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丝瓜果实发育过程中 4-香豆酰辅酶 A 连接酶在管状分子分化中的作用

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摘 要 对丝瓜果实发育过程中 4-香豆酰辅酶 A 连接酶作为初步纯化,并对它的一些理化性质作了研究.结果表明 4-CL 含有两个同工酶,酶 I 纯化 15.68 倍含量为 22.63%;酶 II 纯化 44.23 倍含量为 3.91%.酶 I 对香豆酸和咖啡酸的亲合性大于酶 II 的,酶 I 的最适温度为 40℃,最适 pH 为 8.0.研究表明 4-CL 活性与木质素的生物合成和管状分子分化密切相关,它们之间存在一种顺序关系,4-CL 是管状分子分化的标志酶.

关键词 丝瓜果实,4-香豆酰辅酶 A 连接酶,管状分子,细胞分化

分类号 Q 945

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The Role of 4-coumarate Coenzyme A Ligase in Tracheary Element Differentiation During Fruit Development of *Luffa cylindrica* Roam^{*}

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Abstract The 4-CL was purified roughly from the fruits of sponge gourd and its essential properties were characterized. The results showed that the 4-CL contained two isoenzymes. A 15.68-fold purification of enzyme I and 44.23-fold purification of enzyme II was observed. The purity of enzyme was 22.6% in the case of enzyme I and 3.91% in the case of enzyme II. The affinity of enzyme I to 4-coumarate and caffeic acid was higher than that of enzyme II. The optimal temperature was at 40°C and the pH-optimum was at 8.0 in enzyme I. The study revealed that the activity of 4-CL was related much with the lignin biosynthesis and the TE differentiation, and there was a sequential relation among them.

Keywords fruits of sponge gourd, 4-Coumarate coenzyme A ligase, tracheary element, cell differentiation

Abbreviations 4-CL= 4-coumarate coenzyme A ligase,
OMT= o-methyltransferase, PO= peroxidase,
PAL= phenylalanine ammonia-lyase, TE= tracheary element(s)

The cell differentiation was the base of structural change and formal construction in the plant. Therefore, the research was paid attention to the cell differentiation^[1]. TE can be clearly distinguished from parenchyma cell by the characteristic secondary wall thickening, which is accompanied by lignification. Lignification can be a marker of TE differentiation^[2]. Therefore studying mechanism of cytodifferentiation have been made by TE differentiation and lignin synthesis. The metabolism of phenylpropanoid is sources for biosynthesis of lignin, in which several enzymes, e. g. PAL, OMT, 4-CL and PO were involved^[3]. It has been much reported that PAL, and PO^[1]. 4-CL have been extracted from various plant^[4]. In the present study, we explored relationship each other among 4-CL activity and the biosynthesis of lignins, as is also TE-differentiation using experimental system of the fruits of sponge gourd.

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1 Material and methods

- (1) Plant material The fruits of *Luffa cylindrica* Roem were from the Biological Station of East China Normal University. It was monoecious species. The seeds were sown in batchers at interval ten days. The fruits from tenth node to twentieth node were used.
- (2) Lignin content determination According to the method previously described^[3].
- (3) Isolation and purification of 4-CL: The procedures were referred to method of BI and Ouyang^[5].
- (4) Assay for 4-CL activities: The enzyme activities were determined in a spectrophotometric assay. The optical density was determined with 245 nm and 362 nm respectively in case of ferulic acid and caffeic acid became substances.
- (5) Assay for the physical and chemical properties of 4-CL: The form I and II of enzyme were obtained through chromatograph over DEAE-cellulose 52 column. The following all assays were given as form I of enzyme.
- (6) The temperature-optimum reaction After the form I was incubated in various temperature for 10 minutes respectively. Then the enzyme activity was determined by a direct spectrophotometric method.
- (7) Assay for 4-CL thermostability: The method was as previously described. The form I was separated into three parts. The samples were incubated at thermostatic condition in various temperatures. The enzyme activity was determined once at intervals of 5 minutes.
- (8) Assay for pH-optimum reaction of 4-CL: The enzyme was added into various pH-buffer solutions. After reaction solution was kept for 10 minutes at 40°C, then pH-optimum was determined.
- (9) The counting of the number of tracheary elements: The percent of TE was counted by sectioning and staining method. The sections were stained with the phloroglucinol test. The number of TE were counted using statistics method, and showed with the percentage of TE area for total area of fruit section.
- (10) The protein determination Protein was determined by Bradford method^[6] using BSA as a standard.

Results

- (1) The lignin content changes in developing process of sponge gourd fruits: The content of lignin shown as Fig. 5. It increased slowly until at fifteenth day after flowering. It enhanced rapidly to maximum until at twentieth day after flowering.
- (2) The activity of 4-CL changes in developing process of the fruits of sponge gourd: The p-coumaric acid was used as substrate. The activity change of enzymes shown at Fig. 5. The results indicated that activity change increases with pollination and fruit developing. There is a peak from the fifteenth to twentieth day after flowering.
- (3) The physical and chemical properties of 4-CL: The material was used by fruits at fif-

teenth day after flowering. Two primarily purified isoenzymes of 4-CL were got as shown Fig. 1 and Tab. 1.

Tab. 1 Summary of purification and separation of two forms of 4-CL from sponge gourd fruit

Step	Total activity	Total protein	Specific activity	Purification fold	Yield
Crude extract	10584	8586	1.2	1.0	100
Precipitation in 45% ~ 75% of ammonia sulfate	8820	2916	2.98	2.5	83.3
Dextran G-25, DEAE-25, chromatography, Enzyme					
From I	2396	127.3	18.82	15.68	22.63
From II	414	7.8	63.07	44.23	3.91

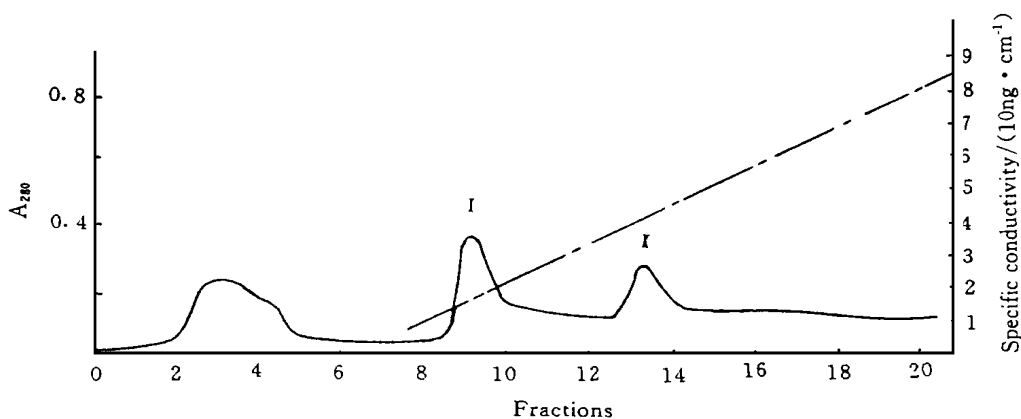


Fig. 1 Chromatography elution profile of 4-CL isoenzymes on DEAE-cellulose 52 Column

(4) The substrate specificity: All enzymes reacted with p-coumaric, ferulic and caffeic acids respectively for assay 4-CL activities. The enzyme II activity was lower than that of enzyme I (Tab. 2).

Tab. 2 Substrate specificity of isoenzymes of 4-CL from sponge gourd fruit

Enzyme	Coumaric acid	Caffeic acid	Ferulic acid
Form I	504	340	98
Form II	107	46.1	8

(5) The temperature optimum reaction: The enzyme I was incubated at various temperature respectively. Then activity was determined after 10 minutes. Optimum temperature was at 40°C or so (Fig. 2).

(6) The 4-CL thermostability: The results indicated that enzyme activity at 30~40°C was stabler than others (Fig. 3).

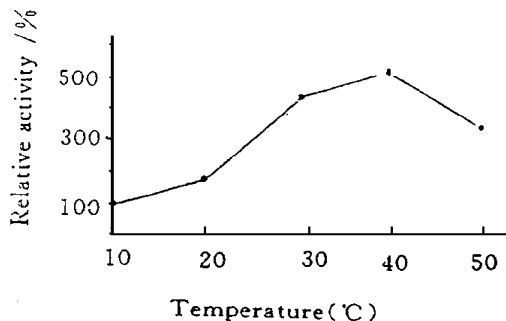


Fig. 2 The temperature optimum of 4-CL from sponge gourd fruit

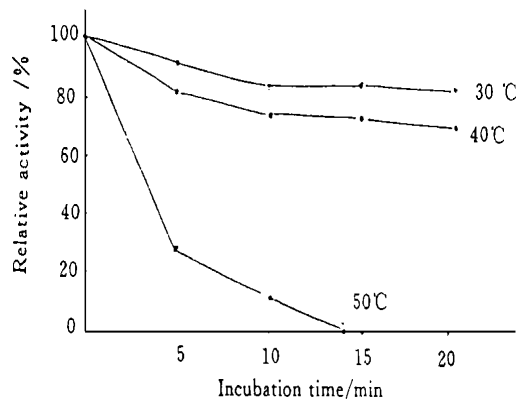


Fig. 3 The thermostability of 4-CL from sponge gourd fruit

(7) pH-optimum reaction The enzyme was incubated in various pH buffer, then its activity was determined, pH-optimum was found about at 8.0 (Fig. 4).

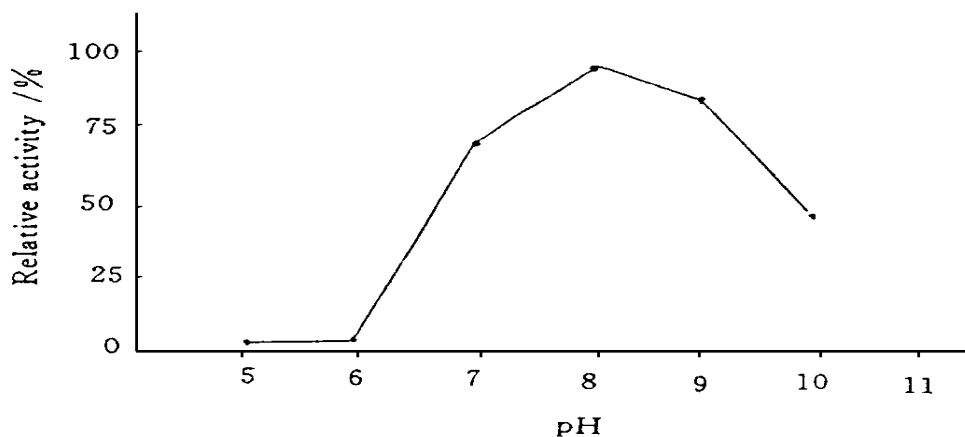


Fig. 4 The pH optimum of 4-CL from sponge gourd fruit

(8) TE number estimation Percentage of TE among total areas was counted by statistics method. The results was shown in Fig. 5. At the twenty-fifth day after flowering, TE percentage was the maximum. In developing process of fruits of sponge gourd the correlation among 4-CL activity, lignin content and TE numbers showed in Fig. 5.

3 Discussion

Only one form coumarate CoA ligase has been characterized in spruce^[7]. However,

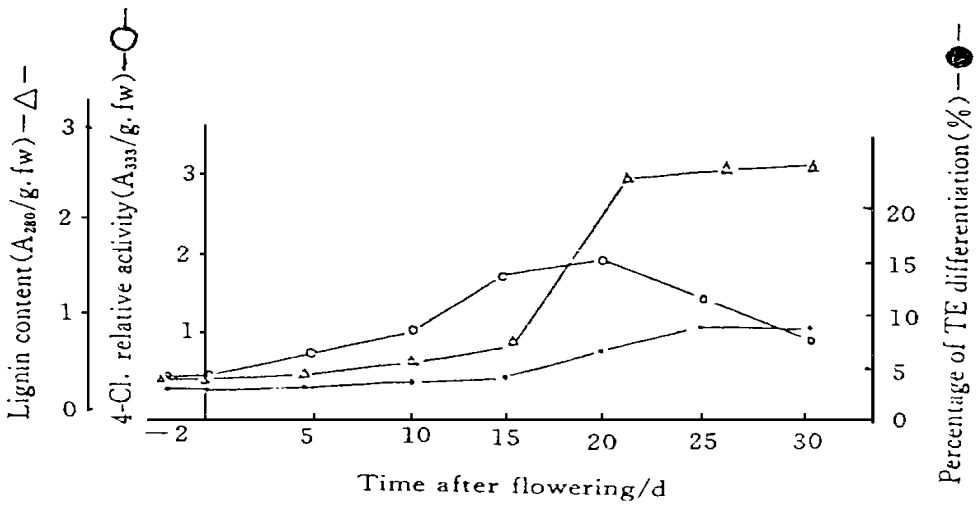


Fig. 5 The change of 4-CL activity, Lignin content and TE number during the fruit development of sponge gourd

the occurrence of multiple forms of this enzyme has been demonstrated in cell suspension of etiolated pea seedling and rice^[5]. In the present study two p-coumarate; CoA ligases were separated from sponge gourd fruits. The results indicated that optimum-temperature was at 40°C. The pH-optimum was 8.0.

In our study two 4-CL isoenzymes were separated from fruit of sponge gourd. The activity reacted of both form I and II enzymes with p-coumarate, caffeic and ferulic acid decreased one after another. The affinity of form I was bigger than that of form II as a whole. But affinity of form I in rice to ferulic acid was absent almost^[5]. Grand et al (1982) indicated once that particular substrate specificities of 4-CL correlated not only with the plant species, but also with the tissues character^[8]. Our study indicated that 4-CL was strongly correlated with both lignin synthesis and tracheary element differentiation. The peak of 4-CL activity appeared previously to the peak of lignin synthesis. Then ratio on TE differentiation raised obviously. There was time sequence relation among these changes. It is in accord with the fundamental rule that material metabolism is always established prior to the morphogenesis. Our study provided the evidence that 4-CL as marker of TE differentiation reported by Church and Gatston^[4]. At 30~40°C 4-CL activity exhibited thermostability and significantly high. The 4-CL thermostability can ensure that TE differentiated largely and lignified speedily, and fruits of sponge gourd ripened promptly in the July and August.