GROWTH PROMOTERS AND THE SYNTHESIS OF PROTEIN IN PLANT MITOCHONDRIA : Part I - Effect of kinetin on the incorporation of amino acids into mitochondrial protein.

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Kinetin stimulates protein synthesis in mitochondria isolated from 48-hr etiolated seedlings of Vigna sinensis (Linn.) Savi. The effect appears at the early stage of incubation and attains the optimum at 10^{-6}M concentration. Kinetin shows a synergistic effect with indole-3-acetic acid in regard to amino acid incorporation.

Kinetin is now known to cause a great number of growth responses and physiological functions of plants[1]. Kinetin promotes growth, so it is quite likely that it should influence protein synthesis, since protein is one major component of growth substances. In the present paper the effect of kinetin on the synthesis of protein by mitochondria isolated from 48-hr etiolated seedlings of Vigna sinensis (Linn.) Savi has been investigated.

Materials and Methods

Germination of seeds and isolation of mitochondria - Germination of seeds and isolation of mitochondria were carried out as reported earlier[2].

Determination of radioactivity in protein - For the measurement of radioactivity, protein was processed as reported earlier[2]. The radioactivity was determined in a gas flow counter, Model D 47 (Nuclear Chicago) and corrections were made for self-absorption and background.

Bacterial counts - Viable bacteria were counted by the plate and dilution method with Eugonagar medium. Addition of 5% defibrinated blood was without any effect.

RESULTS

Effect and optimum concentration of kinetin on the incorporation of amino acids into protein by mitochondria - In the present plant system kinetin stimulated protein synthesis in isolated mitochondria with an optimal concentration of 10^{-6}M (Table 1).

TABLE 1

EFFECT AND OPTIMUM CONCENTRATION OF KINETIN ON THE INCORPORATION OF AMINO ACIDS INTO PROTEINS BY MITOCHONDRIA

Mitochondria were incubated aerobically at 37°C for 2 hr in sucrose (0.4 M)-potassium phosphate (0.06 M) buffer (pH 7.0) containing ^{14}C-labelled algal protein hydrolysate (264858 counts/min initially). A control was also simultaneously run with boiled mitochondria and the data corrected accordingly. The incubation mixture contained 7-10 mg of protein per ml.

Addition	Sp. radioactivity Counts/min./mg. protein
None	7188
Kinetin (10^{-3}M)	9011
Kinetin (10^{-4}M)	9203
Kinetin (10^{-5}M)	9454
Kinetin (10^{-6}M)	10531
Kinetin (10^{-7}M)	9828
Kinetin (10^{-8}M)	8614

Time course study with kinetin - Figure 1 shows the time course study with kinetin. It appears from the figure that the

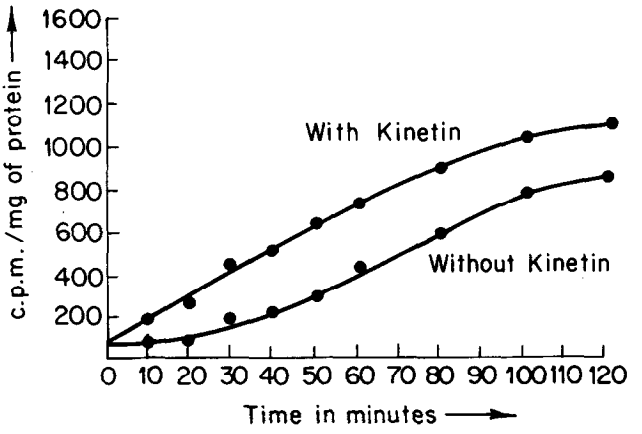

Figure 1. Kinetic study with kinetin.

stimulatory effect of kinetin is exhibited at the early period of incubation.

Bacterial contamination - It is possible that the stimulatory effect of kinetin may be due to the bacterial contamination since it is now known that kinetin influence growth of certain microorganisms. Bacterial counts were determined as described, which allow to conclude that the stimulation of protein synthesis was not due to stimulation of bacterial growth.

Synergism with indole-3-acetic acid - Kinetin stimulates protein synthesis by mitochondria of this particular plant. Protein synthesis is greatly stimulated by the joint action of kinetin and indole-3-acetic acid indicating synergistic effect of hormone pair as shown in Table 2.

DISCUSSION

Little definite is known about the site and mechanism of action of kinins in plant tissues. The ability of kinin to stimulate RNA and protein synthesis in free nuclei[3,4] strongly suggests

TABLE 2

SYNERGISM WITH INDOLE-3-ACETIC ACID

Incubation was carried out as in Table 1.

Addition	Sp. radioactivity Counts/min./mg. protein
None	7203
Kinetin (10^{-6}M)	9817
Indole-3-acetic acid (10^{-4}M)	8754
Kinetin (10^{-6}M) + Indole-3-acetic acid (10^{-4}M)	11426

that one site of kinin action is within the nucleus. Growth studies with enucleated algae cells indicate that an additional site is in the cytoplasm[5]. The ability of kinins to delay senescence in chlorophyll-containing barley leaves, but not in albino barley leaves[6] implicates the chloroplast as a possible site of kinin action. The stimulatory effect of kinetin on protein synthesis in mitochondria isolated from 48-hr etiolated seedlings of Vigna sinensis (Linn.) Savi (Table 1) lends support to the contention that mitochondrion is also a possible site of kinin action. The stimulatory effect of kinetin is maximum at an optimum concentration of 10^{-6}M. Lower incorporation at hypo-optimal concentrations may be due to the lack in saturation of the active centres by the hormone. The hyperoptimal concentrations are found to be self-inhibitory. The effect of kinetin is manifested at the early stage of incubation (Fig. 1).

Kinins modify the action of other hormones. This is illustrated by the work of Skoog et al.[7] Their experiment pertains to the growth in vitro of tobacco callus tissue on a suitable medium containing kinetin alone and in combination with indole-3-acetic

acid. Kinetin alone had little effect but when kinetin and indole-3-acetic acid were applied simultaneously the growth was greatly effected than by a single hormone. Mitochondrial protein synthesis in this particular plant system is greatly effected by the simultaneous application of the hormone pair than by a single hormone (Table 2). The optimum concentration of IAA in this plant system on amino acid incorporation is 10^{-4}M (Unpublished data). This indicates that kinetin has a synergistic effect with indole-3-acetic acid in the present mitochondrial system in regard to protein synthesis.

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