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Degradation of *Solanum xanthocarpum* Leaves Protein

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Inside plant cells, the enzyme ribulose bisphosphate carboxylase/oxygenase (rubisco) forms the bridge between life and the lifeless, creating organic carbon from the inorganic carbon dioxide in the air. But only three carbon dioxide molecules are fixed by rubisco per second. Chloroplasts are filled with rubisco, which comprises half of the protein which contributes upto 50 % soluble protein and 30 % of total leaf nitrogen in leaves of C3 plants. This makes rubisco the most plentiful single enzyme on the earth. In the present study, *Solanum xanthocarpum* leaves protein and carbohydate contents was 12 and 18 %, respectively. It was used for the degradation and the neutralization of it for the nitrogen composition in the environment. Protein degradation considerd when 1 mM SDS and EDTA used under different stress conditions. Protein degradation limits at 60 ºC using tris buffer pH 8.0 at 1 h with stirring. Characterization and degree of dissociation of rubisco/protein was determined by splitting property of bands on gel electrophoresis and discrete spots on TLC.

Key Words: Rubisco, Protein degradation, *Solanum xanthocarpum* L.

INTRODUCTION

Photosynthesis is one of the most heat sensitive function of plants. Exposure of plants to elevated temperatures results in a rapid inhibition of photosynthetic CO_2 fixation, oxygen evolution and photophosphorylation[1]. Rubisco (ribulose 1,5-bisphosphate carboxylase-oxygenase) is the most abundant protein and a major sink for C3 plant nitrogen[2,3]. Protein degradation is required for change in the protein pattern and normal development of plants, homeostasis with final death of it[4]. Rubisco seems to be a main target of many stresses get inactivated by elevating light and temperature.

Solanum xanthocarpum, the Indian night shade, commonly known as 'Kantakari' is found throughout the country, but more abundantly in arid areas. The plant is known to have multiple medicinal properties[5-7]. The extract of various parts of it have been used against agricultural pests as repellant[8], contact poison[9] and as molluscicide[10] in public health. The plant extract is also used in the treatment of diseases like fever, asthma, tuberculosis, kidney disorders, cough, constipation, tooth-ache, sore-throat, rheumatism and gonorrhea[5]. Phytoactive compounds extracted from this plant include solanocarpine, solanocarpidine, disgenin, carpestrol and sitosterol and steroids[11]. In view of the potential medicinal and insecticidal importance of this species, an attempt has been made to study the protein degradation under the different physical and chemical conditions like temperature, stirring, salt, surfactant, chelator *etc*.

EXPERIMENTAL

The *Solanum xanthocarpum* leaves were collected from Ambernath, District-Thane, Maharashtra state, India in April, 2010. It is waste land weed which occurs throughout India.

The aerial parts of *Solanum xanthocarpum* leaves were air dried in the shade for several days at room temperature. After drying the leaves were separated and ground to obtained fine powder. The leaves powder was stored in air-tight container to protect from humidity and light.

Tris buffer, Citron X-100, SDS, urea and EDTA used were of analytical and pure grade obtained from Hi-Media. Deionized and distilled water used throughout the study.

Leaves extract: *Solanum xanthocarpum* leaves powder (10 mg) was extracted with (50 mM) *tris* buffer pH 8.0. The crude was filtered through muslin cloth and supernatant was stored in a refrigerator for further study.

Protein assay: The total protein was determined by the method of Lowry *et al.*[12], using casein as a standard (0.16 corresponds to 0.1 mg of protein). The spectrophotometric protein assay was measured at 280 nm on UV-visible spectrophotometer (Shimadzu UV-1800).

Determination of buffer system: To determine optimum pH and buffer system for maximum rubisco activity; phosphate buffer and *tris* buffer were used. Finally *tris* buffer was selected for the separation of rubisco as maximum activity was observed in *tris* buffer system.

Determination of optimum pH: *Solanum xanthocarpum* leaves powder was extracted in *tris* buffer system for the determination of optimum pH. It was studied at various pH ranging from 5-10 (50 mM) using various buffer system and assay was carried out.

Effect of temparature/heat on *Solanum xanthocarpum* leaves protein: The most conventional method was used to study protein degradation is, supernant incubated at various temperature. The stability of rubisco (protein) was estimated by studying irreversible thermal inactivation at several temperatures from 0-60 ºC. The 100 µL of protein extract were kept in a water-bath at temperature 10-60 ºC using 0 ºC as a control. Protein assay was carried out for the denaturation/stability of rubisco.

Effect of chelator/detergents: Supernatant of *Solanum xanthocarpum* leaves were incubated with Citron X-100 w/v, 1 mM of SDS, 1 mM of urea and 1 mM EDTA separately at 10-60 ºC. Protein assay was carried out by colorimetrically as well as spectrophotometrically.

Effect of change in concentration of SDS and EDTA at 10-60 ºC: Change in concentration of SDS and EDTA incubated with *Solanum xanthocarpum* leaves supernatant separately at various temperature using untreated sample as a control. Protein assay was carried out by colorimetrically as well as spectrophotometrically.

Effect of stirring: The leaves extract was kept on a magnetic stirrer and aliquots of 0.1 mL sample at different time intervals (1-10 h) were used for the degradation of rubisco (protein) and the capacity of Ca-1 phosphate inhibitor. After each stress condition the sample was loaded on TLC and electrophoresis.

Thin layer chromatography: Thin layer chromatography was performed on 20 cm × 20 cm cellulose plates with the solvent *n*-propanol/ammonia/water (6:3:1 v/v).

Electrophoresis: The leaves extract was reacted with SDS solution containing (50 mM) *tris* buffer pH 8.0, (1 mM) EDTA and (1 mM) DTT kept at 25 ºC for 0.5 h. A 20 µL was then separated on 7.5 % polyacrylamide gel and stained with coomassie brilliant blue.

RESULTS AND DISCUSSION

Solanum xanthocarpum leaves have medicinal properties and used to treat a disease which is depending upon the constituents present in the plant. This plant is waste land weed. *Solanum xanthocarpum* leaves contain 12 % protein and 18 % of carbohydrates measured by colourimetrically as well as spectrophotometrically.

When *Solanum xanthocarpum* leaves (10 mg) was extracted with different molar concentrations of various buffer at varying pH. It was observed that 50 mM *tris* buffer at pH 8.0 shows maximum activity of rubisco as compared to phosphate buffer.

When this waste land weed exposed to various temperature at different time interval, it strongly affects senescence and the degradation of chloroplast protein. Rubisco represents an interesting enzyme protein with respect to its catabolism. Rubisco degradation relevant under various stress conditions that changes its pattern. After incubation of *Solanum xanthocarpum* leaves supernatant at various temperatures ranging 0-60 ºC with 0.1 ºC control accuracy; showed 20-30 optimm temperature but it stable up to 60 ºC (Fig. 1). It gives insight of protein degradation in relation to time. However further increase in temperature causes denaturation and change in colour of supernatant was observed. To restore of rubisco in an active state at high temperatures requires faster rates of rubisco inactivation. Rubisco was extremely heat stable and require 5 h stirring for degradation. But it is insufficient to keep pace with the faster rates of rubisco inactivation at high temperatures and stirring. Thus, the activation state of rubisco decreases under heat stress. The poor performance of it at high temperature has been attributed to its relatively low temperature optimum for catalysis caused in part by thermal instability[13].

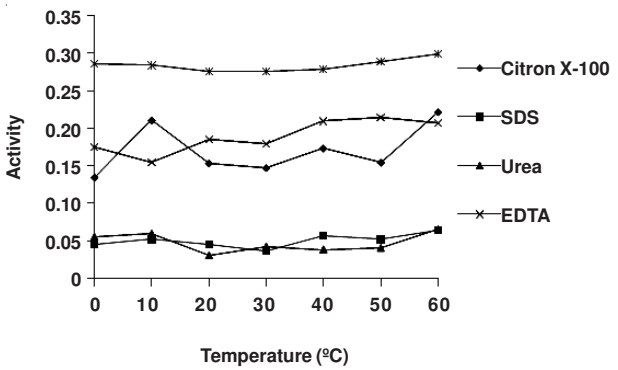

Fig. 1. Effect of temperature in presence of denaturating agents on the dissociation of rubisco: (◆) 1 mM Citron X-100; (■) 1 mM SDS; (▲) 1 mM urea; (×) 1 mM EDTA; (∗) control

Small organic molecules in aqueous solution can have profound effects on protein stability, structure and function. They may affect directly or indirectly, by binding to the protein or by altering the solvent environment. Citron X-100, SDS, urea and EDTA are the highly effective and widely used as protein denaturants (Figs.1 and 2). At every step of analysis control was maintained without using denaturing agents. Leaves protein concentration is high at 30-60 ºC in presence of citron X-100. Rapid increase in concentration of protein observed upto 10 ºC but stable from 20-50 ºC and inclined after 60 ºC. When the solution was incubated with 1 mM SDS and urea separately, there was no change in protein concentration when it treated with and without temperature it indicates

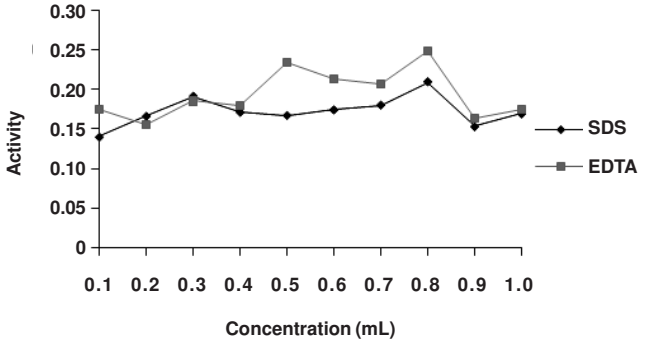

Fig. 2. Effect of SDS and EDTA concentration on the dissociation of protein: (◆) 1 mM SDS; (■) 1 mM EDTA

that citron X-100 is strong denaturating agents as compared to SDS and urea. By incresease in concentrations of EDTA and SDS was incubated separatly with *Solanum xanthocarpum* leaves protein, it gives better degradation of protein.

After 5 h of stirring of *Solanum xanthocarpum* leaves solution showed maximum protein. It probably suggests that stirring with magnet bars may cause mechanical disintegration of protein (Fig. 3). The protein degradation also considerd when 1 mM SDS and EDTA were used at 60 ºC with 1 h stirring. It showed varying R_f values as 0.19, 0.67, 0.73 and splitting pattern on electrogram. Composite of all denaturant with optimum temparature and pH with stirring gave excellent degradation of protein.

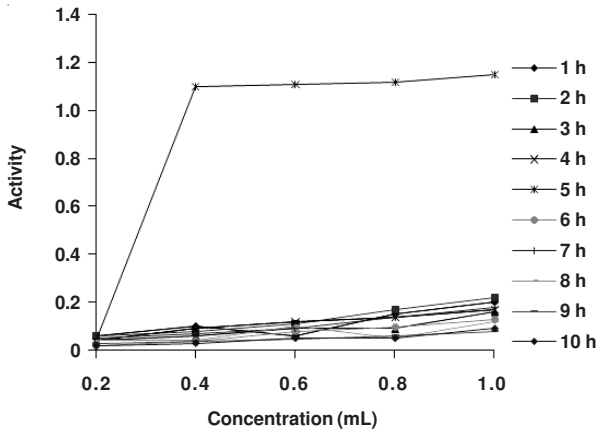

Fig. 3. Effect of stirring on the dissociation of rubisco: (◆) 1 h stirring; (■) 2 h stirring; (▲) 3 h stirring; (×) 4 h stirring; (∗) 5 h stirring; (o) 6 h stirring; (l) 7 h stirring; (–) 8 h stirring; (—) 9 h stirring; (◆) 10 h stirring

Conclusion

All denaturants shock *Solanum xanthocarpum* leaves protein and changes its milieu which might modulates the precise response of photosynthesis. This stresses play the role of dynamic phenomenon inducing the change in conformation of protein, powered by the energy of temparature/sunlight, plants perform this central task of carbon fixation.

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