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# Cell Biological Studies on the Mechanism of Development and Differentiation VIII

## 2. Effects of Peptide on cAMP Level in Corn Endosperm

**Young Dong Cho*, Young Hee Kang, Sun Hi Lee, and Seong Ki Kim**

(Department of Biochemistry* and Department of Biology
College of Science, Yonsei University, Seoul)

## 생체발생 및 분화기구의 세포생물학적 연구 VIII

## 2. 옥수수 배젖에서 Peptide가 cAMP Level에 미치는 영향

趙暎東*·康榮熹·李舜熙·金成淇

(연세대학교 이과대학 생화학과*, 생물학과)

## ABSTRACT

Activities of corn endosperm adenylate cyclase and phosphodiesterase were found right after germination, and phosphodiesterase activity was shown to increase steadily. Protease activity was also found. Corn peptide fraction purified by using Sephadex G-25 column was shown to enhance corn phosphodiesterase activity but inhibit bovine phosphodiesterase activity. And the fraction inhibits corn adenylate cyclase activity. Trypsin-treated peptide fraction was shown to enhance phosphodiesterase activity 80% compared to that of native peptide fraction. However, in case of DNase phosphodiesterase was shown to be innocuous. According to cumulative results, it is more likely that peptide fraction produced by protease inhibits adenylate cyclase activity and enhance phosphodiesterase, decreasing cAMP level.

## INTRODUCTION

C-AMP has major amplifying regulatory functions in prokaryotes (Pastan and Adyhya, 1976) and eukaryotes (Cohen, 1978) but, dispite considerable investigation (Amrheim, 1977; Ashton and Polya, 1978; Cho *et al.*, 1982; Lin, 1974; Polya and Bowman, 1981; Sachar *et al.*, 1975), there is still no clear evidence for an explicit regulatory function for cAMP in higher plant. However, the ubiquitous occurrence and diverse physiological

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role of cAMP in animal tissues have promoted considerable interest over possible analogous functions of this nucleotide in higher plant tissues. Apparently cAMP can elicit physiological responses in plants similar to those evoked by certain plant hormones (Hall and Galsky, 1973). However, there is evidence for the prescence of cAMP in higher plants (Amrheim, 1974; Amrheim, 1977; Ashton and Polya, 1978; Lin, 1974). Adenylate cyclase (EC 4.6.1.1) has yet to be convincingly demonstrated in cell free extract from higher plants (Hintermann and Parish, 1979), but evidence for the presence of cAMP protein kinase (EC 2.7.1.37) from wheat germ with endogenous substrate, T-substrate (Yan and Mao, 1982a and 1982b). There are several reports on the presence of phosphodiesterases (EC 3.1.4.1) that catalyze the hydrolysis of cAMP (Ashton and Polya, 1975; Brewin *et al.*, 1973; Brown *et al.*, 1980; Lin and Varner, 1972). Cyclic AMP level is surely dependent on both activities of adenylate cyclase and phosphodiesterase. We have recently reported that cAMP level decreased drastically in corn after germination (Cho *et al.*, 1982) and the activity of phosphodiesterase in corn increased constantly after germination (Cho *et al.*, 1983). However, phosphodiesterase activity seemed to be not enough for such drastic decrease in cAMP level considering adenylate cyclase, and led us to find if there might be a sort of endogenous regulator for both enzyme as shown in inhibition of phosphodiesterase by peptides (Collier *et al.*, 1982), which could be formed by action of proteases on protein as suggested (Naito *et al.*, 1979) and the amount of protein was also observed to decrease after germination in corn (Cho *et al.*, 1982).

In work reported in this communication activity of adenylate cyclase was observed on time course. The dependence of both adenylate cyclase and phosphodiesterase activities was measured in the presence of peptide fraction obtained from corn. Protease activity possibly responsible for producing peptide fraction from protein was also observed. The actions of trypsin and DNase on corn peptide fraction were checked if there might be any change undertaken in the fraction.

## MATERIALS AND METHODS

**Plant materials.** Seed of corn (*Zea mays* L) were germinated in moist vermiculite at 25°C for days in the dark as described elsewhere (Cho *et al.*, 1982). Endosperm was obtained by using same method (Cho *et al.*, 1982).

**Chemicals.** Most of chemicals, trypsin inhibitor, and bovine phosphodiesterase were purchased from SIGMA Chemical Co., U.S.A.. Chemicals were purified if necessary. [2, 8-$^3$H]-cAMP was obtained from New England Nuclear.

**Determination of cyclic AMP.** 1 g of endosperm tissue was used in each sample if not stated by using the methods (Cho *et al.*, 1982).

**Enzyme preparation.** Method (Rutheford *et al.*, 1976) was slightly modified. The tissue was ground by using a mortar and pestle in $10^{-2}$M Tris buffer (pH 7.0) which contained

0.01 mg/ml $MgCl_2$. The homogenate was then centrifuged for 30 min. at 12,000 *g* and the supernatant was decanted and used as the source of phosphodiesterase. The remaining pellet was resuspended in Tris buffer and passed through on Aminco French Pressure Cell (twice). The resulting slurry was then centrifuge at 12,000 *g* for 30 min. and the supernatant used as the source of adenylate cyclase.

**Assay of enzyme activity.** Phosphodiesterase activity was determined as previous paper (Cho *et al.*, 1980). Adenylate cyclase activity was done as followings. 0.5ml of crude enzyme was added to 0.5ml of a $10^{-2}$M Tris buffer pH 7.4 containing 1.0 $\mu$Ci of[$^3$H] adenosine triphosphate. The reaction mixture was then placed in a water bath at 37°C and allowed to react for 15 min. At the end of this time period a 60 $\mu$l aliquot was spotted on Whatman 3 MM chromatography paper using a solvent system which contained 1M ammonium acetate and 95% ethanol (3:7). Standard of cyclic AMP and ATP were also spotted. The chromatogram was allowed to develop for 7hrs after which time the standard spots were identified using a UV hand lamp. The corresponding spots from the reaction mixture clute were then cut out and placed in scintillation vials containing toluene and 2,5-diphenyloxazole. The vials were counted in Packard Tricard-300 scintillation counter.

**Corn peptide fraction and effect on enzymes.** Corn peptide fraction was obtained as described elsewhere (Cho *et al.*, 1983). It was purified by using Sephadex G-25 column, and checked by UV absorbance. Each fraction was 5ml. The purified peptide fraction was added to reaction mixtures of adenylate cyclase and phosphodiesterase, respectively as described elsewhere (Cho *et al.*, 1983; Collier *et al.*, 1982). Before the assay of both enzyme activities, peptide fraction was preincubated for 24 hrs at 30°C with gentle shaking with trypsin(EC 3.4.21.4) from bovine pancrease, and DNase II (EC 3.1.21.1) from bovine pancrease. After incubation the action of trypsin was stopped by adding trypsin inhibitor from bovine pancrease. The reaction of DNase II was terminated by boiling for 10 min. Both enzyme activities from corn and bovine phosphodiesterase activity were measured in the presence of various amount of trypsin and DNase treated peptide fraction (Collier *et al.*, 1982).

**Protease activity.** Protease activity of corn was checked on time course. The preparation and measurement were done by using the method described elsewhere (Naito *et al.*, 1979).

## RESULTS AND DISCUSSION

Peptide fraction from 7 days old corn was purified using sephadex G-25 column and each fraction were checked if there was any effect on corn phosphodiesterase activity (Fig. 1). Fraction number 10 was shown to have maximum effect on the enzyme. This result confirmed a previous result regarding enhancement of the enzyme activity by crude peptide fraction (Cho *et al.*, 1983). However, the same fraction was also shown to inhibit the activity of bovine phosphodiesterase (Fig. 2). This result is in contrast to enhancement

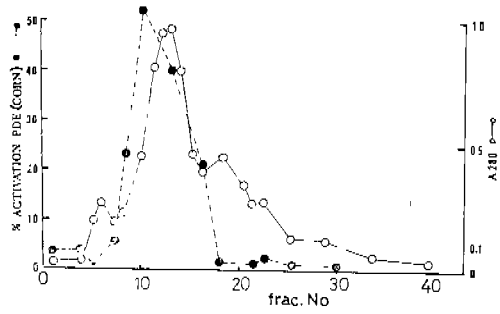


Fig. 1. Activation of corn phosphodiesterase by corn peptide fractions obtained from Sephadex G-25 column.

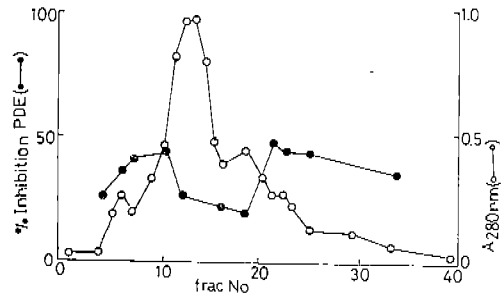


Fig. 2. Inhibition of bovine phosphodiesterase by corn peptide fraction obtained from Sephadex G-25 column.

Table 1. Enhancement of corn phosphodiesterase activity by native peptide and hydrolyzed peptide by trypsin, chymotrypsin, and DNase II

| Treatment | PDE Activity(DPM) |
|---|---|
| None | 58800(100%) |
| Trypsin | 50100( 80%) |
| Chymotrypsin | 50200( 82%) |
| DNase | 55500( 94%) |

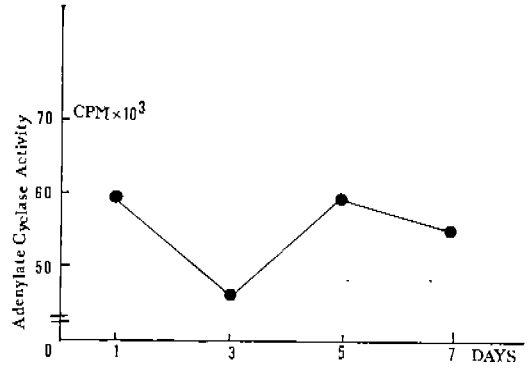


Fig. 3. Activity of adenylate cyclase on the time course.

of bovine phosphodietserase activity by endogenous peptide fraction from rat brain (Collier *et al.*, 1982). Trypsin-treated peptide fraction was shown to enhance phosphodiesterase activity 80% compared to that by native peptide fraction. However, DNase seemed to be innocuous for the fraction (Table 1), possibly excluding other compound responsible for enhancing phosphodiesterase activity. Adenylate cyclase activity was checked on the time course (Fig. 3). Although there is a fluctuation in the enzme activity, clearly it is hard to explain decrease in cAMP level after germination even if there are the precedence of phosphodiesterase activity over adenylate cyclase activity and enhancement of phosphodiesterase activity by peptide fraction. Inhibition of adenylate cyclase at various days by corn peptide fraction was shown to decrease steadily (Fig. 4). Accordingly peptide amount produced by hydrolysis seems to be so critical for adenylate cyclase activity and phosphodiesterase activity that protease activity was checked on time course (Fig. 5). After germination, protease activity was steadily decreased. In case of germinating barley grain, large pool of small peptides and amino acids have been identified in both endosperm and embryo (Higgins and Payne, 1981). The peptide amount was also shown to increase

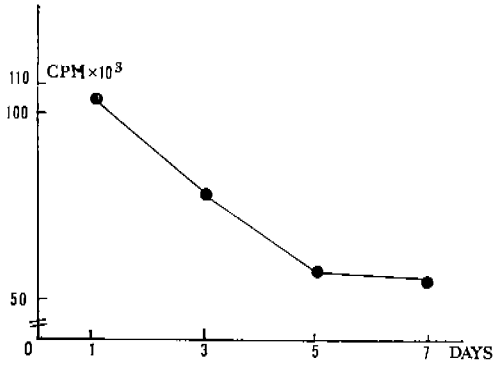


Fig. 4. Inhibition of adenylate cyclase by corn peptide fraction obtained from Sephadex G-25 column.

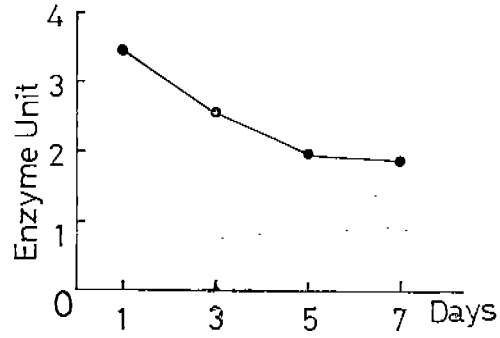


Fig. 5. Corn protease activity on time course.

during the first 3 days of germination and subsequently decrease. Protease possibly with other protease seems to involve in hydrolyzing proteins and peptides in turn are transfer from the endosperm to the embryo as claimed (Higgins and Payne, 1981). Although protease activity seems to be relatively low (Naito *et al.*, 1979), the enzyme activity is possibly enough considering the fluctuation of peptide amount if same case with barley. However, protease activity observed is to be studied more in detail if it might play critical role, as far as both enzymes are concerned.

Cumulative results suggest that protease in corn produces peptides from proteins or storage proteins and peptides in turn enhance phosphodiesterase activity and inhibit adenylate cyclase activity, decreasing cAMP level. Such suggestion is supported by our unpublished data that peptide and amino acid levels increase right after germination. Increase in peptide amount is clearly important because it can have an effect on adenylate cyclase and phosphodiesterase activities, which regulate cAMP level in corn and cAMP in turn activates protein kinase as shown in wheat germ (Yan and Mao, 1982a) and possibly lipase. Although a potent endogenous substrate for protein kinase was found in wheat germ (Yan and Mao, 1982b), the role of protein kinase in plant is very ambiguous. Therefore, cyclic AMP dependent protein kinase promotes considerable interest over possible analogous function of this enzyme in higher plants.

## 摘 要

옥수수 배젖에서 adenylate cyclase 및 phosphodiesterase activitiy는 발아후 곧 나타났고 phosphodiesterase activity는 점점 증가하였다. Protease activity도 발아후 곧 있었다. Sephadex G-25 column으로 정제한 corn peptide fraction은 corn phosphodiesterase activity를 높혔으나 bovine phosphodiesterase activity는 저하시켰다. 또한 adenylate cyclase activity도 저하시켰다. Trypsin으로 처리한 peptide fraction은 trypsin으로 처리하지 않은 peptide fraction에 비하여 20% 적게 phosphodiesterase activity

를 상승시켰다. DNase II로 처리한 경우는 phosphodiesterase activity에는 영향이 없었다. 이상의 결과로 corn peptide는 protease에 의하여 생성되고, 생성된 peptide는 adenlylate cyclase activity를 저해하고 phosphodiesterase activity를 상승시켜서 옥수수 배젖 cAMP level을 저하시키는 것으로 사료된다.

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