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REGULATION OF CELLULASE SYNTHESIS IN MYCELIAL FUNGI: PARTICIPATION OF ATP AND CYCLIC AMP

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SUMMARY

ATP and cAMP in 4 strains of mycelial fungi were determined by luciferin-luciferase system and HPLC respectively. Cellulase synthesis was subject to the dual control of ATP and cAMP. No matter what carbon sourse was used, cellulase synthesis was repressed if intracellular ATP concentration was over 10^{-7}mg/ml. Exogenous cAMP could increase cellulase synthesis under depression conditions.

INTRODUCTION

Cellulases in mycelial fungi are adaptive enzymes, whose biosynthesis is induced by the presence of inducers but becomes repressed once a low-molecular-weight, easy metabolizable carbon source becomes available[1]. In Trichoderma [2,3], the phenomenon of carbon catabolite repression of cellulase synthesis is well documented[4,5]. However, the mechanism of carbon catabolite control of cellulase formation is unknown. Although the molecular mechanism of carbon catabolite control of enzyme synthesis in eukaryotes differs from that of prokaryotes[6], several common phenomena may provide the clues to know the essence of catabolite control in eukaryotes, such as ATP is the end product of saccharides metabolite, and cAMP may be involved in the depression of enzyme synthesis[7]. Since the change of ATP and the level of cAMP in the metabolic process of fungi are too small to be determined accurately, it has become necessary to develop more sensitive techniques for their measurement.

In our present paper,we determined the intracellular ATP amount precisely using the luciferin-luciferase system, which was believed to be the most specific, sensitive determination method, and cAMP by HPLC. This makes it possible to study the effect of ATP and cAMP levels on the regulation of cellulase synthesis.

MATERIALS AND METHODS

Strains:

Trichoderma reesei Rut C30, obtained from American Army Natick lab.

Trichoderma pseudokoningii S38, wild type obtained from soil in our lab[8].

Penicillium decumbens JU-A10, selected in our lab [9].

Aspergillus niger L22, selected in our lab.

Culture conditions:

Strains were cultured in the batches of the medium of Mandels salt solution[10], adding 0.5% (w/v) various carbon sources at 30℃ on a gyratory shaker at 160rpm.

Determination of ATP:

Using the luciferin-luciferase system[11], ATP was determined by a Liquid Scintillation Counter(Backman Co.), with the commodity ATP (Sigma Co.) as the standard.

Determination of cAMP:

Using HPLC with a column of Spheriosorb S5 SAX(Waters Co.)at the elution rate of 1ml/min[12], cAMP amount was determined with the commodity cAMP (Sigma Co.) as the standard.

Filter paper activity (FPA) assay:

According to the references[13].

RESULTS AND DISCUSSION

Change of intracellular ATP with various concentration of glucose.

Batches of the medium with various concentration of glucose as the carbon source were inoculated with the four strains seed for about 14 days respectively. The relationships between the intracellular ATP amount and FPA synthesis were observed (Fig.1. A and B).

With 0.5 % (w/v) glucose as the carbon source, the intracellular ATP of the four strains were below 10^{-8} mg/ml from beginning to end. At the same period, the FPA synthesis was over the amount of 1.5IU/ml except for strain L22.

With 2 % (w/v) glucose as the carbon source, the intracellular ATP was higher. Within 2 days of inoculation, the ATP of the four strains was over 10^{-7}mg/ml. At the same period, the FPA synthesis was below 0.05IU/ml except for the strain C30.

The FPA synthesis started to increase gradually after 6 days; at that time, the intracellular ATP decreased below the concentration of 5×10^{-8}mg/ml.

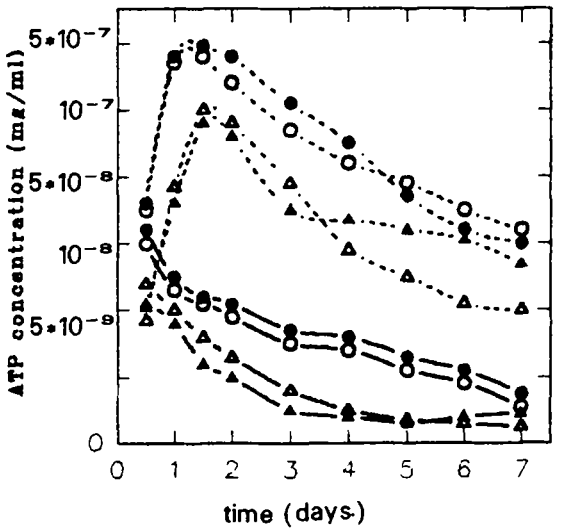

Fig.1A Intracellular ATP level under various concentration of glucose
Symbols: ●S38, ○C30, ▲A10, △L22
—— 0.5% Glu,- - - 2% Glu

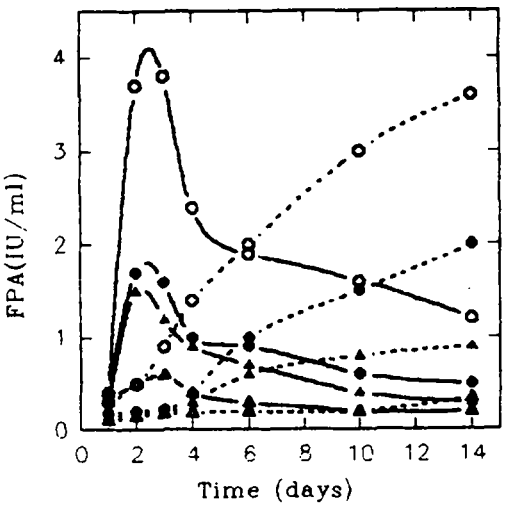

Fig.1B Effect of various concentration of glucose on FPA synthesis. (the symbols are the same as that of Fig.1A.)

Change of intracellular ATP amount incubated with cellulose powder.

Batches of the medium with 2% (w/v) microcrystalline cellulose powder (70μ m particle size) were inoculated with the seed strains. The intracellular ATP of the strains were relatively stable and below the concentration of 5×10^{-8}mg/ml from beginning to end(Fig.2), and the FPA synthesis could carry on normally.

Above results indicate that cellulase synthesis is interrelated with the intracellular ATP though various strains have different sensitivity. No matter what carbon source is used, cellulase is repressed if the intracellular ATP exceeds 10^{-7} mg/ml. The excessive accumulation of intra-cellular ATP is the essential reason for enzyme repression.

Effect of exogenous ATP on enzyme repression

With various carbon sources (0.5%) adding exogenous ATP to a final concentration 0.5 mg/ml, the intracellular ATP increased exceeding the concentration of 10^{-7}mg/ml which caused the enzyme repression happened (Table.1). This indicates that exogenous ATP can affect the amount of intracellular ATP. But exogenous ATP can only increase the amount of intracellular ATP limitedly, the higher intracellular ATP does not exceed 10^{-6} mg/ml even if the concentration of exogenous ATP reaches 5 mg/ml. In addition, exogenous ATP alone can not be used as carbon source and affect the amount of intracellular ATP. This may show that it needs a specific system for ATP transfering from extracellular to intracellular.

Table 1 Effect of various carbon sources or/and extracellular ATP on intracellular ATP amount on day 4 of inoculated

carbon sources	C30		S38		A10		L22	
	ATP	FPA	ATP	FPA	ATP	FPA	ATP	FPA
Glucose	0.5	2.4	1.7	1.1	0.2	1.0	0.2	0.3
Glycerol	0.7	2.0	0.8	0.8	0.3	0.4	0.1	0.2
Cellobiose	0.2	2.9	0.2	1.8	0.2	1.5	0.5	1.2
Cellulose powder	1.1	1.8	1.5	2.1	1.0	1.1	8.0	1.6
Glucose+ATP	55	0.3	45	0.1	30	0.3	50	0.02
Glycerol+ATP	65	0.1	48	0.1	40	0.05	46	0
Cellobiose+ATP	45	0.4	48	0.2	25	0.4	44	0.05
Cellulose powder+ATP	48	0.3	60	0.1	35	0.2	62	0.3
ATP	-	-	-	-	-	-	-	-

Value: ATP, mg/ml($\times 10^{8}$); FPA, IU/ml

Effect of exogenous cAMP on enzyme synthesis under nonrepression condition.

Exogenous cAMP (final concentration was 10^{-3}mol/L) could increase FPA synthesis obviously with 0.5% glucose as carbon source (Fig.3). However, with sophorose and cellulose powder as the carbon source respectively, the increase of FPA synthesis was higher than that of incubating with glucose as carbon source(Fig.4). Since sophorose and

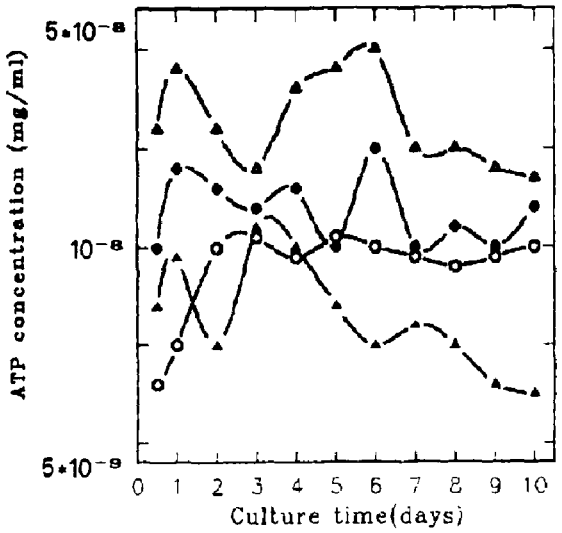

Fig.2 Change of intracellular ATP during the strains cultivated by cellulose powder.

Symbols: ●S38, ○C30, ▲A10, △L22

cellulose were believed to be the potential inducers of cellulase synthesis[1,5], this results may indicate that although the regulation of enzyme synthesis in eukaryotes is very complicated, the mechanism of cAMP on regulating the enzyme gene expression in eukaryotes is similar to a certain degree to that of prokaryotes[6,14].

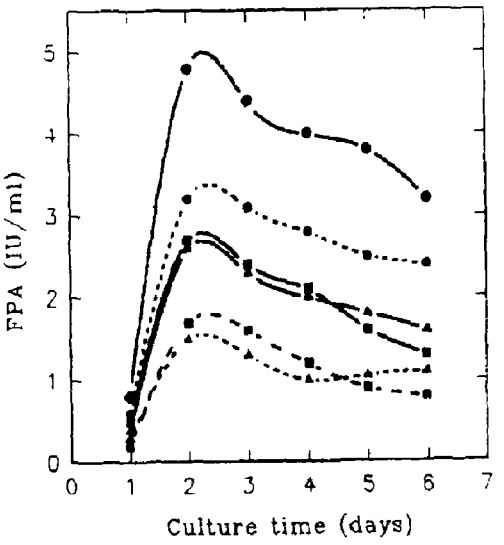

Fig.3 Effect of exogenous cAMP on FPA synthesis
Symbols: ■ S38, ● C30, ▲ A10
- - - 0.5% Glu,—— adding cAMP.

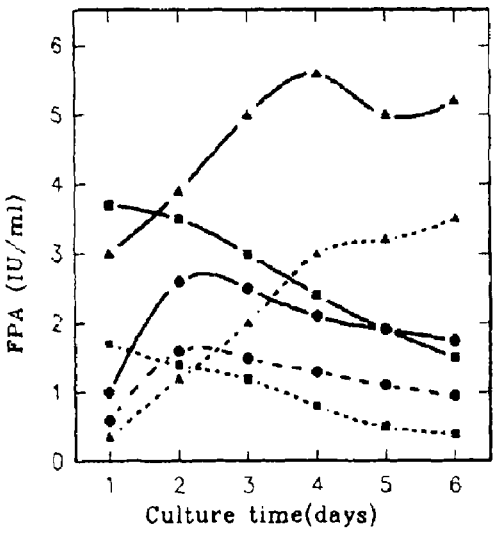

Fig.4 Effect of cAMP on A10 FPA synthesis on sophorose or cellulose powder.
Symbols: ●Glu, ■Sop, ▲Cellulose powder
- - - 0.5% Glu,—— adding cAMP

Cellulase synthesis is subject to the dual control of induction and repression. Repression play a decisive function in regulation of cellulase synthesis. The intracellular ATP level may be the essential reason resulting in the repression of enzyme synthesis. If repression of enzyme is removed, the overproduction of cellulase can be obtained. According to our results, cellulase can be synthesized normally by controlling the intracellular ATP below the concentration of 5×10^{-8}mg/ml. On the other hand, the addition of exogenous cAMP (or similar substances) can increase cellulase synthesis.

Acknowledgments

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