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EFFECTS OF LIGHT AND KINETIN ON AMARANTHIN SYNTHESIS INDUCED BY *c*AMP*

MARINA GIUDICI DE NICOLA, VINCENZO AMICO and MARIO PIATTELLI

Istituto di Chimica Organica dell'Università di Catania, Catania, Italia

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Key Word Index—*Amaranthus tricolor*; *A. caudatus*; Amarantaceae; biosynthesis; light; phytochrome; *c*AMP; kinetin; betalains; amaranthin.

Abstract—The effects of *cyclic* 3′,5′-adenosine monophosphate (*c*AMP) on amaranthin synthesis in the dark, or in the presence of kinetin or light were investigated in isolated cotyledons of *Amaranthus tricolor* and *A. caudatus*. The results suggest that sites or modes of action of *c*AMP and kinetin are not separated and differ from those of light and that the nucleotide cannot be considered a messenger involved in amaranthin formation stimulated by kinetin or by light.

INTRODUCTION

In higher plants *cyclic* 3′,5′-adenosine monophosphate (*c*AMP) mimics the action of some phytohormones, such as gibberellins [1–3] and auxins [4, 5]. Recently, it has been reported that *c*AMP induces synthesis of amaranthin in *A. paniculatus* in the dark [6].

The present study was designed to determine the effects of the administration of *c*AMP in combination with other inducers which are known to promote the synthesis of the pigment, that is light and kinetin.

RESULTS AND DISCUSSION

Experiments were carried out using two *Amaranthus* species which respond differently to kinetin and light, *A. tricolor* cv. Illumination and *A. caudatus*. Etiolated seedlings of the former species lack amaranthin, whose synthesis can be induced by kinetin [7, 8], or white (W) light [9] but not by far-red (FR) irradiation [10, 11], while seedlings of *A. caudatus* produce in darkness a substantial amount of pigment, which is increased by W, FR [6] and kinetin [10, 11]. In all experiments excised cotyledons were used and *c*AMP was administered as the N^6, $O^{2'}$-dibutyryl derivative, in the presence of theophylline (1 mg/ml) which reduces the rate of its destruction [12].

The responsiveness of *A. tricolor* and *A. caudatus* cotyledons to the application in the dark of *c*AMP at various concentrations, as assessed by amaranthin production, is given in Table 1. The sensitivity to *c*AMP may be specific for this nucleotide, since adenosine or ATP administration was ineffective. From a comparison of the effects of *c*AMP and kinetin in *A. tricolor* cotyledons with those in *A. caudatus*, it is apparent that the extent to which these species are affected by both compounds differs widely, and this can be ascribed to the different status of activation of the genes controlling amaranthin synthesis.

The induction of amaranthin synthesis by *c*AMP or kinetin, which requires a lag-phase of nearly 8 hr (Fig. 1), is inhibited by actinomycin D or puromycin (Table 1). This is evidence that both *c*AMP and kinetin act at the level of the genes coding for the enzymes involved in pigment synthesis.

A combination of *c*AMP and kinetin at optimum concentration promotes amaranthin accumulation of the same magnitude of that pro-

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Table 1. Amaranthin synthesis induced by kinetin (10 μg/ml) and cAMP in isolated cotyledons of *A. tricolor* and *A. caudatus*

Treatment	Absorbance at 537 nm* *A. tricolor*	*A. caudatus*
cAMP (5×10^{-3} M)	0·185	0·049
cAMP (10^{-3} M)	0·295	0·085
cAMP (5×10^{-4} M)	0·274	0·079
cAMP (10^{-4} M)	0·165	0·061
cAMP (5×10^{-5} M)	0·108	0·055
cAMP (10^{-5} M)	0·070	
Adenosine (5×10^{-4} M)	0·00	0·00
ATP (5×10^{-4} M)	0·00	0·00
Kinetin	0·287	0·095
cAMP (5×10^{-4} M) + actinomycin D (10 μg/ml)	0·115	
cAMP (5×10^{-4} M) + puromycin (100 μg/ml)	0·055	
Kinetin + actinomycin D (10 μg/ml)	0·115	
Kinetin + puromycin (100 μg/ml)	0·053	
cAMP (10^{-3} M) + kinetin	0·292	0·084
24 hr FR	0·00	0·098
24 hr FR + kinetin	0·185	0·195
24 hr FR + cAMP (5×10^{-4} M)	0·068	0·188

* Increase over dark control.

Table 2. Effects of white light on amaranthin synthesis induced by kinetin (10 μg/ml) and cAMP on isolated cotyledons of *A. tricolor* and *A. caudatus*

Treatment	Absorbance at 537 nm* *A. tricolor*	*A. caudatus*
15 min W light	0·052	
6 hr W light	0·190	0·108
Kinetin	0·287	0·095
Kinetin + 15 min W light	0·537	
Kinetin + 6 hr W light	0·778	0·270
cAMP (5×10^{-3} M)	0·185	0·049
cAMP (5×10^{-3} M) + 15 min W light	0·277	
cAMP (5×10^{-3} M) + 6 hr W light	0·441	0·174
cAMP (10^{-3} M)	0·295	0·085
cAMP (10^{-3} M) + 15 min W light	0·455	
cAMP (10^{-3} M) + 6 hr W light	0·552	0·303
cAMP (5×10^{-4} M)	0·274	0·079
cAMP (5×10^{-4} M) + 15 min W light	0·423	
cAMP (5×10^{-4} M) + 6 hr W light	0·540	0·282
cAMP (10^{-4} M)	0·165	0·061
cAMP (10^{-4} M) + 15 min W light	0·258	
cAMP (10^{-4} M) + 6 hr W light	0·430	0·217

* Increase over dark control.

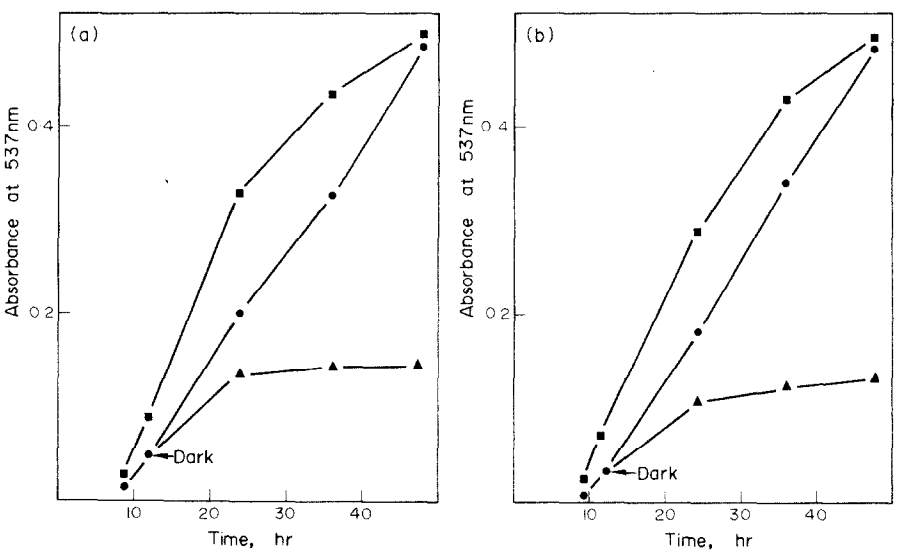

Fig. 1. Time course of amaranthin synthesis in *Amaranthus tricolor* induced by (a) cAMP or (b) kinetin. ■ In the presence of (a) cAMP or (b) kinetin; ▲ in the presence of (a) cAMP or (b) kinetin and under continuous FR; ● in the presence of (a) cAMP or (b) kinetin and under FR until 12 hr.

duced individually by the two inducers. This and the essential identity of the kinetics of amaranthin formation induced by *c*AMP and by kinetin exclude the possibility that one of these factors could act as inducer of the other and suggest that their site(s) or modes of action are not separated.

Amaranthin formation induced by *c*AMP or by kinetin is consistently enhanced by 6-hr W irradiation (Table 2). Since this increase is also observed when *c*AMP is administered at optimum (5×10^{-3} M) concentration, there is evidence that the nucleotide cannot be considered a "second messenger" in the light-induced amaranthin synthesis, as claimed by Rast *et al.* [6].

Photoactivation of phytochrome by 15 min W (Table 2) produces an increase of the amaranthin synthesis induced by *c*AMP or kinetin but a much smaller one than that produced by a longer irradiation period (6 hr). Furthermore, amaranthin formation promoted in cotyledons from dark-grown seedlings of *A. caudatus* by continuous FR, which maintains a low but constant level of P_{730}, is increased by the simultaneous application of *c*AMP or kinetin (Table 1). On the contrary, in *A. tricolor* under the same conditions of irradiation no pigment is formed and the synthesis induced by *c*AMP or kinetin is strongly inhibited; in a subsequent period of darkness amaranthin synthesis resumes at the normal rate (Fig. 1). These observations show that in amaranthin synthesis *c*AMP cannot replace phytochrome, as suggested by Rast *et al.* [6].

In conclusion, the results above provide evidence that *c*AMP and kinetin induce amaranthin synthesis by way of gene activation, probably through the same mechanism, and contradict the assumption that the nucleotide may act as a "second messenger" of light action.

EXPERIMENTAL

Cotyledons from 2-day germinated seeds of *Amaranthus tricolor* cv. Illumination and *A. caudatus* were used. They were incubated in 5-cm Petri dishes with the appropriate solutions until the end of the experiments (24 hr). When required, cotyledons were illuminated with fluorescent white (W) light 5000 lx at cotyledons level) or with FR of 1·9 mW/cm irradiation intensity [10]. At the end of the light treatment, cotyledons were returned to darkness and pigment extraction was carried out 24 hr after the onset of the experiment. Amaranthin was determined as reported previously [13]. Six replicates were used in all experiments and each experiment was repeated at least 6 times. The experimental error was normally 4% or less in any given experiment.

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