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PORPHYRIN BIOSYNTHESIS IN THE SOYBEAN CALLUS TISSUE SYSTEM—XVIII. LEVELS OF SUCCINYL CoA SYNTHETASE, CYSTHATIONASE, RHODANESE, AMINOLEVULINATE SYNTHETASE AND AMINOLEVULINATE DEHYDRATASE IN CLONES OF DIFFERENT AGE

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Abstract—1. The activity of Succinyl CoA Synthetase (Suc CoA-S), Cysthationase, Rhodanese, Aminolevulinate Synthetase (ALA-S) and Aminolevulinate Dehydratase (ALA-D) was studied in old (405–407 subcultures) and young (34–36 subcultures) soybean callus clones as a function of the days of growing.

2. Suc CoA-S, ALA-S and ALA-D activities were much lower in old than in young callus, while the activity of Cysthationase and Rhodanese was higher in old callus.

3. ALA-S reached its maximum activity when Rhodanese and Cysthationase their minimum, on the 11th day of growth. It is suggested that the cellular content of a possible thio-compound which would regulate ALA-S activity, is controlled through its degradation by Rhodanese.

INTRODUCTION

For many years we have been interested in the study of tetrapyrrole biosynthesis in soybean callus tissue cultures, a highly dividing system which either dark or light-grown fails to synthesize chlorophyll in amount equivalent to that found in mature leaves (Batlle *et al.*, 1975 and references there in; Wider de Xifra & Batlle, 1976, 1978; Batlle *et al.*, 1976a,b; Wider de Xifra *et al.*, 1978).

One of the important findings that has emerged from these studies has been the direct measurement of Aminolevulinate Synthetase (EC 2.3.1.37) (ALA-S) in a vegetable like tissue. It was found that enzyme activity reached a sharp maximum on the 11th day of growth (Wider de Xifra *et al.*, 1971). Some properties of the soybean callus ALA-S resemble those of the *Rh. spheroides* enzyme (Marriot *et al.*, 1969; Tuboi *et al.*, 1969) and the presence of a compound which seems to control ALA-S activity was also detected in these cultured cells, which could account for the changes in activity during aging of crude extracts or supernatants.

Succinyl CoA Synthetase (Suc CoA-S) (EC 6.2.1.5) and Aminolevulinate Dehydratase (ALA-D) (EC 4.2.1.24) were also studied in soybean callus, but their activities were not significantly modified on aging (Wider de Xifra *et al.*, 1971).

On the other hand, it has been demonstrated that in *Rh. spheroides*, cystine trisulphide is an activator of ALA-S, that both Cysthationase (EC 4.2.1.15) and Rhodanese (EC 2.8.1.1) are involved in the formation and degradation respectively on this trisulphide and consequently on the control of ALA-S activity (Wider de Xifra *et al.*, 1976).

Furthermore, after studying these tissue cultures for nearly 15 hr, we have observed that some of their properties were modified as the clone of callus was physiologically older; it was found that the longer the time colourless callus was cultured in the dark, the less was the activity of the enzymes involved in porphyrin biosynthesis.

Therefore, it was considered appropiate to reinvestigate in older callus, some of the phenomena already studied and compare with data obtained from younger callus.

In this paper we will then report some results, studying the activity of Suc CoA-S, Cysthationase, Rhodanese, ALA-S and ALA-D in both old and young soybean callus clones, as a function of the days of growing, with the view of correlating the levels of ALA-S, Cysthationase and Rhodanese activities in clones of different age and their role, if any, in the regulation of soybean callus porphyrin biosynthesis.

MATERIALS AND METHODS

Unless otherwise indicated all chemicals were purchased from Sigma Chemical Co.

Source material of enzymes

Undifferentiated callus cultures from soybean seeds were obtained and grown according the procedure of Miller (1963). Two soybean callus clones were used in this study, old and young. Old callus refers to cultures that were grown in the dark for 15 yr with regular 14 days subculturing (405–407 subcultures), and young callus corresponds to cultures which have been subcultured 34–36 times (13 months old). The growth medium and cultured conditions were those reported by Tigier *et al.* (1970).

Protein concentrations were determined by the method of Lowry *et al.* (1951).

Assay of enzymic activities

Homogenate of crude extract (50% wet wt/vol) of wound callus was prepared in a Potter–Elvehjem type homogenizer with 0.1 M Tris–HCl buffer, pH 9.0.

Extracts were immediately centrifuged at 4° for 10 sec at 6000 *g* and different activities were determined in the supernatant.

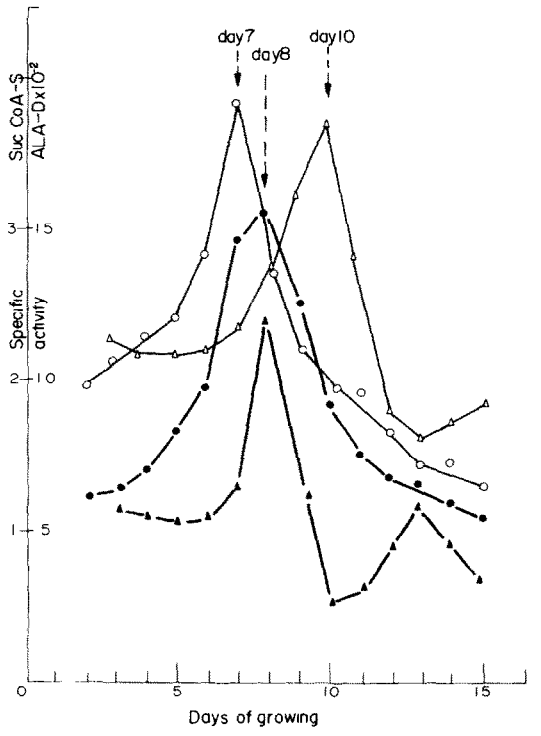

Fig. 1. Variation of Suc CoA-S (○, ●) and ALA-D (△, ▲) activities in old (●, ▲) and young (○, △) soybean callus at different days of growth. Each point represents the average value of three separate experiments with triplicate samples.

Suc CoA-S was assayed as described by Wider de Xifra & Tigier (1971).

ALA-S was assayed according to Wider de Xifra *et al.* (1971).

ALA-D was measured as described by Tigier *et al.* (1970).

Cysthationase and Rhodanese were assayed following the procedures described by Wider de Xifra *et al.* (1976) slightly modified.

Enzyme units

1 unit of enzyme activity is defined as the amount of enzyme which catalyses the formation of 1 nmol of product in 60 sec under the standard conditions. Enzymic activities are expressed as units per mg of protein.

RESULTS AND DISCUSSION

As can be seen in Fig. 1, Suc CoA-S and ALA-D activities were much lower in old than in young callus; they were also dependent on the days of growing; Suc CoA-S reached its maximum on the days 7th and 8th for young and old callus respectively, while ALA-D activitiy was highest on the 10th and 8th day of growth. Maxima for young callus do not apparently show any clear correlation; however those for old callus were coincident, which might suggest that they could be related to some extent with the content of ALA in the cell.

When we measured Cysthationase and Rhodanese at different days of growth, we observed (Fig. 2) that the activity of these enzymes was highest in old callus. One possible explanation for this phenomenon is that

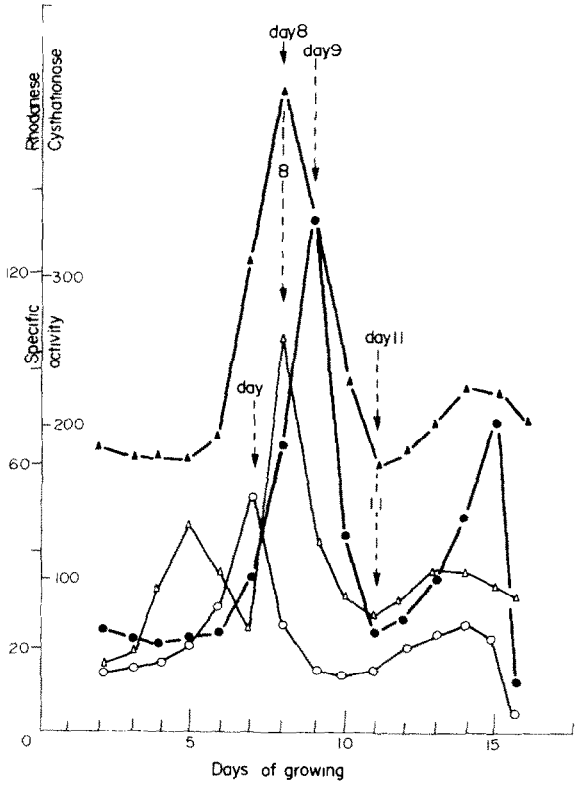

Fig. 2. Variation of Cysthationase (○, ●) and Rhodanese (△, ▲) activities in old (●, ▲) and young (○, △) soybean callus at different days of growth. Each point represents the average value of three separate experiments with triplicate samples.

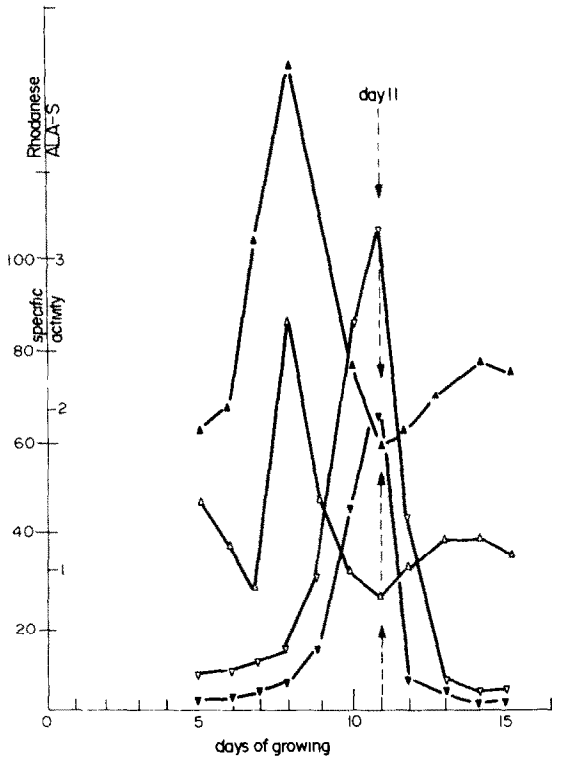

Fig. 3. Variation of Rhodanese (△, ▲) and ALA-S (▽, ▼) activities in old (▲, ▼) and young (△, ▽) soybean callus at different days of growth. Each point represents the average value of three separate experiments with triplicate samples.

the metabolism of sulphur compounds could be related with the oldness process of the tissue after prolonged culture and perhaps that might be the reason why the activity of Cysthationase and Rhodanese increased as the clone of callus gets physiologically older. A maximum on the 8th day of growth was also found for Rhodanese in both old and young cells, while Cysthationase reached its peak on the 9th and 7th days in old and young callus respectively. A very interesting finding was that in all tissues tested, the activities of these enzymes involved in sulphur metabolism showed minimum activity on the 11th day while, ALA-S reached its sharp maximum on the same day (Fig. 3) but as expected, ALA-S was more active in young callus.

These results might indicate that a similar scheme to that proposed by Wider de Xifra *et al.* (1976) for the control of ALA-S in *Rh. spheroides* is also operating in soybean callus. However, as the levels of Cysthationase activity were much higher than those of Rhodanese, we can further postulate that the cellular content of a thio-compound which would actually regulate ALA-S activity, is in fact controlled through its degradation by Rhodanese. If this assumption is valid, at the lowest activity of Rhodanese would correspond a maximum for ALA-S. In some preliminary experiments it has been found that ALA-S in old callus was activated by cystine trisulphide, supporting the hypothesis that poli-sulphides can also be involved in the regulation of this enzyme in soybean cultured cells.

Finally, these findings have also confirmed early work indicating that metabolic changes occur in these tissues cultures after prolonged culture; here, it was found that the length of time previously spent by the callus undergoing subculture diminishes its capacity to synthesize the enzymes involved in porphyrin biosynthesis but increases that related to the enzymes responsible for sulphur metabolism. Therefore when working with plant tissue cultures it is important to establish the age of the cultures and if possible to perform comparative studies in clones of different age.

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