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Oxadiargyl induced conformational transition of cystatin isolated from yellow mustard seeds: Biophysical and biochemical approach

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ABSTRACT

Phytocystatins are thiol proteinase inhibitors crucial due to their inhibitory activity in plants. These play important roles in improving crop yield, protection against insects and pathogens and modulation of apoptosis. In this chemical era, various pesticides are being used globally to increase the crop biomass. These pesticides accumulate in plant body and produce harmful effects on plants itself by interacting with essential proteins. In this present study, we have monitored the interaction of a herbicide; oxadiargyl, with phytocystatin isolated from yellow mustard seeds (YMP) by employing spectroscopic techniques viz. UV, fluorescence, FTIR and CD spectroscopy and Isothermal titration calorimetry (ITC). UV and fluorescence spectroscopy shows YMP transformation from native to non-native form apparent by decreased absorbance and decreased fluorescence. FTIR and CD spectroscopy further confirmed secondary structural disruption of YMP. Anti-papain activity assay was also carried out; a reduction in activity was observed in presence of oxadiargyl. Thermodynamic parameters obtained from ITC shows affinity of oxadiargyl towards phytocystatin. Oxadiargyl was also found to induce ROS generation in YMP as evident by DNPH assay. Thus oxadiargyl binds to phytocystatin causing structural alterations reducing its physiological benefits and altering its functionality and ultimately leading to reduced crop yield.

1. Introduction

Cystatins act as competitive inhibitors that which synchronize the biological activity of C1 class (papain-like) cysteine proteases; implicated in proteolytic process and are ubiquitously distributed in every form of life viz. vertebrates as well as invertebrates [1]. Presently cystatins can be categorised in to four families: stefins, cystatins, kininogens and phytocystatins [2–4]. All the cystatins contain three conserved regions unravelled by mutagenesis and X-ray crystallographic studies. These are an N-terminal glycine, a glutamine valine-glycine (Q-X-V-X-G) loop and a second c-terminus hairpin loop consisting of proline tryptophan (PW) residues [5]. Though the importance of cystatins has been explored meticulously in animal and plant system, some still remain unexplored. Cystatins have many beneficial properties for plants and humans. Cystatins function in the native host-plant defense system and they are expressed in response to wounding and pest infestation. Cystatins can also control fungal and viral pathogens acting against viruses by affecting their replication which requires cysteine proteinase activity [6]. Phytocystatins are involved in variety of essential processes; still these have been given little importance up till now and needs to be explored. Cystatins are further involved in the regulation of plant developmental processes ranging from seed germination [7] to natural and abiotic stress-induced senescence [8]. Expression of an exogenous cystatin in a genetically modified transgenic tobacco plant limits chilling and drought sensitivity [9] and in soybean, controls shoot branching and plant growth [10]. Exogenous cystatin expression also represses potato tuber sprout growth associated with loss of apical dominance and formation of an increased number of small buds at the skin surface [11]. A number of transgenic plant lines expressing exogenous cystatins were successfully produced and tested over the last 15 years for various protease targets. Studies have shown increased expression of phytocystatins in response to biotic and abiotic stress. Plant pathogens such as fungi, insects, nematodes and many more invade into tissues by utilising cysteine proteases and disturb the homeostasis of plant residential proteins. Apart from pathogens, excessive weeds also reduce the growth of desired plant and compete for the available resource. Some weeds also show allelopathic effect, hence affect optimum crop production. The pathogens act as parasites and continuously damage plant body that will lead to compromised yield or ultimately death. To escape such incidences,

Abbreviations: YMP, yellow mustard phytocystatin; CD, circular dichroism; ITC, isothermal titration calorimetry; ROS, reactive oxygen species.

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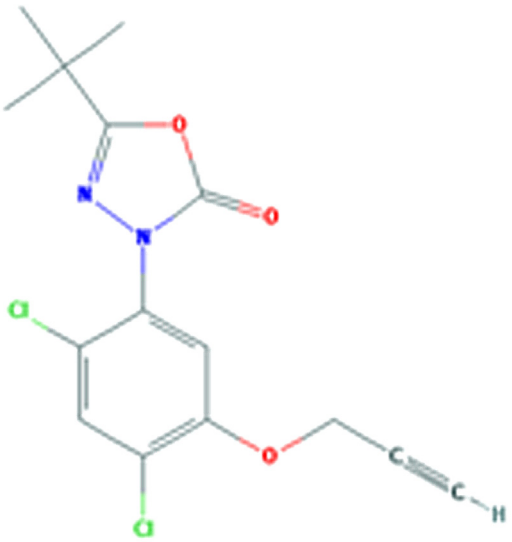

Fig. 1. Molecular structure of oxadiargyl.

use of pesticides is on a hike. In this era of chemical advances, there is a frequent use of fertilizers, herbicides, fungicides, insecticides, nematicides by cultivators to prevent plants from attack by these pathogens. There are many pesticides which are routinely used in cropland such as pendimethalin, methoxyfenozide, CuII hydroxide, chlorpyrifos, mancozeb, carbamates, glyphosate, methamidophos, iprodione, procymidone and chlorothalonil [12–15].

Remarkable paybacks have been observed after employing pesticides in agriculture, forestry, domestic sphere as well as in public health. Various vector borne diseases are nearly eradicated by killing vectors with the help of pesticides. Use of pesticides is profitable to improve crop

Our present study aims to see the effect of herbicide; oxadiargyl on cystatin isolated from yellow mustard. In our present work, we have analyzed in vitro interaction of herbicide: oxadiargyl with cystatin isolated from yellow mustard. The work is carried out to highlight the side effects caused by the usage of herbicides in yellow mustard plants. Oxadiargyl is a herbicide of oxadiazoles chemical group, an active constituent in protecting plants from weeds (Fig. 1). It is an inhibitor of protoporphyrinogen oxidase the most significant action target of large group of herbicides [17].

2. Materials and methods

2.1. Materials

Oxadiargyl, papain, 8-Anilino-1-naphthalenesulfonic acid, and Dinitrophenyl hydrazine were purchased from Sigma Chemical Co, USA. Casein, EDTA, disodium hydrogen phosphate, monosodium dihydrogen phosphate, copper sulfate, L-cysteine, sodium dodecyl sulfate, tetramethylethylenediamine, trichloroacetic acid, Trifluoroacetate, and Folin-Ciocalteu phenol reagent were obtained from Sisco Research Lab, India. YMP was isolated and purified as documented earlier [18]. All other chemicals used were of highest purity grade.

2.2. Sample preparation

YMP stock solution (10 μM) was prepared in 50 mM sodium phosphate buffer solution of pH 7.50. To avoid any microbial contamination sodium azide (0.02%) is added to all the preparation and were filtered before use. Oxadiargyl stock of 2 mg/ml was prepared in organic solvent (ethanol). Aliquots were analyzed at different time intervals (1–8 h) for structural and functional changes. All the experiments are done in triplicates for accuracy.

2.3. Isolation of YMP

Isolation of phytocystatin was done by employing mainly two-step process including ammonium-sulphate saturation and size-exclusion chromatography from yellow mustard seeds as reported earlier [18]. The isolated phytocystatin is named as yellow mustard phytocystatin (YMP).

2.4. Anti-papain activity assay

Inhibitory activity of YMP alone and YMP with different concentration (10–40 μM) of oxadiargyl was carried out based on its ability to inhibit caseinolytic activity of papain by method of Kunitz [19]. Activity of phytocystatin alone was taken as 100%. Ani-papain activity was also determined in the presence of oxadiargyl for different time intervals (1, 4 and 8 h).

2.5. UV spectroscopic measurement

The UV measurements of YMP in the presence and absence of varying concentration (10–40 μM) of oxadiargyl was made and spectra were recorded in the range of 190–400 nm. The inhibitor (YMP) concentration was kept (4 μM). The UV spectra were taken by making use of spectrophotometer (Shimadzu, JAPAN) with a cuvette of 1.0 cm length.

2.6. Trp fluorescence measurement

Trp fluorescence emission spectra were recorded in 300–400 nm rangeon a Shimadzu RF-5301spectrofluorophotometer (Tokyo, Japan) with excitation wavelength at 295 nm [20]. Path length was kept 5 mm quartz cell. The final concentration of YMP in the aliquots was 4 μM.

2.7. Anilino-1-naphthalene-sulphonic acid (ANS) fluorescence measurement

ANS binding was measured by fluorescence emission spectra with excitation wavelength of 380 nm and emission range was taken from 400 to 600 nm. Typically, the concentration of ANS was 50 molar excess of the protein concentration and concentration of protein YMP was 4 μM in 50 mM sodium phosphate buffer, pH 7.5 [21].

2.8. Fourier transformed infra-red spectroscopy (FTIR spectroscopy)

ATR-FTIR spectra were taken with a PerkinElmer FTIR (PerkinElmer Spectrum 100) in deuterated buffer of pH 7.5 of 50 mM sodium phosphate buffer in the range of 1600–1700 cm^{-1} at room temperature. Fourier transform infrared spectra of YMP (10 μM) and YMP with different concentrations of oxadiargyl (10–40 μM) were recorded. All the spectra were average of three independent spectra.

2.9. Circular dichroism

Far-UV CD examination was performed by a Jasco spectro polarimeter, model Jasco 815 and serial no B069061168, calibrated with ammonium D-10-camphorsulfonate. Spectra of control YMP and YMP along with oxadiargyl were taken in the range 200–250 nm. The path length was 1 mm and concentration of YMP