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Allicin from Garlic Strongly Inhibits Cysteine Proteinases and Cytopathic Effects of *Entamoeba histolytica*

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The ability of *Entamoeba histolytica* trophozoites to destroy monolayers of baby hamster kidney cells is inhibited by allicin, one of the active principles of garlic. Cysteine proteinases, an important contributor to amebic virulence, as well as alcohol dehydrogenase, are strongly inhibited by allicin.

Allicin [*S*-(2-propenyl)2-propene-1-sulfinothioate], which is one of the active principles of freshly crushed garlic (*Allium sativum*) homogenates, has been shown to have a variety of antimicrobial and antitumor activities (1–3, 17). One of the interesting findings reported by this laboratory some years ago (12) was the impressive growth-inhibitory effect of allicin on *Entamoeba histolytica*, the protozoan parasite that each year causes more than 50 million symptomatic cases of intestinal amebiasis. Allicin's antimicrobial properties were suggested to be due to its specific interference with the sulfhydryl enzymes (18). In the current report we present evidence of the remarkable inhibitory effect that pure allicin has on the ability of amebic trophozoites to destroy monolayers of baby hamster kidney (BHK) cells and on the inactivation of the various cysteine proteinases, enzymes which have been shown to be important contributors to amebic virulence and to be important for the degradation of food particles within the amebic vacuoles (5, 6, 9, 15, 16).

Pure allicin was produced by interaction of the synthetic substrate alliin [(+)*S*-2-propenyl L-cysteine *S*-oxide) with purified alliinase isolated from garlic cloves as described previously (8). Aqueous stock solutions of allicin (10 mM) were preserved at 4°C. Allicin was found to strongly inhibit the destruction of BHK cell monolayers by intact trophozoites (Fig. 1). BHK cell monolayers were incubated with 10^5 freshly harvested trophozoites of *E. histolytica* HM-1:IMSS for 1 h at 37°C as described previously (4) in the presence of various concentrations of allicin in Dulbecco's modified Eagle's medium. After the interaction, the trophozoites were removed from the wells by washing with cold phosphate-buffered saline (PBS) buffer. The percentage of remaining living BHK cells in the wells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT)-based assay (13). At 10 μM allicin, there was a 50% inhibition of the destruction of the monolayer, and at 50 μM allicin, no destruction could be observed. Experiments with controls incubated in the absence of trophozoites indicated that with 50 μM allicin there was minimal damage (<10%) to the viability of the mammalian cell monolayer (Fig. 1).

Allicin was also found to strongly inhibit the cysteine proteinase activities of intact and prelysed *E. histolytica* trophozoites. Trophozoites of *E. histolytica* HM-1:IMSS (10^6), grown and harvested as described previously (7), were suspended in 1 ml of PBS (pH 7.4) and were exposed before or after lysis with Nonidet P-40 (1% in PBS) for 10 min at 37°C to different concentrations of allicin. Intact trophozoites exposed to allicin were lysed immediately after the exposure under the same conditions. Cysteine proteinase activity was determined in the lysates by following the digestion of the chromophoric substrate benzyloxycarbonyl-L-arginyl-L-arginine-*p*-nitroanilide (Z-Arg-Arg-pNA; Bachem) (11). One unit of activity is defined as the number of micromoles of substrate digested per minute per milligram of protein. When the activity was determined in the presence of allicin, the reaction was started by adding the substrate 1 min after the addition of allicin. Short exposure of intact trophozoites to allicin (10 min at 100 μM) followed by detergent lysis of the amebae revealed an 80% decrease in total cysteine proteinase activity compared to that found in nonexposed cells (Fig. 2). Significantly lower concentrations of allicin (1 μM) were required to completely inhibit the cysteine proteinase activity of prelysed trophozoites. Treatment of the lysates with dithiothreitol (DTT) at 5 mM fully restored the proteinase activity (data not shown). Cathepsin B, a well-known cysteine proteinase which has been reported to have some substrate similarity to certain amebic cysteine protein-

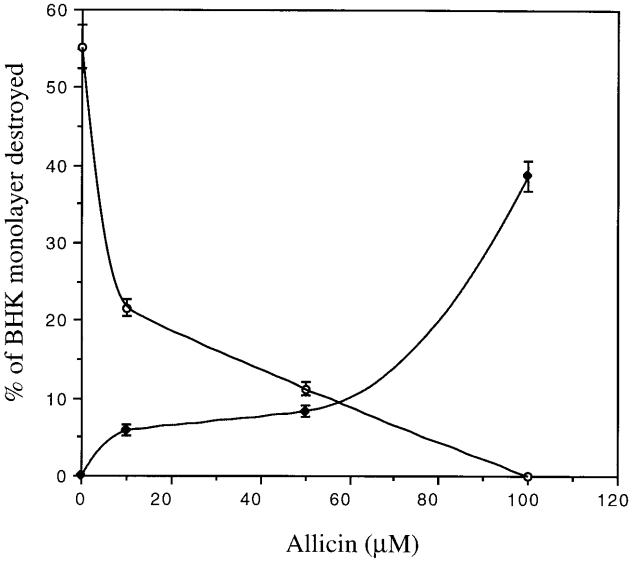

FIG. 1. Effect of allicin on BHK cell monolayer destruction by *E. histolytica* HM-1:IMSS. Open symbols, assays performed with *E. histolytica* HM-1:IMSS trophozoites (10^5); filled symbols, assays performed without added trophozoites. The percentage of live BHK cells remaining after the experiment was determined by the MTT-based assay (13).

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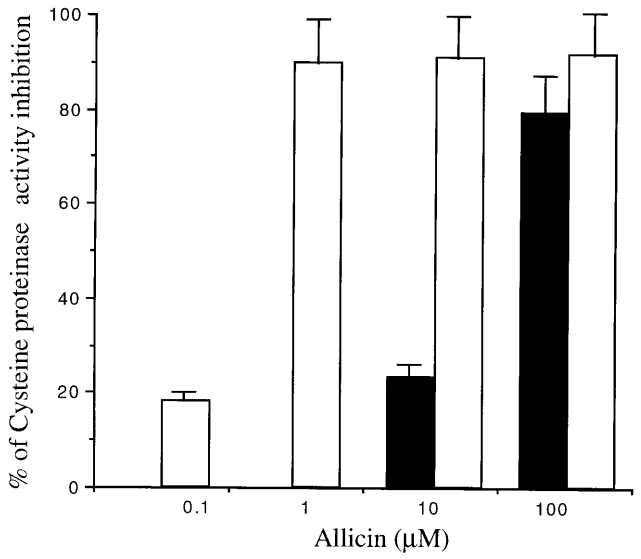

FIG. 2. Dose-response of allicin on intact versus disrupted trophozoites of *E. histolytica*. Symbols: open boxes, cysteine proteinase activity in crude trophozoite lysates exposed to various concentrations of allicin for 1 min; filled boxes, cysteine proteinase activity in lysates after intact trophozoites were exposed to various concentrations of allicin for 10 min. Cysteine proteinase activity (100%) determined in the absence of allicin was 54 U. One unit of activity was defined as the number of micromoles of substrate (Z-Arg-Arg-pNA) digested per minute per milligram of protein. Bars indicate standard deviations of the means.

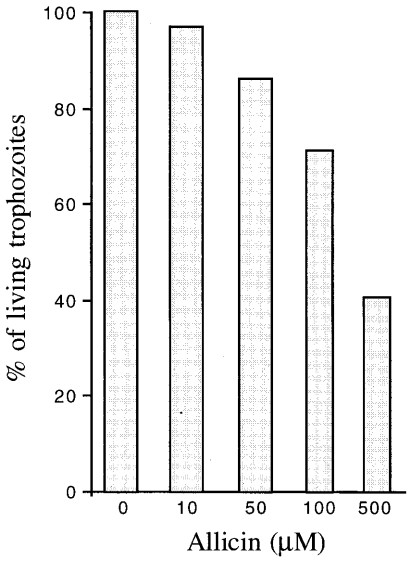

FIG. 4. Effects of allicin on the viability of *E. histolytica* HM-1:IMSS trophozoites. The percentage of live trophozoites remaining after their exposure to allicin (10 min) was determined by the MTT-based assay (13).

ases (10), was also strongly inhibited by allicin (10 μM). Inhibition could be fully reversed with 5 mM DTT (data not shown). Proteinase K, a protease belonging to the group of serine proteases, was not inhibited by allicin (1 mM) (data not shown).

The activities of the various cysteine proteinases of the ameba were also detected by observing in nondenaturing sodium dodecyl sulfate (SDS)-polyacrylamide gels-gelatin the different protein band digestion areas as described previously (14). SDS-polyacrylamide gel-gelatin analyses of the cysteine proteinases in the amebic lysates revealed that all the protein bands with visible gelatinase activity were significantly inhibited for the allicin-treated amebae (Fig. 3). This confirmed that allicin inhibits without discrimination all the cysteine proteinases which have gelatinase activity.

Recently, we have shown that allicin reacts with L-cysteine molecules by chemically modifying their free SH group in an S-thiolation reaction which can be reversed by DTT (14a). The same mechanism would be in agreement with the cysteine proteinase inhibition by allicin and can also explain the reversibility of inhibition with DTT.

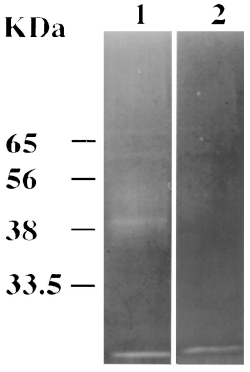

FIG. 3. Differences in the gelatinase activities of lysates of *E. histolytica* trophozoites (10^5) after exposure of intact amebae to allicin (100 μM). Lane 1, non-treated amebae; lane 2, amebae exposed to 100 μM allicin.

We have previously shown that intact trophozoites are rapidly killed by allicin (12). The percentage of living trophozoites remaining after a short (10-min) exposure to different concentrations of allicin was determined by estimating their dehydrogenase activity by the MTT-based assay (13) (Fig. 4). The concentration of allicin necessary to reduce by 60% the level of MTT activity was rather high (500 μM). A similar concentration of allicin was required to inhibit by 50% the activity of isopropanol dehydrogenase, which was assayed separately as described previously (11). This is fivefold higher than the concentration of allicin needed to inhibit more than 80% of the cysteine proteinase activity (Fig. 3). These results indicate that the dehydrogenases are not the preferred target of allicin and that the inhibition of destruction of monolayers of BHK cells is mainly due to inhibition of cysteine proteinase activity. The inhibition of dehydrogenases may contribute, however, to the overall lethal effect of allicin on intact trophozoites.

Our present findings shed more light on the remarkable mode of action of allicin on *E. histolytica* and lend further support to the reasons for the widespread use of allicin and fresh garlic extracts since ancient times as broad-spectrum, natural antimicrobial agents.

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