Biochimica et Biophysica Acta 1840 (2014) 350–356

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

# Nucleic acid binding properties of allicin: Spectroscopic analysis and estimation of anti-tumor potential

Gunjan Tyagi [a], Shrikant Pradhan [b], Tapasya Srivastava [b,*], Ranjana Mehrotra [a,**]

[a] Quantum Optics and Photon Physics, CSIR—National Physical Laboratory, Dr. K.S. Krishnan Marg, New Delhi 110 012, India
[b] Department of Genetics, University of Delhi (South Campus), New Delhi 110 021, India

ARTICLE INFO

*Article history:*
Received 21 April 2013
Received in revised form 3 August 2013
Accepted 6 September 2013
Available online 13 September 2013

*Keywords:*
Deoxyribonucleicacid
Ribonucleicacid
Allicin
Fourier transform infrared spectroscopy
MTT

ABSTRACT

*Background:* Allicin has received much attention due to its anti-proliferative activity and not-well elucidated underlying mechanism of action. This work focuses towards determining the cellular toxicity of allicin and understanding its interaction with nucleic acid at molecular level.
*Methods:* MTT assay was used to assess the cell viability of A549 lung cancer cells against allicin. Fourier transform infrared (FTIR) and UV-visible spectroscopy were used to study the binding parameters of nucleic acid-allicin interaction.
*Results:* Allicin inhibits the proliferation of cancer cells in a concentration dependent manner. FTIR spectroscopy exhibited that allicin binds preferentially to minor groove of DNA via thymine base. Analysis of tRNA allicin complex has also revealed that allicin binds primarily through nitrogenous bases. Some amount of external binding with phosphate backbone was also observed for both DNA and RNA. UV visible spectra of both DNA allicin and RNA allicin complexes showed hypochromic shift with an estimated binding constant of $1.2 \times 10^4$ $M^{-1}$ for DNA and $1.06 \times 10^3$ $M^{-1}$for RNA binding. No major transition from the B-form of DNA and A-form of RNA is observed after their interaction with allicin.
*Conclusions:* The results demonstrated that allicin treatment inhibited the proliferation of A549 cells in a dose-dependent manner. Biophysical outcomes are suggestive of base binding and helix contraction of nucleic acid structure upon binding with allicin.
*General significance:* The results describe cytotoxic potential of allicin and its binding properties with cellular nucleic acid, which could be helpful in deciphering the complete mechanism of cell death exerted by allicin.


## 1. Introduction

Allicin, diallylthiosulfinate, is an organosulfur compound known for its effective medicinal properties [1] (Fig. 1). It is regarded as a principle biological active component of garlic. Allicin was isolated in the middle of 20th century and was first recognized for its antibacterial properties [2]. Discovery of allicin is regarded as a leap forward in deciphering the health benefits associated with garlic. So far much research has been conducted on this medicinal compound and it is known for its antibacterial, anti-inflammatory and anti-oxidant properties along with cardiovascular benefits [3–5]. Since last few decades several studies have also linked allicin to tumor inhibition. Scientific investigations have demonstrated that allicin is capable of arresting rapidly dividing cells and can elicit their death. It has been reported to induce cell death by triggering apoptosis through mitochondrial pathway in HL60 and U937 cells [6]. Allicin was found to induce apoptosis in human cervical cancer SiHa cells and mouse fibroblast-like L-929 cells, through the appearance of characteristic apoptotic bodies, DNA fragmentation, and activation of caspases [7]. Growth of human epithelial carcinoma cell lines was also inhibited by allicin through a caspase-independent pathway, mediated by protein kinase A (PKA) activation and release of flavoprotein, AIF (apoptotic-inducing factor) from mitochondria [8]. Many other studies also attribute the cell death in various cancer cell lines and inhibition of induced tumor in animals to the anticancer action of allicin. However, the underlying mechanism of cell death caused by allicin has not been precisely and completely interpreted.

Several lines of evidence have indicated that allicin and its related compounds exert significant effect on cellular nucleic acid as a part of their anti-cancer mechanism. It is known to induce DNA fragmentation in various cell lines. S. Oommen et al. have shown the decrease in the rate of DNA synthesis as a function of increased concentration of allicin [7]. Several DADS (di allyl disulfide) analogues have been recognized to bind DNA [9]. It has also been realized that allicin reacts with RNA polymerase [10] and induces suppression of certain mRNA levels in human monocytes [11]. On the basis of these observations we have assessed the activity of allicin against A549 human non-small cell lung adenocarcinoma cell line and investigated the possibility of allicin interaction

* Corresponding author. Tel.: +91 11 24112761.
** Corresponding author. Tel.: +91 11 45608366; fax: +91 11 45609310.
*E-mail addresses:* tapasya@south.du.ac.in (T. Srivastava), ranjana@nplindia.org (R. Mehrotra).

0304-4165/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbagen.2013.09.007

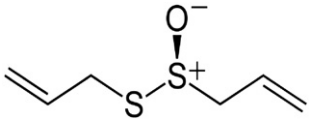

**Fig. 1.** Chemical structure of allicin.

with DNA and RNA by using FTIR difference spectroscopy and UV-vis absorption spectroscopy. FTIR spectroscopy has solved various problems related to biological macromolecular complexes [12–14]. It has emerged as an efficient tool in characterizing the nature of various biomolecules and their complexes, particularly for their structural information [15]. Precisely, it has shown tremendous potential in deciphering the binding parameters of various DNA-ligand and RNA-ligand complexes [16–19]. Hence in this study we have used the potential of FTIR and UV-vis spectroscopy to analyze various binding parameters of allicin DNA and allicin RNA complexes.

## 2. Materials and methods

### 2.1. Materials

Calf thymus DNA (Type I, sodium content 6%) and tRNA from Baker's yeast were purchased from Sigma-Aldrich chemicals (USA). Allicin was procured from Allicin International Ltd. Purity of DNA and RNA were estimated by recording the UV absorbance at 260 and 280 nm and calculating ratio, $A_{260}/A_{280}$. The ratio was found to be more than 1.9, indicating that the DNA and RNA are free from protein [20]. Deionized water from the Millipore water purification system was used in the preparation of desired aqueous solutions. Other chemicals and reagents used in the study were of analytical grade and used as supplied.

### 2.2. Preparation of stock solutions

Solution of DNA and RNA sodium salt was prepared in 10 mM Tris-HCl buffer (pH 7.4) and kept at 8 °C for 24 h. The solution was stirred at frequent intervals to ensure its homogeneity. Concentration of DNA and RNA stock solutions was measured spectrophotometrically using excitation coefficient of 6600 $cm^{-1}$ $M^{-1}$ and 9250 $cm^{-1}$ $M^{-1}$ respectively [21]. The final concentration of DNA and RNA stock solutions was 25 mM. For FTIR studies allicin–DNA and allicin–RNA complex solutions were prepared, so as to attain the allicin/DNA and allicin/RNA molar ratios ($r$) of 1/150, 1/80 and 1/20 with varying concentration of allicin and constant concentration of DNA and RNA. UV visible studies were carried out using allicin concentrations ranging from 0.025 mM to 0.5 mM and DNA and RNA concentration of 2.5 mM.

### 2.3. Cell culture and cell viability

The human non-small cell lung adenocarcinoma cell line A549 was used in the study. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Sigma, USA) supplemented with 10% fetal bovine serum (Himedia, India) and 10 μg/ml ciprofloxacin (Sigma, USA). Cells were incubated at 37 °C in 5% $CO_2$ atmosphere.

Cell viability was measured using the MTT assay. A549 cells were plated at a density of $5 \times 10^3$ cells per well in 96 well plates. After overnight culture, cells were treated with different concentrations of allicin (1, 10, 20, 40, 60, 80, 100, 120, 150 μg/ml) and incubated in cell culture conditions for 72 h at 37 °C. Then, medium in each well was discarded and 100 μl media containing 200 μg/ml MTT was added in each well and the plates were incubated at 37 °C for 4 h. The medium containing MTT was discarded and 200 μl DMSO was added to dissolve the insoluble purple formazan product to colored solution. Absorbance was recorded at 570 nm. Allicin mediated cell death was assessed by comparing the viability of treated cells with that of untreated control cells using the following formula.

$$\text{Cell Viability } \% = \frac{[\text{Absorbance of a well with cells}] - [\text{Mean absorbance of media blank well}]}{\text{Mean absorbance in } [\text{control wells} - \text{media blank well}]} \times 100$$

The experiments were performed in three biological replicates.

### 2.4. FTIR spectral measurements

FTIR spectra were recorded with Varian 660-IR spectrophotometer, equipped with DTGS (deuterated triglycine sulphate) detector and KBr beam splitter. All spectra were recorded in 10 milli molar Tris-HCl buffer as a solvent (pH of 7.4). Liquid samples were analyzed in attenuated total reflectance mode with ZnSe crystal. Ambient humidity of 45% RH was maintained during the experiment. A total of 256 scans were recorded for each sample in the spectral range of 4000-650 $cm^{-1}$ with a resolution of 4 $cm^{-1}$. Background spectra were collected before each measurement. A spectrum of buffer solution was recorded and subtracted from the spectra of DNA, RNA and allicin DNA and allicin RNA complexes. A satisfactory buffer subtraction was considered to be achieved when the intensity of water combination band at about 2200 $cm^{-1}$ became zero in all the spectra recorded [22]. FTIR difference spectra were produced by subtracting the spectrum of free DNA from the spectrum of allicin DNA complex ((DNA solution + allicin solution) − (DNA solution)).

### 2.5. UV–visible spectral measurements

The UV–visible spectra were recorded on Perkin Elmer spectrophotometer, Lambda 35. Quartz cuvettes of 1 cm path length were used for measurement. Spectra were recorded for free DNA, RNA and for various allicin–DNA and allicin–RNA complex solutions. For the calculation of the binding constant of the reaction occurring between allicin and DNA and allicin and RNA the method described by Kanakis et al. [23] is used. It is presumed that after the interaction between ligand [L], which is allicin and substrate [S] which is DNA and/or RNA in aqueous solution, the complex [SL] forms [24].

It is also assumed that the substrate and the ligand follow beer's law for the absorbance of light. The absorbance of substrate solution at its total concentration with a path length [$l$] of 1 cm is

$$A_o = \varepsilon_s l S_t \tag{1}$$

$\varepsilon_s$ is the molar absorptivity of DNA.

The absorbance of solution consisting of total concentration of substrate along with total concentration of ligand is

$$A_L = \varepsilon_S l[S] + \varepsilon_L l[L] + \varepsilon_{SL} l[\mathrm{SL}]. \tag{2}$$

[$S$] is the concentration of uncomplexed substrate.
[$L$] is the concentration of uncomplexed ligand.
[SL] is the concentration of substrate-ligand complex.
$\varepsilon_L$ is the molar absorptivity of ligand.

After combining with the mass balance of substrate and ligand, the absorbance equation can be written as

$$A_L = \varepsilon_S l S_t + \varepsilon_L l L_t + \Delta\varepsilon_{SL} l[\mathrm{SL}] \tag{3}$$

$$\Delta\varepsilon_{SL} = \varepsilon_{SL} - \varepsilon_S - \varepsilon_L.$$

The absorbance of solution measured against the total concentratin of ligand as reference is

$$A = \varepsilon_S l S_t + \Delta\varepsilon_S l[\mathrm{SL}] \tag{4}$$