subsequent pulverization. According to the manufacturer, it is a pure garlic product with no preservatives or chemicals added. Powder with the same lot number was used throughout the study. Aqueous extract was prepared by dissolving 1 g of powder in 40 ml of yeast nitrogen base (Difco Laboratories, Detroit, Mich.) supplemented with 5% glucose to a final concentration of 25 mg/ml. The suspension was mixed well and allowed to stand for 15 min at room temperature. With a filtration system (Fleaker; Corning Glass Works, Corning, N.Y.), the suspension was filtered through three layers of disposable wipers (Kimwipes; Kimberly-Clark Corp., Neenah, Wis.) and then through no. 1 filter paper (Whatman, Inc., Clifton, N.J.) to remove undissolved particles. The filtrate was sterilized by filtration with a 0.45-μm-pore-size cellulose membrane (Millipore Corp., Bedford, Mass.). This extract was stable for up to 10 weeks at 4°C (2).

A stock solution of garlic extract, prepared as described above with distilled water in place of yeast nitrogen base, contained 1.23 mg of protein per ml as determined by the method of Lowry et al. (12). The UV-visible spectrum of a 1:10 dilution of this extract gave a single A_{218} peak (2.84 absorption units) and a shoulder between 265 and 285 nm.

Eight clinical isolates of *C. albicans* were obtained from the Clinical Laboratory of Loma Linda University, Loma Linda, Calif. The MIC of garlic extract against these isolates ranged between 1:160 and 1:320 (6.25 and 3.12 mg/ml, respectively). All cultures were maintained on Sabouraud dextrose agar (Difco) at 25°C until used. For metabolic experiments, yeast nitrogen base supplemented with 5% glucose was used to cultivate the cells at 30°C in a water bath shaker.

The effect of garlic extract on macromolecular synthesis was determined on three *C. albicans* strains for which the MIC was 1:160 (6.25 mg/ml). The results with these strains were essentially identical. Protein synthesis was measured by adding 10 μCi of L-[4,5-^{3}H]leucine (specific activity, 120 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) to 60 ml of culture growing aerobically at 30°C in the presence of unlabeled DL-leucine (0.02 mg/ml) to give a final specific activity of 0.17 μCi (0.154 μmol/ml). A 30-ml sample of the culture received 6 ml of garlic extract stock solution to achieve a final dilution of 1:240 (4.17 mg/ml), which allowed slow growth of the organisms. The other 30 ml of the culture received only yeast nitrogen base supplemented with 5% glucose (6 ml). Samples (1 ml) were taken in duplicate every 15 min for 2 h, and each sample was mixed with 0.1 ml of 5% (wt/vol) perchloric acid and stored on ice until all the samples were collected. Each sample then received 5 ml of 5% (wt/vol) cold perchloric acid and was kept on ice for 30 min. The sample was filtered with 0.2-μm-pore-size filter membranes (Nuclepore Corp., Pleasanton, Calif.) and washed with 5% cold perchloric acid. The filters were dried overnight at 37°C and placed in a Filmware tube (Nalge Co., Rochester, N.Y.) containing 4 ml of CytoScint (WestChem, San Diego, Calif.), and the radioactivity was measured in a scintillation counter.

Nucleic acid was determined in a similar fashion, except that the incorporation of [^{32}P]phosphate (2 μCi/ml, carrier free; Amersham) was measured in the hot perchloric acid-soluble fraction from garlic-treated and control cultures. After a 30-min incubation of each fraction at 0°C with 5% (wt/vol) perchloric acid, the cells were washed and incubated at 70°C for 20 min in 5% (wt/vol) perchloric acid. Lipid

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synthesis was measured by adding 25 μCi of [1-^{14}C]acetate (specific activity, 55 mCi/mmol; Amersham) to 50 ml of culture growing in the presence of unlabeled sodium acetate (15 μg/ml) to give a final specific activity of 0.5 μCi (0.183 μmol/ml). Lipids were extracted by a chloroform-methanol mixture as described by Ames (4). Garlic extract was used at the final 1:180 dilution.

The results are shown in Fig. 1 and 2. Because garlic extract inhibits growth, it was expected that all macromolecular synthesis would be inhibited. The extent of protein and nucleic acid synthesis inhibition indeed paralleled growth inhibition (Fig. 1a and b), but lipid synthesis was completely blocked (Fig. 2).

The inhibition of lipid synthesis was not due to an inability of the cells to take up radioactive acetate. This was evidenced in the amount of free [^{14}C]acetate found in the cold acid-soluble pool of garlic-treated cells, an amount comparable to that of the control (results not shown).

Other workers have established that allicin reacts stoichiometrically with sulfhydryl compounds such as glutathione,

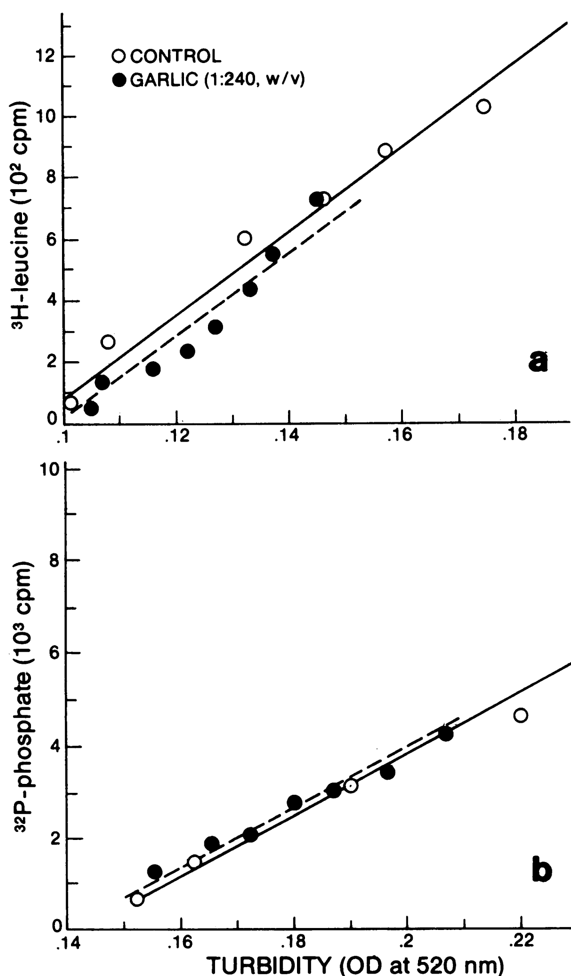

FIG. 1. Effect of garlic extract on protein and nucleic acid biosyntheses in *C. albicans*. (a) [^{3}H]leucine was incorporated into cold acid-insoluble fractions to estimate the amount of protein synthesis in the presence or absence of garlic extract. (b) [^{32}P]phosphate was incorporated into a hot acid-soluble fraction to determine the amount of nucleic acid synthesis in the presence or absence of garlic. Samples in both experiments were taken at 15-min intervals. OD, Optical density.

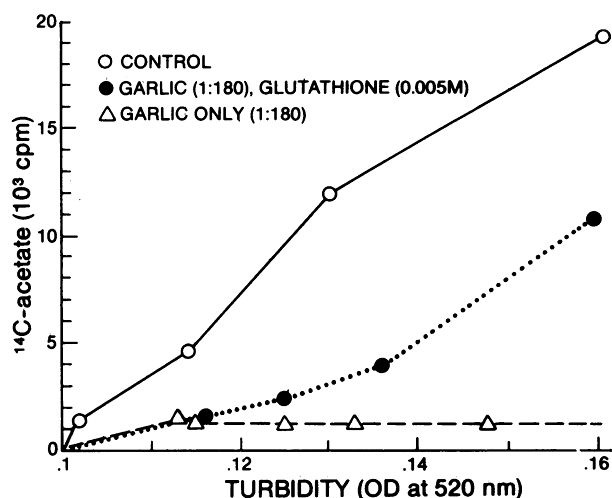

FIG. 2. Lipid biosynthesis by *C. albicans* in the control culture, culture containing garlic, and culture containing garlic pretreated with glutathione. Radioactivity in the chloroform-methanol extract of each 0.8-ml sample was used to estimate lipid synthesis. OD, Optical density.

resulting in the loss of all or most of the inhibitory activity of allicin (8). In this study, pretreatment of garlic extract with glutathione reduced the effectiveness of allicin in the inhibition of lipid synthesis (Fig. 2), supporting the notion that a disulfide-containing component such as allicin is the main growth inhibitor in garlic extracts (3, 7, 13, 16).

The residual growth of *C. albicans* in the presence of garlic resembled the behavior of glycerol-deprived *Escherichia coli*, which continues its growth for about one doubling in the absence of lipid synthesis (9).

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LITERATURE CITED

1. **Adetumbi, M. A., and B. H. S. Lau.** 1983. *Allium sativum* (garlic)—a natural antibiotic. Med. Hypotheses **12**:227–237.
2. **Adetumbi, M. A., and B. H. S. Lau.** 1986. Inhibition of in vitro germination and spherulation of *Coccidioides immitis* by *Allium sativum*. Curr. Microbiol. **13**:73–76.
3. **Amer, M., M. Taha, and Z. Tosson.** 1980. The effect of aqueous garlic extract on the growth of dermatophytes. Int. J. Dermatol. **19**:285–287.
4. **Ames, G. F.** 1968. Lipids of *Salmonella typhimurium* and *Escherichia coli*: structure and metabolism. J. Bacteriol. **95**:833–843.
5. **Amonkar, S. V., and A. Banerji.** 1971. Isolation and characterization of larvicidal principle of garlic. Science **174**:1343–1344.
6. **Amonkar, S. V., and E. L. Reeves.** 1970. Mosquito control with active principle of garlic, *Allium sativum*. J. Econ. Entomol. **63**:1172.
7. **Appleton, J. A., and M. R. Tansey.** 1975. Inhibition of growth of zoopathogenic fungi by garlic extract. Mycologia **67**:882–885.
8. **Barone, F. E., and M. R. Tansey.** 1977. Isolation, purification, synthesis and kinetics of activity of the anticandidal components of *Allium sativum* and hypothesis for its mode of action. Mycologia **69**:793–825.
9. **Bell, R. M.** 1974. Mutants of *Escherichia coli* defective in membrane phospholipid synthesis: macromolecular synthesis in an sn-glycerol 3-phosphate and acyltransferase K_m mutant. J. Bacteriol. **117**:1065–1076.

10. **Cavallito, C. J., and J. H. Bailey.** 1944. Allicin, the antibacterial principle of *Allium sativum*. I. Isolation, physical properties and antibacterial action. J. Am. Chem. Soc. **66**:1950–1951.
11. **Delaha, E. C., and V. F. Garagusi.** 1985. Inhibition of mycobacteria by garlic extract (*Allium sativum*). Antimicrob. Agents Chemother. **27**:485–486.
12. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
13. **Moore, G. S., and R. D. Atkins.** 1977. The fungicidal and fungistatic effects of an aqueous garlic extract on medically important yeast-like fungi. Mycologia **69**:341–348.
14. **Sharma, V. D., M. S. Sethi, A. Kumar, and J. R. Rarotra.** 1977. Antibacterial property of *Allium sativum* Linn.: in vivo and in vitro studies. Indian J. Exp. Biol. **15**:466–468.
15. **Stoll, A., and E. Seeback.** 1951. Chemical investigations on alliin, the specific principle of garlic. Adv. Enzymol. **11**:377–400.
16. **Tansey, M. R., and J. A. Appleton.** 1975. Inhibtion of fungal growth by garlic extract. Mycologia **67**:409–413.
17. **Wills, E. D.** 1956. Enzyme inhibition by allicin, the active principle of garlic. Biochem. J. **63**:514–520.