Journal of Cellular Biochemistry 58:481–489 (1995)

Light and Electron Microscopic Immunocytochemical Localization of Two Major Proteins in Garlic Bulb

G.Y. Wen, A. Mato, H.M. Wisniewski, M.N. Malik, E.C. Jenkins, A.M. Sheikh, and K.S. Kim

New York State Institute for Basic Research in Developmental Disabilities, Staten Island, New York 10314 (G.Y.W., H.M.W., M.N.M., E.C.J., A.M.S., K.S.K.); Tottenville High School, Staten Island, New York 10312 (A.M.)

Abstract Garlic is known as a potent spice and a medicine with broad therapeutic properties ranging from antibacterial to anticancer, antidiabetic, and anticoagulant. Two major proteins of 40 KD and 14 KD constituting approximately 96% of total garlic proteins have been recently purified at our Institute. This immunocytochemical and ultrastructural study revealed that the 40 KD protein was localized in the parenchyma sheath cells (PSC) of garlic bulbs, whereas the 14 KD protein was present in the cortical cells (CC). Immunogold electron microscopy study indicated that the 40 KD protein was specifically localized in the globular granules of the cytoplasmic area of PSC. Each globular granule was amorphous and homogenous with membrane limiting its outermost layer. The yellowish color of PSC in freshly cut slices of garlic bulb suggested that PSC may have sulfur-containing compounds such as allicin, the primary contributor of the pungency and medicinal properties of garlic. Ellman's reagent test quantitatively revealed that there were 17.8 n moles sulfhydryl (SH)/ml of 40 KD garlic protein. Microtubule tubulin in mitotic figures from PHA-stimulated human short-term whole blood cultures reacted strongly with antitubulin antibody but reacted negatively with anti-40 KD garlic protein antibodies and therefore was not related to the 40 KD garlic protein immunocytochemically. © 1995 Wiley-Liss, Inc.

Key words: garlic proteins, parenchyma sheath cell, cortical cell, microtubule, tubulin

Garlic (*Allium sativum*) is classified as a member of the lily family [Mann, 1952]. It has been widely used not only as a spice/food but also as a folk medicine since ancient times in India, Egypt, Greece, Rome, and China for varieties of ills, including abdominal pain (intestinal disorders, ulcer), parasitic infection, insect and snake bites, hemorrhoids, rheumatism, and other ailments [Walker, 1986; Bordia and Bansal, 1973]. Garlic has also been thought to possess many other therapeutic properties such as the following: it lowers the blood-sugar level in diabetics [Bordia and Bansal, 1973]; it acts as an anticoagulant by action of ajoene (rearrangement of product of sulfur-containing allicin), the antiplatelet compound derived from garlic [Rendu et al., 1989; Walker, 1986; Heinerman, 1993]; it is antiinflammatory; it has exhibited antibiotic effects against both gram-positive and gram-negative bacteria; it inhibits growth of animal tumors by modulating macrophage and T-lymphocyte function related to antitumor immunity [Lau et al., 1991]; it lowers blood cholesterol by inhibiting cholesterol biosynthesis [Gebhardt, 1991; Jain et al., 1973; Bordia and Bansal, 1973; Pool, 1992]; it possesses an antifungal activity against a wide range of fungi [Amer et al., 1980; Bilgrami, 1992]. As indicated above, the characteristics of garlic are multifaceted. However, there is presently no information available on the composition and localization of proteins in garlic. A recent study [Malik et al., in preparation] revealed that garlic is composed of two major proteins with molecular weights of 40 KD and 14 KD, which constitute 96% of the total garlic protein. We have developed both polyclonal and monoclonal antibodies to these two major proteins and have described their histiotypic as well as their subcellular localization in this report.

MATERIALS AND METHODS

Both commercially available and homegrown garlic bulbs were used for this study. A mature/dry garlic bulb is a storage structure particularly well adapted to dry and semidried condi-

Received August 30, 1994; accepted December 22, 1994.

Address reprint requests to G.Y. Wen, Dept. Pathological Neurobiology, NYS Institute for Basic Res. in Devel. Disab., 1050 Forest Hill Road, Staten Island, NY 10314.

© 1995 Wiley-Liss, Inc.

tions for storage. It usually has 10–20 cloves. Each of the cloves consists of a protective leaf, a storage leaf (greatly thickened sheath), and a short stem. The outermost leaf, termed the protective leaf of the clove, is a dead, dry, bladeless sheath. The storage leaf accounts for about 80% of the fresh weight of the clove. All of the foliage leaves from the current season's growth, and the stem and roots as well, die, leaving only the cloves to continue the plant for the next season [Mann, 1952]. Therefore, the tissue being described here is from the storage leaf.

Antibody Preparation

Polyclonal antibodies to purified 40 KD and 14 KD proteins of garlic were raised in New Zealand white rabbits by a series of intramuscular injections with 250 μg of purified 40/14 KD protein in Freund's complete adjuvant initially and then two to three booster injections in Freund's incomplete adjuvant at intervals of 2–4 weeks. Antibodies were detected using an enzyme-linked immunosorbent assay (ELISA) procedure. Antisera giving >50% maximum color response after 30 min at 1,000-fold dilution were used as sources for the IgG fraction. IgG was purified using a column packed with Avid AL (BioProbe International, Inc., Tustin, CA). The anti-40/14 KD antibodies were then purified from the total IgG fraction on a 40/14 KD garlic proteins–agarose affinity column. Monoclonal antibodies (G1E5 and B5D6) to 40 KD and 14 KD proteins were produced with protocols described previously [Kim et al., 1990]. Antitubulin polyclonal antibody was obtained commercially (Sigma Chemical Co., St. Louis, MO).

Immunocytochemistry

Garlic bulbs were longitudinally and transversely cut into 1–2 mm thick slices and fixed in 10% neutral buffered formalin for 4–7 days and processed for 6 μm paraffin sections. Immunocytochemical protocols used by Munoz-Garcia et al., [1986] were modified. Briefly, garlic sections were deparaffinized, hydrated and washed in 0.05M Tris buffer saline (TBS), pretreated with formic acid for 5 min to expose the buried epitopes of antigens [Kitamoto et al., 1987] in order to maximize the reactivity with antibodies, washed with tap water for 10 min (three changes), treated with 3% hydrogen peroxide for 20 min to inhibit the endogenous activities, washed with TBS for 5 min, and treated with normal goat serum containing 10% fetal bovine serum for 30 min to block the nonspecific binding. The sections were then incubated with primary antibody (polyclonal antibodies against 40 KD (diluted at 1:15,000) and 14 KD (diluted at 1:15,000) garlic proteins and monoclonal antibody (Mab), G1E5, to 40 KD (diluted at 1:100) and Mab, B5D6, to 14 KD (diluted at 1:100) garlic protein) in a humidified chamber at 4°C overnight and washed with TBS. Host-matching secondary antibody (biotinylated goat antirabbit IgG for polyclonal antibody produced in rabbit or biotinylated horse antimouse Mab produced in mouse [Vectastain Elite rabbit/mouse ABC kit; Vector Lab. Inc., Burlingame, CA]) was added for 30 min at room temperature. The slides were rinsed again with TBS, exposed to Avidin-biotinylated peroxidase (Vectastain elite ABC kit) for 45 min, rinsed with TBS, visualized with 3, 3′-diaminobenzidine tetrahydrochloride (DAB) (Sigma Chemicals Co., St. Louis, MO) for 2–15 min, rinsed with TBS, dehydrated through 25%, 50%, 80%, two changes of 100% ethanol and 100% xylene, and mounted with permount. Since both 40 KD and 14 KD proteins/antigens were from garlic, antibody to 40 KD served as a positive control for antibody to 14 KD and vice versa. Immunoreactivity was evaluated and photographed with the light microscope. In addition, freshly cut unstained thin slices of garlic bulb were also examined with the light microscope.

Electron Microscopy (EM)

Garlic bulbs from the same source mentioned above were used in this EM study. Garlic bulbs were longitudinally and transversely cut into 1 mm thick slices and immediately fixed in 4% glutaraldehyde in 0.1 M Sorenson's phosphate buffer at pH 7.4 for 4 days instead of 2 h. Then the fixed slices were rinsed with the same buffer at pH 7.4 for 2 h, postfixed in 1% osmium tetroxide for 4 days, rinsed in the same buffer for 2 h, dehydrated through 70%, 85%, 95%, and 100% ethanol, and placed in propylene oxide (2×) for 1 h each; followed by infiltration in a 1:1 mixture of propylene oxide and Spurr's medium [Spurr, 1969] for 2 h and then placement in pure Spurr's medium for 4 days. The sample was then embedded and polymerized in a 70°C oven for 24 h. Then 1 μm sections were cut with a glass knife and stained with toluidine blue and counterstained with basic fuchsin, and the area of interest was selected for ultrathin sectioning

with a diamond knife. A Hitachi 7000 transmission electron microscope operated at 75 KV was used.

Immunogold Electron Microscopy Study Using LR White Embedded Sections

Garlic bulbs were cut into 1 mm thick slices and immediately fixed in 2% paraformaldehyde and 0.25% glutaraldehyde for 60 min, rinsed in 0.1 M phosphate buffer at pH 7.4 for 30 min, dehydrated through 50% and 70% ethanol for 15–20 min each, placed in a 1:1 mixture of 70% ethanol and LR White acrylic resin, medium grade (E.F. Fullam Inc., Latham, NY) for 60 min, and then placed in pure LR White resin overnight. Garlic slice samples were embedded in gelatin capsules and polymerized in a 52°C oven for 3 days. Then 1 μm sections were cut and stained with the same procedures for EM study as described above. For immunogold EM study, relatively thick EM sections of 90–100 nm (gold interference color to obtain greater signal for immunogold labelling) were cut and placed on noncoated 300 mesh nickel grids. Protocols for immunogold labelling were carried out as follows: The EM sections were immersed in a blocking solution of 20% normal goat serum (NGS) + 1% bovine serum albumin (BSA)/0.1 M phosphate buffer saline (PBS)/0.2% NaN_3 in a staining well for 30 min. The sections were then placed in either anti-40 KD (G1E5) or anti-14 KD (B5D6) garlic protein primary monoclonal antibody (diluted at 1:15) at 4°C overnight. Colloidal immunogold Auroprobe EM GAM (goat antimouse) IgG 30 nm gold particles (Amersham Life Science, Arlington, IL) were spun at 1,000–1,200 RPM in an Ependorff microfuge for 20 min to remove the undesired colloidal clumps producing nonspecific labelling. The supernatant containing suspended gold particles was diluted at 1:20× and used as a secondary antibody and incubated for 60 min or longer at room temperature. The EM sections on grids were then jet washed with 30 drops of 1% BSA/0.1 M PBS/0.2% NaN_3, followed by immersion in three changes of the same solution for 5 (3 × 5) min each; they then were jet washed with double-distilled water and dried on filter paper. After the sections were totally dried, they were stained with 2% aqueous uranyl acetate for 5 min. The EM sections were examined with a Hitachi 7000 electron microscope operated at 75 KV. Since both 40 KD and 14 KD proteins/antigens were from garlic, antibody to 40 KD served as a positive control for antibody to 14 KD and vice versa.

Immunogold EM Study With Etching of Spurr's Embedded Sections

Etching of Spurr's ultrathin sections and immunogold labelling were carried out to overcome the cell wall/membrane splitting of garlic tissues embedded in LR White resin. Spurr's ultrathin sections at 90–100 nm (gold interference color) were placed on 300 mesh uncoated nickel grids and rinsed with double distilled water for 10 min. In order to expose the antigens, sections were etched for 10 min with the supernatant of the saturated sodium metaperiodate centrifuged at 14,000 rpm in an Ependorff microfuge for 5 min, then rinsed with double-distilled water for 10 min, blocked with 10% NGS for 15 min, and then applied with primary monoclonal antibody, G1E5 (1:15× diluted with 1% BSA/PBS/0.2% NaN_3 buffer) at 4°C overnight. The other monoclonal antibody, B5D6 (diluted at 1:15), and polyclonal antibodies (diluted at 1:100) were also used. Sections were rinsed with PBS for 4 × 5 min, placed in GAM-30 (goat antimouse 30 nm) IgG immunogold particles (1:20× dilution) for 120 min or longer at room temperature, rinsed with 1% BSA/PBS/0.2% NaN_3 buffer 2 × 5 min, rinsed with PBS for 3 × 5 min, postfixed with 2% glutaraldehyde/PBS for 30 min, washed with PBS 2 × 5 min, jet washed again with 30 drops of double-distilled water, dried with filter paper, stained with 2% aqueous uranyl acetate for 5 min, rinsed with Millipore filtered (0.2 μm pore size) distilled water for 50 s, and dried again with filter paper. Examination of each section was carried out on the Hitachi 7000 electron microscope operated at 75 KV.

Quantitative analysis of the numbers of gold particles was performed by counting gold particles/μm^2 area from 10 different locations of globular granules of PSC of garlic bulb in the LR White embedded and in Spurr's medium embedded sections to obtain their mean ± standard deviation (SD) in order to select an appropriate immunogold EM method for this study.

Measurement of Sulfhydryl/SH Group in Garlic Protein With Ellman's Reagent

The presence of sulfhydryl/SH group in 40 KD garlic protein was detected by adding 250 μl of purified 40 KD garlic protein to the mixture of 50 μl of Ellman's reagent solution prepared by

dissolving 4 mg of Ellman's reagent to 1 ml of reaction buffer (0.1 M Na_2HPO_4, pH 8.0) according to the protocol provided with the package (22582×; Pierce Co., Rockford, IL) [Ellman, 1959] and 2.5 ml of reaction buffer (0.1 M) and incubating the mixture solution for 15 min at room temperature prior to measurement of absorbance at 412 nm and comparing with Ellman's reagent standard curve by preparing various concentrations of cysteine standards: 1.5 mM (2.634 mg/10 ml), 1.25 mM (2.195 mg/10 ml), 0.75 mM (1.317 mg/10 ml), and 0.25 mM (0.439 mg/10 ml). Based on the absorbance value of sample after subtracting the blank, the amount of sulfhydryl/SH can be quantitatively calculated according to the protocol provided with the package.

Comparison of Garlic Proteins and Microtubule Tubulin

As a source of mitotic figures, human whole blood was cultured for 4 days at 37°C in a 5% CO_2, water-jacketed incubator, in complete medium composed of RPMI-1640 base, 15% fetal bovine serum (GIBCO, Grand Island), 0.2% phytohemagglutinin-P (DIFCO, VWR, Piscataway, NJ), 1 mm glutamine, and 10,000 U-mcg/ml penicillin-streptomycin (GIBCO). At the end of 4 days, each 4 ml culture was washed in HANKS balanced salt solution (GIBCO), fixed, and washed several times in 3:1 methanol:acetic acid (Fisher, Springfield, NJ). The fixed mitotic cells were placed on clean, cold slides and allowed to air-dry. These slides were then immunocytochemically stained with either antitubulin or anti-40 KD garlic protein antibodies as described above.

RESULTS

Immunocytochemistry

Examination of garlic bulb sections immunocytochemically stained with anti-40 KD antibody and anti-14 KD antibody individually showed that strong positive immunoreactivities of both 40 KD and 14 KD proteins with each corresponding antibody were located in two distinctively different cell types of the garlic bulb. The 40 KD protein was located in the parenchyma sheath cells (PSC) (Fig. 1), whereas the 14 KD protein was present in the cortical cells (CC) (Fig. 2). The parenchyma sheath (PS) is the outermost part of vascular bundle of the storage leaf of garlic bulb and surrounding phloem parenchyma. The xylem is situated in the central region of the vascular bundle. The PSC (Figs. 3, 4) is long and sausage-like, containing cytoplasmic protein, lipid, starch granules, mitochondria, endoplasmic reticulum, vacuoles, and other organelles. A group of PSC forms a PSC bundle surrounded by CC. As compared with PSC, the CC (Figs. 1, 2) were loosely packed cells containing a relatively small amount of remnant cytoplasm, mitochondria, other substances, and a large area of empty space. All three antibodies, Mab G1E5 against 40 KD and polyclonal antibodies against both 40 KD and 14 KD proteins, revealed strong positive immunoreactivities to each corresponding protein in garlic tissue. Mab B5D6 against 14 KD, however, showed a specific but weak immunoreactivity.

Numerous yellowish spots were observed in the freshly cut unstained thin slices of garlic bulb. Light microscopic examination confirmed that these yellowish spots were equivalent to PSC/bundles.

Immunogold Electron Microscopy

EM study revealed that the immunogold particles representing 40 KD protein were specifically localized in globular granules of various sizes (1–20 μm in diameter) in the cytoplasmic area of garlic bulb PSC (Fig. 4). These globular granules contained amorphous homogenous contents and were membrane-bound at their outermost layers (Fig. 5). Gold particles were not observed in the mitochondria, starch granules, and cell walls (Figs. 4). Gold particles were not observed in CC (Fig. 6).

Numerous immunogold particles were observed in the LR White embedded sections of garlic tissues, but severe cell wall/membrane splitting (not shown) was observed. Comparison of two different immunogold EM methods using either LR White or Spurr's medium as embedding material conducted in this study (Table I) showed that Spurr's embedded garlic tissue displayed excellent preservation of cell wall and other cellular structures without any splitting problem (Fig. 5). Quantitative analysis (Table I) showed that the numbers of gold particles in the etched Spurr's embedded section is approximately 10% (5.5 ± 2.01 vs. 58.7 ± 7.2) of those in the LR White embedded section.

Measurement of Sulfhydryl/SH in Garlic Protein With Ellman's Reagent

A 250 μl purified 40 KD garlic protein sample mixed with 2.5 ml of reaction buffer and 50 μl of Ellman's reagent solution gave an absorbance

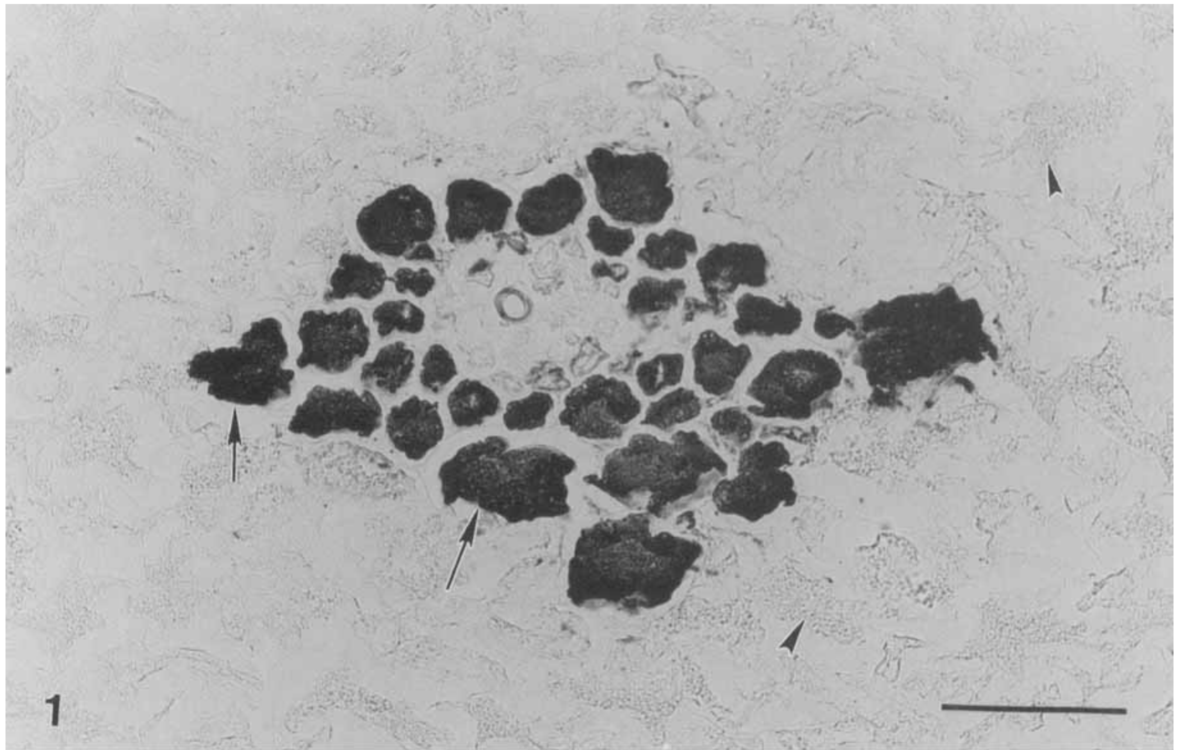

Fig. 1. Strong immunoreactivity to a monoclonal antibody (G1E5) against 40 KD garlic protein is present in the parenchyma sheath cells (arrows) as shown in the transverse section of garlic bulb. The cortical cells **(arrowheads)** surrounding the parenchyma sheath cells are not reactive to this anti-40 KD antibody. Bar, 100 μm. Note: Anti-40 KD polyclonal antibody also revealed a strong immunoreactivity with parenchyma sheath cells (not shown).

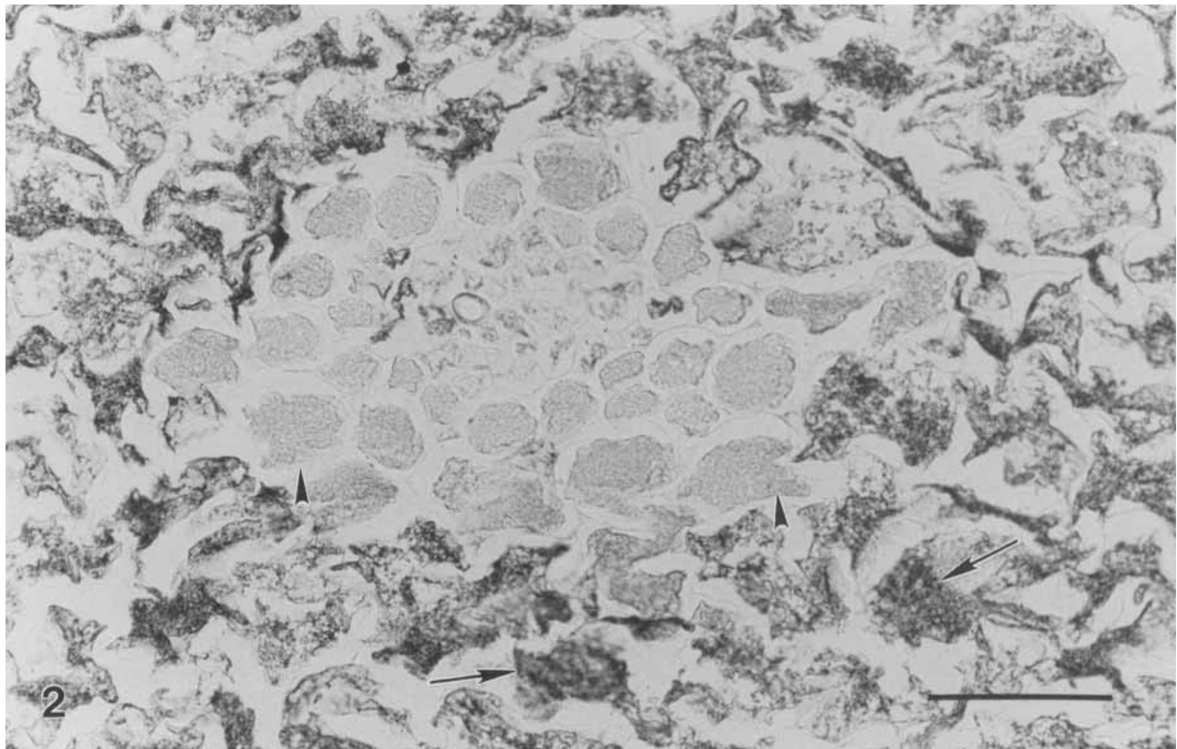

Fig. 2. Positive immunoreactivity to a polyclonal antibody against 14 KD garlic protein is located in the cortical cells **(arrows)** as shown in the transverse section of garlic bulb. The parenchyma sheath cells **(arrowheads)** are not reactive to this anti-14 KD antibody. Bar, 100 μm.

(at 412 nm) of 0.0228. Another sample of 40 KD garlic protein gave an absorbance of 0.0222. Based on the mean absorbance of 0.0225, there were 17.8 n moles sulfhydryl/ml 40 KD purified garlic protein.

Comparison of Garlic Proteins and Microtuble Tubulin

Mitogenically PHA-stimulated lymphocytes from human short-term whole blood culture were rich in microtubule tubulin, as they consistently reacted positively to antitubulin antibody (Fig. 7). Other mitotic cells from the same culture reacted negatively with anti-40 KD gallic protein antibody (Fig. 8).

DISCUSSION

Results of this immunocytochemical study revealed that 40 KD garlic protein was localized in the PSC of garlic bulb, whereas 14 KD garlic protein was present in the CC of garlic bulb. Using immunogold electron microscopy with monoclonal antibody (G1E5) to 40 KD protein, the 40 KD protein was further specifically localized in the globular granules of PSC. The monoclonal antibody (B5D6) to 14 KD protein revealed a specific but weak immunoreactivity at the light microscopic level.

In this study, numerous yellowish spots were observed in the freshly cut cross-sections of garlic bulbs. Light microscopic examination confirmed that these yellowish spots were equivalent to PSC of garlic bulb. The location of 40 KD protein in the PSC as shown in this study suggested that 40 KD protein may be associated with allicin, a sulfur-containing substance. The presence of 17.8 n moles sulfhydryl/ml purified 40 KD garlic protein quantitatively measured by Ellman's reagent test confirmed that 40 KD garlic protein contained sulfur. Allicin, reported as a sulfur-containing compound in the Merck Index [Budavari, 1989], is a yellow liquid and gives garlic its characteristic odor. The specific location of 40 KD protein in the globular granules within the PSC indicated that 40 KD protein was probably synthesized locally by these cells. The homogenous content of the globular granule may imply that each granule was made up of one component, the 40 KD protein. Immunogold EM localization of 14 KD protein using both Mab and polyclonal antibodies against 14 KD did not localize the 14 KD protein subcellularly. This may be related to the paucity of cytoplasm and the abundance of "empty" space in the CC because this type of CC may have contributed to the insufficient signals for immunogold labelling in 90–100 nm EM thin sections in comparison to 6 μm (6,000 nm) thick paraffin sections.

Etching of Spurr's ultrathin sections and immunogold labelling were carried out to overcome the cell wall/membrane splitting of garlic tissue embedded in LR White resin. This is the explanation for including a comparison between LR White and Spurr's embedding media. Cell wall/membrane splitting results in potential alteration of cellular structural integrity. This could change the distribution of antigen/gold particles. By using Spurr's medium, we now know that the results obtained with LR White medium were valid. Without demonstrating the structural integrity by using Spurr's medium, the distribution of gold particles may have been different. Therefore, the comparison between these two media was critical to this part of our study.

The commercially available garlic bulbs used in this study were in the semidried storage condition (for sale in the supermarket), and their cells were not in the stage of active cell division. Electron micrographs revealed that there was no obvious microtubule-like profile in CC. The immunogold particles of 40 KD protein were present in the globular granules of PSC. These globular granules contained amorphous homogenous uniform content without a trace of microtubule tubulin. Tubulin is known to be the major protein present in nearly all eukaryotic cells, with a molecular weight of around 40–50 KD. However, the immunocytochemical and morphological evidence in this study proved that 40 KD garlic protein was not related to microtubule tubulin.

In conclusion, this immunocytochemical study revealed that two major proteins of garlic were present in two distinctively different regions of the garlic bulb. The 40 KD protein was localized in the PSC, whereas the 14 KD protein was localized in the CC. Furthermore, 40 KD protein was specifically localized in the globular granules within the cytoplasmic area of PSC. The correlation of 40 KD protein location with the yellowish appearance of the PSC suggests that 40 KD protein may be a sulfur-containing protein or related to allicin, responsible for the pungency and some medicinal properties of garlic. The significance of the locations of these two proteins will be determined in future studies.

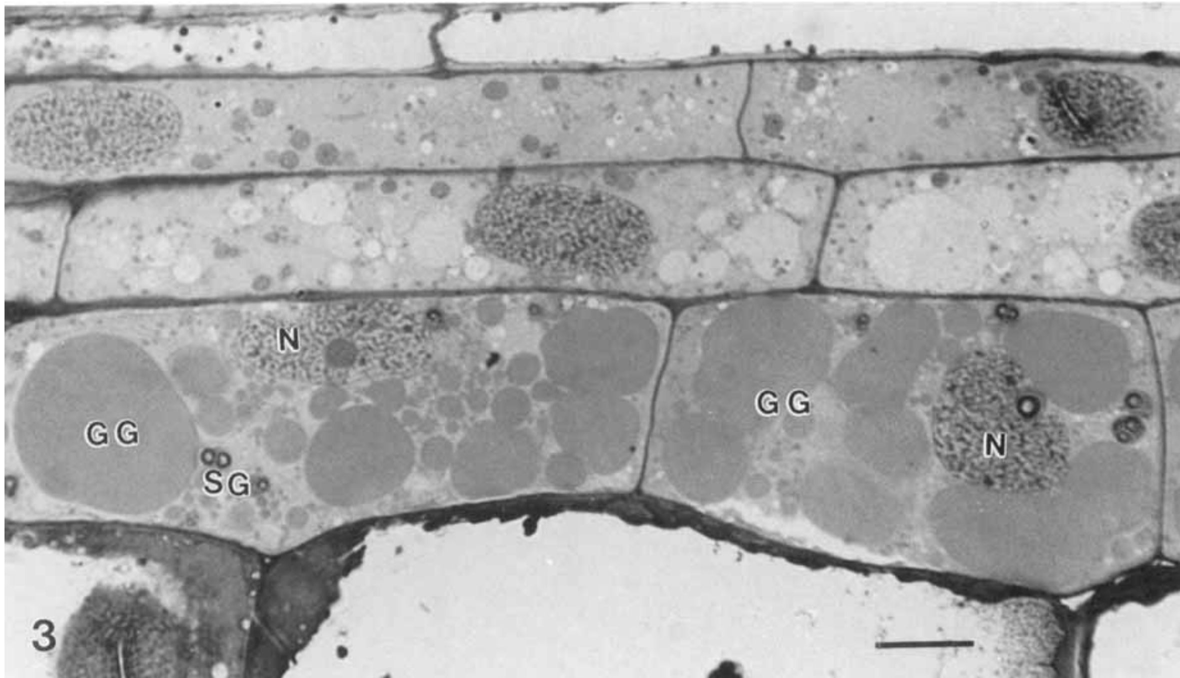

Fig. 3. A toluidine blue-stained 1 μm longitudinal plastic section of garlic bulb showing a parenchyma sheath cell containing globular granules (GG), starch granules (SG), nucleus (N), and nucleolus. Bar, 10 μm.

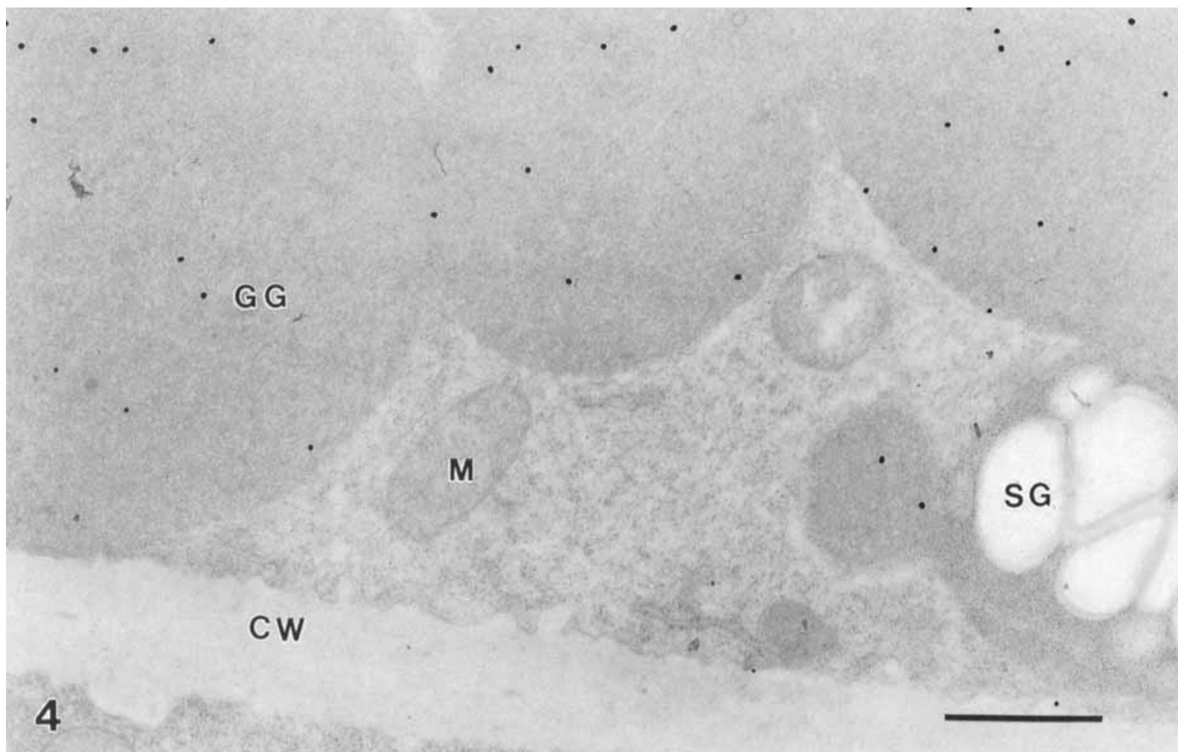

Fig. 4. Electron micrograph of longitudinal section of garlic cells showing that immunogold particles (representing the 40 KD garlic protein) are only present in the globular granules (GG) of parenchyma sheath cell. No gold particles were observed in the starch granules (SG), mitochondria (M), and cell wall (CW) of parenchyma sheath cell. Bar, 1 μm.

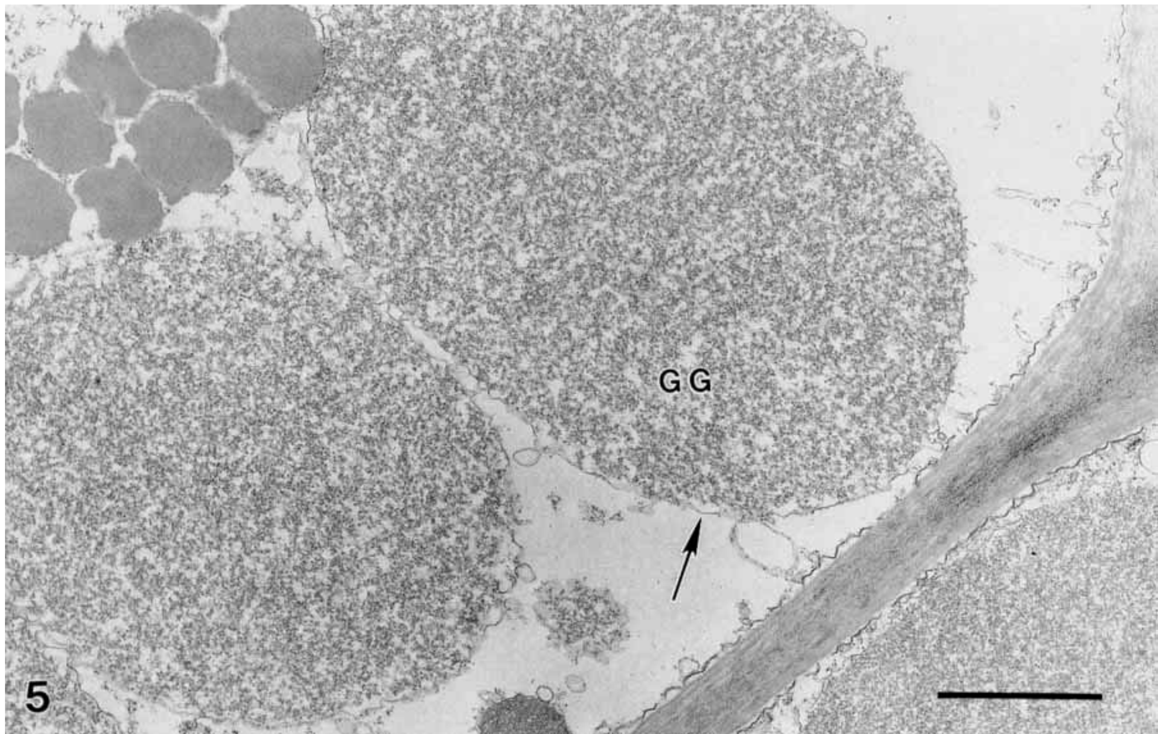

Fig. 5. Electron micrograph of cross-section of a parenchyma sheath cell showing the presence of internal uniform content and outermost membranes (**arrow**) in the globular granules (**GG**). Bar, 2 μm.

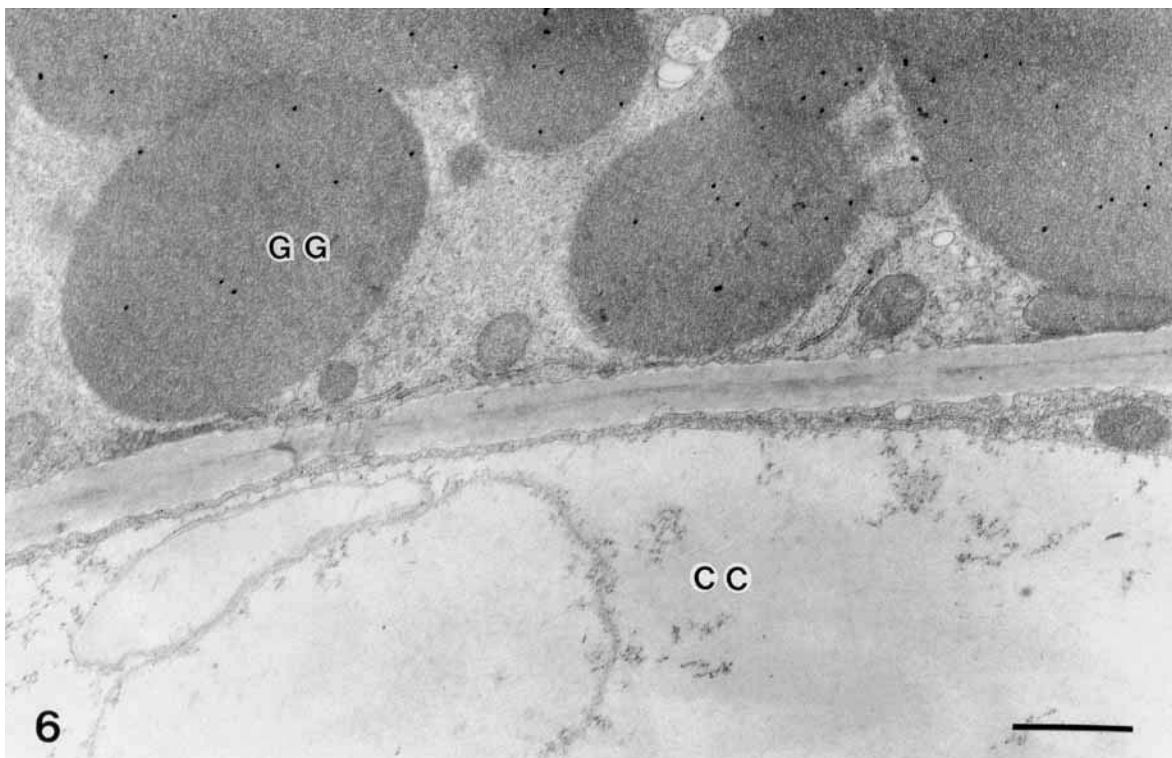

Fig. 6. Electron micrograph of cross-section of garlic cells showing that immunogold particles are present in the globular granules (**GG**) of parenchyma sheath cell but absent in the cortical cell (**CC**). Bar, 1 μm.

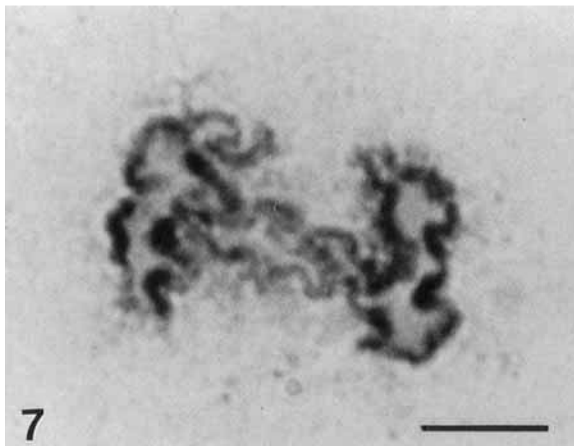

Fig. 7. Light micrograph showing positive immunoreactivity of a mitotic figure from a PHA-stimulated short-term human whole blood culture after exposure to an antitubulin antibody. Bar, 10 μm.

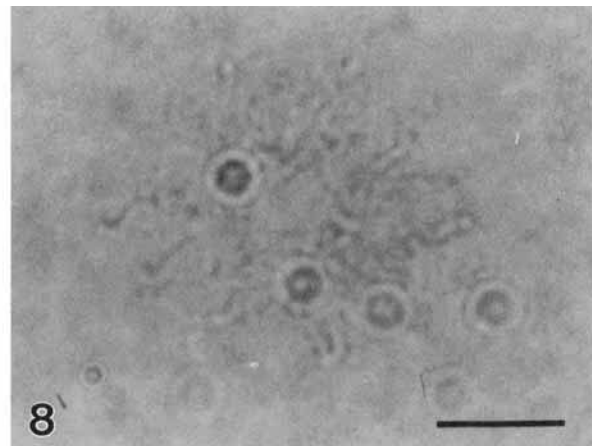

Fig. 8. Light micrograph of another mitotic figure showing negative immunoreactivity to an anti-40 KD garlic protein antibody (G1E5), showing that the 40 KD garlic protein was not related to tubulin present in the mitotic figure. Bar, 10 μm.

TABLE I. Comparison of the Numbers of Immunogold Particles/μm² Area in the Globular Granules of Parenchyma Sheath Cells Using the Same Concentration of Primary Monoclonal Antibody (G1E5) and Secondary Immunogold Antibody But Different Embedding Media

Replication number	LR White embedding medium	Spurr's embedding medium
1	65	4
2	70	5
3	60	3
4	58	8
5	59	7
6	48	4
7	55	4
8	56	8
9	49	4
10	67	8
Mean ± SD	58.7 ± 7.2	5.5 ± 2.01

ACKNOWLEDGMENTS

We thank Mrs. Janis Kay for secretarial assistance in preparing this manuscript. This work was supported by the Office of Mental Retardation and Developmental Disabilities of New York State.

REFERENCES

Amer M, Taha M, Tosson Z (1980): The effect of aqueous garlic extract on the growth of dermatophytes. Int J Dermatol 19:285–287.

Bilgrami KS (1992): Inhibition of aflatoxin production and growth of aspergillus flavus by eugenol and onion and garlic extracts. JAMA 268:3068.

Bordia A, Bansal HC (1973): Essential oil of garlic in prevention of atherosclerosis. Lancet 29:1491–1492.

Budavari S (1989): "The Merck Index," 11th ed. Rahway, NJ: Merck & Co.

Ellman GL (1959): Tissue sulfhydryl groups. Arch Biochem Biophys 82:70–77.

Gebhardt R (1991): Inhibition of cholesterol biosynthesis by a water-soluble garlic extract in primary cultures of rat hepatocytes. Arzneimittelforschung 41:800–804.

Heinerman J (1993): "Healing Power of Herbs." Boca Raton, Florida: New York: Globe Communication Corporation, pp 41–42.

Jain RC, Vyas CR, Mahatma PO (1973): Hypoglycaemic action of onion and garlic. Lancet 29:1491.

Kim KS, Wen GY, Bancher C, Chen CMJ, Sapienza VJ, Hong H, Wisniewski HM (1990): Detection and quantitation of amyloid β-peptide with 2 monoclonal antibodies. Neurosci Res Commun 7:113–122.

Kitamoto T, Ogomor K, Tateishei J, Prusiner SB (1987): Formic acid pretreatment enhances immunostaining of cerebral and systemic amyloids. Lab Invest 57:230–236.

Lau BHS, Yamasaki T, Gridley DS (1991): Garlic compounds modulate macrophage and T-lymphocyte functions. Mol Biother 3:103–107.

Mann LK (1952): Anatomy of the garlic bulb and factors affecting bulb development Hilgardia 21:195–251.

Munoz-Garcia D, Pendlebury WW, Kessler JB, Perl DP (1986): An immunocytochemical comparison of cytoskeletal proteins in aluminum-induced and Alzheimer-type neurofibrillary tangles. Acta Neuropathol 70:243–248.

Pool R (1992): Wrestling anticancer secrets from garlic and soy sauce. Science 257:1349.

Rendu F, Daveloose D, Deboucy JC, Bourdeau N, Levy-Toledanos S, Jain MK, Apitz-Castro R (1989): Ajoene, the anti-platelet compound derived from garlic specifically inhibits platelet release reaction by affecting the plasma membrane internal microviscosity. Biochem Pharmacol 38:1321–1328.

Spurr AR (1969): A low viscosity epoxy resin and embedding medium for electron microscopy. J Ultrastruct Res 26:31–43.

Walker M (1986): "Garlic: Nature's Healer." Greenwich, CT: Devin-Adir Publishers.