ENHANCEMENT OF POLYRIBOSOME FORMATION BY GIBBERELLIC ACID AND 3',5'-ADENOSINE MONOPHOSPHATE IN BARLEY EMBRYOS

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Summary

The formation of polyribosomes in barley embryos was enhanced by both 3',5'-adenosine monophosphate and gibberellic acid. Cyclic nucleotide was more rapid in its effect. No clear-cut relationship between the two compounds was observed at the level of rapidly labeled polyribosome-associated RNAs. Cyclic AMP- and gibberellic acid-induced polyribosome formation was inhibited by abscisic acid and this inhibition was largely reversed by the cytokinin, kinetin. A combination of cyclic nucleotide and gibberellic acid did not result in a synergistic or additive effect on polyribosome formation. Evidence presented supports the conclusion that cyclic AMP mediates the gibberellic acid enhancement of polyribosome formation.

Cyclic AMP* is known to be involved in the modulation of hormonal effects. According to the current concept hormones catalyze the conversion of ATP to cyclic AMP which then induces the target tissues to elicit a response (1,2). In plants cyclic AMP has been shown to mimic the actions of GA_3 (3,4,5,6), auxin (7,8) and cytokinin-like hormones (9). Plant hormones affect processes which regulate the level of cyclic AMP within the cell (5,7,9).

In the data presented here both cyclic AMP and GA_3 promoted the formation of polyribosomes in barley embryo and that this action of cyclic AMP was more rapid than GA_3.

MATERIAL AND METHODS

Polyribosome isolation. Twenty grains of barley (Hordeum vulgare L. cv. Himalaya) were incubated in 3 ml of hormone solution at 25°C for periods indicated. The embryos were isolated and incubated with shaking for 90 min in a medium (3 ml) containing the hormone(s) and 20 µg/ml chloramphenicol and 20 µCi ^3H uridine (1 mCi/m mole). Embryos were frozen in dry ice, powdered and homogenized in 8 ml of 0.25 M sucrose, 0.05 M Tris-HCl, pH 8.0, 0.45 M KCl, 0.02 M magnesium acetate and 0.005 M mercaptoethanol plus 0.5 ml of Triton X-100. The homogenate was centrifuged at 20,000g for 15 min. The supernatant was layered

*Abbreviations: Cyclic AMP, 3',5'-adenosine monophosphate; GA_3, Gibberellic acid; ABA, Abscisic acid.

over 1.8 M (1 ml) and 0.5 M (1.5 ml) sucrose pad containing 0.05 M Tris-HCl, pH 8.0, 0.2 M KCl, 0.01 M magnesium acetate and 0.005 M mercaptoethanol and centrifuged at 129,000g for 2 hr in a Spinco 65 Fixed angle rotor. The ribosomal pellet was resuspended in a 1 ml resuspension solution of 0.05 M Tris-HCl, pH 8.0, 0.2 M KCl, 0.01 M magnesium acetate and 0.005 M mercaptoethanol. The ribosomal resuspension was layered over a 10-34% sucrose density gradient (made in resuspension solution) and centrifuged at 148,000g for 2 hr in SW 41 Ti rotor in a Spinco model L2 65B ultracentrifuge. The distribution of polyribosomes was determined by recording the absorbance at 254 nm in ISCO Model 640 density gradient fractionator.

Polyribosome-associated RNAs. To the 24 drop fractions, collected from the gradients, were added 0.1 ml of yeast RNA (1 mg/ml) and 0.5 ml of 20% TCA. After overnight storage in the cold, RNA was collected on glass fiber filters, dried and radioactivity counted in a Nuclear Chicago Unilux II system. The composition of RNAs associated with the polyribosomes was determined by pooling the gradient fractions of polyribosome regions, sedimenting and isolating the RNA by the phenol method as described previously (10).

RESULTS

Cyclic AMP consistently stimulated polyribosome formation in barley embryos from grains soaked for 18 hr (Fig. 1B). GA_3 at this stage of imbibition had little or no effect on polyribosome formation (Fig. 1C). The polyribosome-associated RNA synthesis at this time, however, was inhibited by cyclic AMP and slightly promoted by GA_3 as determined by ^{3}H-uridine incorporation (Fig. 1B,C). At 30 hr incubation, cyclic AMP caused only a slight promotion in polyribosome formation while GA_3 enhanced it to a greater extent (Fig. 1F,G). The level of uridine incorporation into RNA at this time was much greater in cyclic AMP-treated embryos than those treated with GA_3. Cyclic AMP appeared to promote uridine labeling only in the monomer and dimer region (Fig. 1E,F). When cyclic AMP and GA_3 were used together, the enhancement of polyribosome formation was about the same as in the presence of cyclic nucleotide alone (Fig. 1B,D,F,H). Furthermore, the inhibitory effects of cyclic AMP and GA_3 on polyribosome-associated RNAs at 18 hr and 30 hr respectively were reversed by a combination of the two (Fig. 1B,D,G,H).

The effect of ABA alone and in combination with kinetin on cyclic-AMP-induced polyribosome formation (30 hr) is shown in Fig. 2. ABA inhibited polyribosome formation by itself (Fig. 1E,2A) as well as that induced by cyclic AMP Fig. 1F,2C) and this inhibition was reversed to a marked extent by kinetin

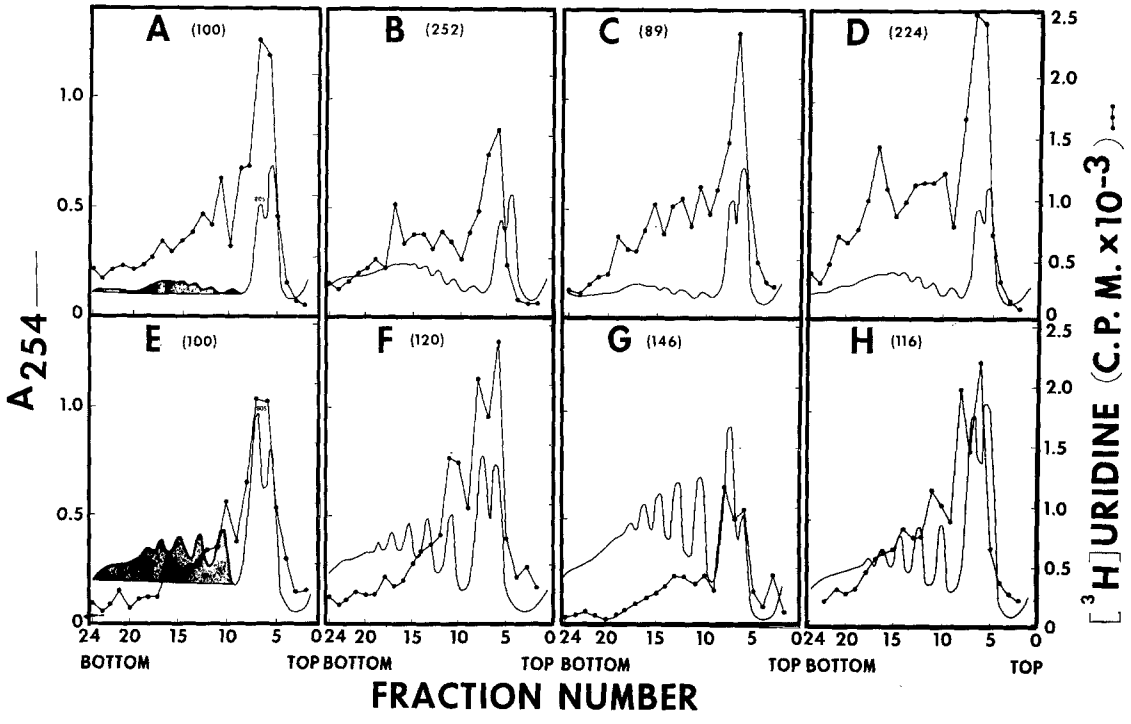
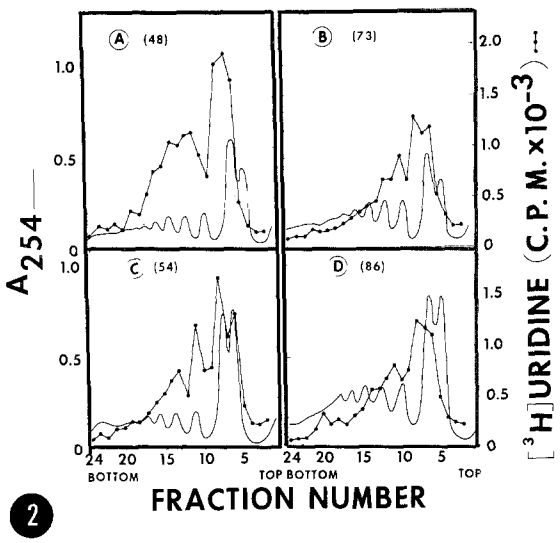


Fig. 1. Effect of cyclic AMP (1 mM) and GA_3 (20 µM) on polyribosome formation and polyribosome-associated RNA synthesis. Embryos were isolated after 18 and 30 hr of incubation of barley grains, labeled for 90 min with ^{3}H-uridine and polyribosomes isolated as described in "Materials and Methods." Polyribosome profiles were quantified by drawing a line from the base of the monosome to the end of the profile and cutting and weighing the area (P) as shown in treatments A and E. Numbers in parentheses are polyribosome contents, expressed as percent of control. Treatments are: top row A to D,18 hr; bottom row E to H,30 hr; A & E, control; B & F, cyclic AMP; C & G, GA_3; D & H, cyclic AMP + GA_3.

(Fig. 2B,D). Kinetin alone had no effect on polyribosome formation and kinetin in combination with cyclic AMP had the same effect as that by cyclic nucleotide alone (data not shown). ABA inhibition of cyclic AMP-induced polyribosome formation was accompanied by an increase in the specific radioactivities of polyribosome-associated RNAs (Fig. 2A,C). On the other hand, a kinetin reversal of ABA inhibition of polyribosome formation was associated with a slight decrease in the specific radioactivities of polyribosome-associated RNAs (Fig. 2A,B,C,D). Similar results were obtained when GA_3 was substituted for cyclic AMP (data not shown).

The composition of rapidly labeled RNAs associated with polyribosomes did



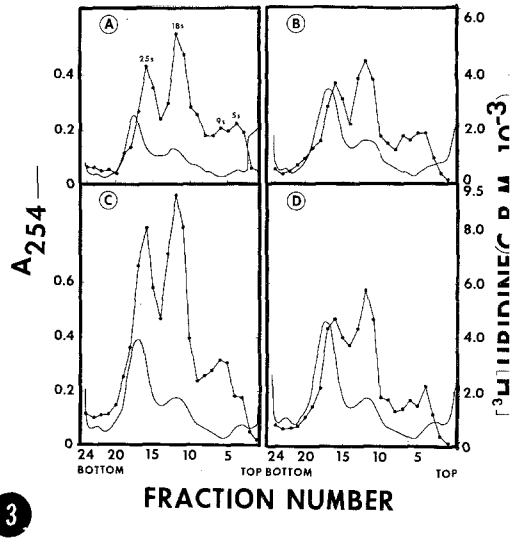


Fig. 2. Effect of ABA (20 μM) alone and in combination with cyclic AMP (1 mM) and kinetin (20 μM) on polyribosome formation and polyribosome-associated RNA synthesis. Embryos were isolated after 30 hr incubation and labeled for 90 min with ^{3}H-uridine as described in "Materials and Methods." Quantification of polyribosome profiles was done as indicated in Fig. 1. Numbers in parentheses are polyribosome contents expressed as percent of control, Fig. 1E. Treatments: A, ABA; B, kinetin + ABA; C, cyclic AMP + ABA; D, cyclic AMP + kinetin + ABA.

Fig. 3. Sucrose density gradient sedimentation of RNAs (phenol-extracted) associated with the polyribosomes of embryos (30 hr grains). Fractions corresponding to polyribosomal region were pooled and ^{3}H-uridine-labeled (90 min) RNAs extracted as described in "Materials and Methods." Treatments: A, control; B, GA_3 (20 μM); C, cyclic AMP (1 mM); D, GA_3 + cyclic AMP.

not show any qualitative differences in RNAs synthesized in the presence of cyclic AMP and GA_3 (Fig. 3). It was of interest to note however, the presence of a non-ribosomal peak (9S) in all treatments. This peak was most prominent in cyclic AMP-treated embryos. The significance of this RNA is being explored.

DISCUSSION

The data presented here show that both cyclic AMP and GA_3 enhance polyribosome formation in barley embryo, but the effect of cyclic AMP is more rapid. The rapidity of the nucleotide effect, compared to hormone, is consistent with the concept that cyclic AMP mediates the action of hormone and that it is closer to the target tissue than the hormone itself (1,2). However, the response of

cyclic AMP at a later period (30 hr) is of lower magnitude compared to GA_3. The inability of cyclic AMP to elicit a response comparable to GA_3 even at the high concentration used here has been reported in other systems (3,6) and can be attributed to the small amounts of nucleotide reaching the target.

There was some stimulation of polyribosome-associated RNA synthesis by GA_3 at 18 hr indicating that its influence on RNA synthesis is different from that on polyribosome formation. At 30 hr, a decreased level of RNA synthesis in response to GA_3 is indicative of large pools of nucleosides and nucleotides which would decrease the specific radioactivity of RNA precursors available for incorporation into RNA. An increase in RNase and nucleotidase levels have been shown in embryo and aleurone of barley by GA_3 (11,12). Although the effects of cyclic AMP in polyribosome-associated RNAs, both at 18 and 30 hr, were different from that of GA_3, some interaction between these two compounds is indicated. Cyclic AMP counteracted the effect of GA_3 on polyribosome associated RNAs at 30 hr. The polyribosome-associated rapidly labeled RNAs showed no qualitative differences in GA_3 and cyclic AMP treatments. A higher specific radioactivity in 18S rRNA in all treatments is perhaps indicative of the presence of non-ribosomal RNAs other than 9S RNA co-sedimenting with 18S rRNA. Presence of non-ribosomal rapidly labeled RNAs associated with polyribosomes have been reported earlier (13).

Hormonal interaction studies further indicated a similarity in the actions of cyclic AMP and GA_3. The cyclic AMP-induced polyribosome fomation was inhibited by ABA and this inhibition was largely reversed by kinetin. Similar results were obtained when GA_3 was used (data not shown). Cytokinin-ABA antagonism in GA_3-induced processes has been shown by Khan (14).

The level of cyclic AMP and its function may be unrelated to the type of tissue being examined. In a highly differentiated and non-growing tissues, such as cereal aleurones, cyclic AMP may have a different role, even when present or supplied in large quantities, than in a rapidly growing tissue, such as cereal embryo. Both adenyl cyclase and phosphodiesterase, which regulate the level of cyclic AMP, are present in plant tissues (9,15,16). There is growing evidence

that increased adenyl cyclase activity in response to certain hormones is associated with inhibition of cellular proliferation and cells showing high mitotic activity possess low level of cyclic AMP (17,18). More work will be needed to elucidate the varied effects of GA_3, cyclic AMP and other plant hormones in various plant tissues.

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