Meir Wilchek

Talia Miron
Aharon Rabinkov
David Mirelman

Allicin from garlic and its numerous applications for the benefit of Man

Department of Biological Chemistry

Tel. 972 8 934 3808 **Fax.** 972 8 946 8256
E-mail: meir.wilchek@weizmann.ac.il

Objectives of Research

Allicin is the main biologically active molecule in crushed garlic. Our objective is to investigate if pure Allicin can be useful for the treatment of various human diseases as well as for environmental and agricultural uses. The main problems which we aim to overcome stem from the very high reactivity, sensitivity and short life time of allicin. This requires the development of unique delivery systems and chemical stabilization studies.

Recent findings

Continuous production of pure allicin in aqueous solutions. Allicin is produced due to the interaction of the garlic enzyme, alliinase, with the substrate alliin. Alliinase was isolated, chemically stabilized and coupled to a solid matrix, thus enabling the efficient conversion of synthetic, nature-identical alliin to allicin. Aqueous solutions of allicin can be stored in the cold at 4°C for months (Fig. 1).

Binary capsules generate pre-determined doses of allicin in the small intestine. Most of the available garlic capsules contain very small, if any, amounts of allicin. A binary entero-coated capsule was recently prepared consisting of predetermined amounts of alliinase and alliin, which are kept physically separated by a special formulation. When the ingested capsules reach the small intestine, alliin dissolves and interacts with the alliinase to produce a predictable amount of allicin.

Thiol-modifying activity of allicin. Allicin specifically reacts with free thiol groups of numerous proteins, resulting in inactivation of a variety of enzymes such as papain, caspases, ACE, and alcohol dehydrogenases. Allicin also reacts with free thiol-containing molecules such as glutathione to yield allylmercaptoglutathione.

Allyl mercaptocaptopril (Captosal®), a novel antihypertensive and antilipidemic drug (with Drs. T. Rosenthal and E. Peleg, TAU-Med). Allicin lowers blood pressure and triglycerides in hypertensive and hyperlipidemic rats . The reaction of allicin with Captopril, commonly used as a hypotensive drug that contains free SH, yielded quantitative amounts of a novel and stable compound, allyl-mercaptocaptopril. This compound lowered blood pressure and triglycerides in hypertensive rats at smaller doses (2-fold) than those used for Captopril.

Allicin uptake prevents arterial plaque formation in genetically modified mice models (with Drs. D.Haratz, A. Shaish and A. Gonen, TAU-Med). Allicin was found to bind to serum LDL fraction and to inhibit its subsequent uptake by macrophages. Daily oral administration of allicin to Apo-E or LDL-receptor knockout mice caused a dramatic reduction in the formation of coronary arterial plaques.

Novel anti-cancer therapy: Alliinase-mAb conjugates generate allicin at target sites.

Allicin is toxic to various cancer cell lines. Allicin short half-life prevents its use in vivo. Conjugates consisting of pure alliinase ligated to a mAb specific for cancer marker ErbB2 retain their specific receptor recognition and enzymatic activity. Addition of the substrate alliin to conjugate-treated cell culture generates allicin, which kills the target cells without damaging normal cells devoid of the mAb receptor. Preliminary experiments in mice clearly demonstrated the high anti-tumor activity of the conjugates (Fig. 2) (animal experiments done with Dr. M Mironchik).

Allicin for soil fumigation in greenhouses and crop protection (with Drs. I. Chet and M. Abramski, HUJ-Agri). Methyl bromide has been recently banned from use as a soil fumigating agent

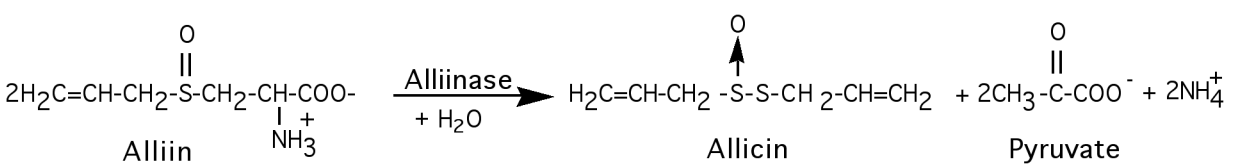

Fig. 1 *Synthesis of allicin from alliin*

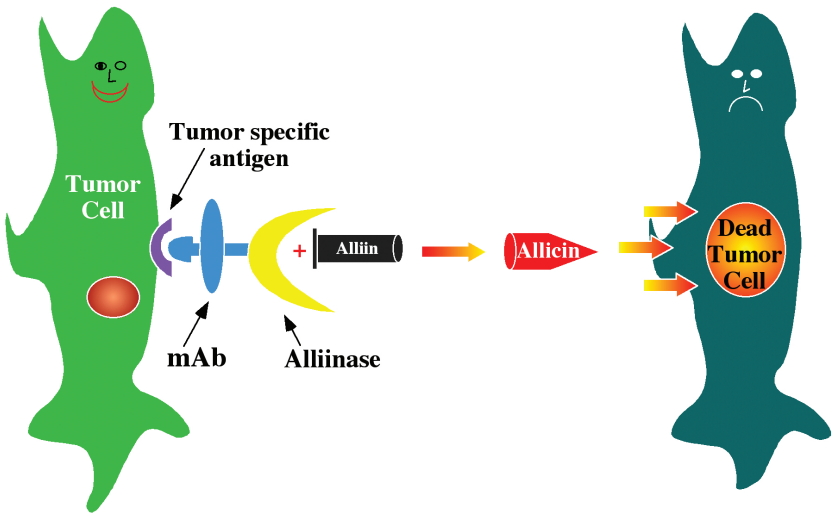

Fig. 2 *Monoclonal antibody-alliinase conjugates for anticancer therapy.*

due to its effect on the ozone layer. Irrigation of soil with low doses of allicin prior to seeding has been shown to protect plants from soil pathogens such as fungi and nematodes.

Selected Publications

Ankri, S., Miron, T., Rabinkov, A., Wilchek, M., and Mirelman, D. (1997) Allicin from garlic strongly inhibits cysteine proteinases and cytopathic effects of Entamoeba histolytica. Antimicrob. Agents & Chemother. 41, 2286-2288.

Rabinkov, A., Miron, T., Konstantinovski, L., Wilchek, M., Mirelman, D. and Weiner, L. (1998) The mode of action of Allicin: Trapping of radicals and interaction with thiol containing proteins. Biochem. Biophys. Acta 1379, 233-244.

Miron, T., Rabinkov, A., Mirelman, D., Weiner, L. and Wilchek, M. (1998) A spectrophotometric assay for allicin and alliinase (alliin lyase) activity: Reaction of 2-nitro-5-thiobenzoate with thiosulfinates. Anal. Biochem. 265, 317-325.

Abramovitz, D., Gavri, S., Harats, D., Levkovitz, H., Mirelman, D., Miron, T., Eilat-Adar, S., Rabinkov, A., Wilchek, M., Eldar, M., and Vered, Z. (1999) Allicin induced reduction in fatty streak formation (atherosclerosis) in mice fed with cholesterol rich diet. Coron. Art. Dis. 10, 515-519.

Miron, T., Rabinkov, A., Mirelman, D., Wilchek, M. and Weiner, L. (2000) The mode of action of allicin: its ready permeability through phospholipid membranes may contribute to its biological activity. Biochem. Biophys. Acta 1463, 20-30.

Rabinkov, A., Miron, T., Mirelman, D., Wilchek, M., Glozman, S., Yavin, E., and Weiner, L. (2000) S-Allylmercaptoglutathione: The reaction product of allicin with glutathione possesses SH-modifying and antioxidant properties. Biochim. Biophys. Acta 1499, 144-153.

Hirsch, K., Danilenko, M., Giat, Y., Miron, T., Rabinkov, A., Wilchek, M., Levy, J., and Sharoni, Y. (2000) The effect of allicin, a major ingredient of garlic, on cancer cell proliferation. Nutr. & Cancer 38, 245-254.

Elkayam, A., Mirelman, D., Peleg, E., Wilchek, M., Miron, T., Rabinkov, A., Sadetzki, S., and Rosenthal, T. (2001) The effects of Allicin and Enalapril in fructose-induced hyperinsulinemic hyperlipidemic hypertensive rats. Am. J. Hypertension 14, 377-381.

Acknowledgements

The research was supported by grants from the Levine Center and Yeda Co. Ltd. at the Weizmann Institute.

M.W. holds the Marc R. Gutwirth Chair of Molecular Biology.