Plant Physiol. (1977) 59, 45-47

Two Effects of Cytokinin on the Auxin Requirement of Tobacco Callus Cultures[1]

Received for publication May 18, 1976 and in revised form September 8, 1976

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ABSTRACT

Cytokinin affects the requirement for auxin of a strain of tobacco callus (*Nicotiana tabacum*) which is cytokinin-autotrophic when grown on Murashige and Skoog medium with 11.4 μM of indole-3-acetic acid but requires cytokinin 6-(3-methyl-2-butenylamino)purine (i⁶Ade) when grown on the same medium with <3 μM indole-3-acetic acid. As the exogenous concentration of cytokinin (i⁶Ade) is increased, the concentration of indole-3-acetic acid required for growth is decreased. A second effect of cytokinin, observed sporadically in cultures with 2.5 μM or 5 μM i⁶Ade, is the transformation of some of the callus pieces to auxin-autotrophic growth. Strains, both callus-forming and bud-forming tissues, that arise in this manner are not permanently altered in their auxin requirement because subcultures on medium without cytokinin still require exogenous auxin.

In vitro reponses of tobacco callus tissue (*Nicotiana tabacum* cv. Wisconsin No. 38) to cytokinin can be divided into those caused by low concentrations, *i.e.* the minimum requirement for growth (10), and those dependent on much higher cytokinin levels, *i.e.* induction of organ formation (11) and the requirement for growth on medium lacking thiamine (1, 2, 7). This paper describes two distinct effects of cytokinin (6-[3-methyl-2-butenylamino]purine, i⁶Ade)[2] on the auxin requirement of a cytokinin-autotrophic tobacco callus strain. One of these effects is a decreased requirement for exogenous auxin by callus grown on medium with ≤ 1 μM i⁶Ade. The other effect is a transformation of callus pieces to auxin-autotrophic tissue when grown on medium with > 1 μM i⁶Ade. This transformation occurs sporadically among replicates and results in morphologically different strains.

MATERIALS AND METHODS

The origin and maintenance of stock cultures of cytokinin-autotrophic tobacco callus on RM 1965 medium containing 11.4 μM IAA have been described (3, 6). The IAA, obtained from Sigma Chemical Company, and the i⁶Ade, synthesized by N. J. Leonard and S. M. Hecht, were added prior to autoclaving. Fresh weight yields were determined after specified growth periods.

RESULTS

Requirement for Exogenous IAA. On medium devoid of cytokinin, the tissue required 3 μM IAA to grow, and the optimum yield was reached with about 12 μM IAA (Fig. 1). The auxin requirement was markedly influenced by exogenous cytokinin (i⁶Ade). As shown in Figure 2, i⁶Ade concentrations ≤2.5 μM enabled cytokinin-autotrophic tissue to grow on medium supplemented with <3 μM IAA. With 0.5 μM i⁶Ade, growth occurred in the presence of 0.2 μM IAA; with 1 μM i⁶Ade, growth occurred in the presence of 0.03 μM IAA; and with 2.5 μM i⁶Ade, growth was obtained on medium containing as little as 0.005 μM IAA. Tissue pieces planted on media devoid of auxin generally did not grow. Therefore, the effect of the applied cytokinin concentrations, apparently, was to enable more efficient utilization of the exogenous auxin.

Transformation to Auxin-autotrophic Growth. In response to 2.5 μM and 5 μM i⁶Ade, a small percentage of the tissue pieces grew in the absence of added IAA (Table I). Two strains of tissue that arose in this manner (designated I and II in Table I) were subcultured for 15 bimonthly passages on medium without IAA but containing 5 μM i⁶Ade. Table II shows that auxin-autotrophic growth was not due to an irreversible transformation because even after extended subculturing on high cytokinin medium, tissue pieces transferred to a medium lacking both auxin and cytokinin failed to grow. On high i⁶Ade medium, strain I cultures routinely formed compact callus which gave rise to slow growing shoot axes, but cultures of strain II grew as a loose, glistening white callus. Growth in response to high i⁶Ade concentrations on medium lacking auxin, therefore, can occur in undifferentiated callus and need not be a consequence of organogenesis.

DISCUSSION

The strain of tobacco callus, designated as cytokinin-autotrophic, synthesizes sufficient cytokinin for its growth provided that the IAA concentration is ≥3 μM. The experiments demonstrate that in the presence of <3 μM IAA concentrations,

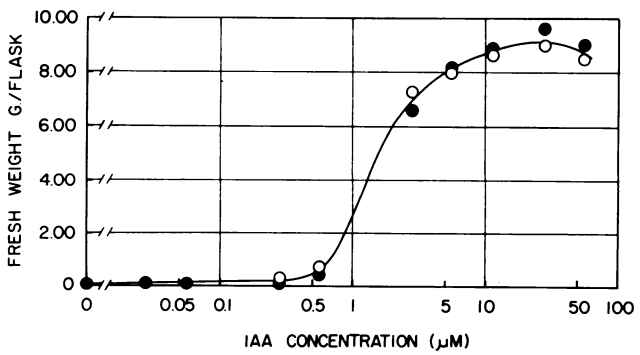

FIG. 1. Auxin requirement of cytokinin-autotrophic callus in the absence of exogenous i⁶Ade. Results of two independent experiments. Each point represents average fresh weight in five flasks; 20 ml of medium and three tissue pieces/flask. Growth period: 40 days.

[1] This work was supported by National Science Foundation Research Grant BMS72-02226-A03 to F. Skoog, University of Wisconsin.

[2] Abbreviation: i⁶Ade: 6-(3-methyl-2-butenylamino)purine.

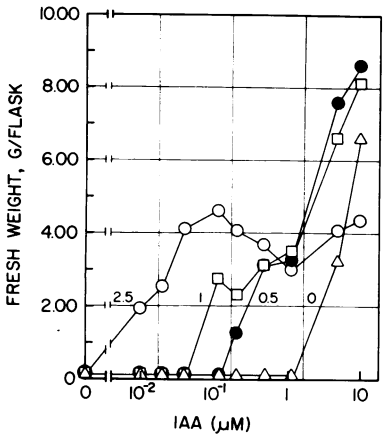

FIG. 2. Effect of i^6Ade concentration on the auxin requirement of cytokinin-autotrophic callus. △: no cytokinin; ●: 0.5 μM i^6Ade; □: 1 μM i^6Ade; ○: 2.5 μM i^6Ade. Growth period: 40 days.

Table I. Transformation of Tobacco Callus Tissue to Auxin-Autotrophy by i^6Ade

Each flask contained one tissue piece, 10 mg per piece. Growth period was 40 days.

i^6Ade (μM)	Fresh weight (g/flask)	No. of flasks
0	less than 0.05	49
	0.05 - 0.15	1
0.1	less than 0.05	49
	0.05 - 0.15	1
0.5	less than 0.05	48
	0.05 - 0.15	2
2.5	less than 0.05	43
	0.05 - 0.15	5
	0.27	1
	1.27	1
5.0	less than 0.05	31
	0.05 - 0.15	12
	0.16	1 (I)
	0.54	1 (II)
	0.91	1
	1.40	1
	1.51	1
	1.93	1
	2.00	1

Table II. Auxin and Cytokinin Requirements of Transformed Auxin-Autotrophic Tobacco Callus Strains

Growth period was 40 days.

	Added Growth Factors (μM)							
	IAA	i^6Ade	IAA	i^6Ade	IAA	i^6Ade	IAA	i^6Ade
	0	0	11.4	0	11.4	5	0	5
Strain	(g per flask)							
I	0.04 ± 0.01		1.20 ± 0.13		6.77 ± 0.50		6.35 ± 0.35	
II	0.02 ± 0.02		9.08 ± 0.08		7.23 ± 0.54		6.65 ± 0.23	

growth is cytokinin-dependent and, in fact, increased concentrations of exogenous i^6Ade are needed for growth when the IAA concentrations are decreased. This mutual interdependence between cytokinin and auxin concentrations and requirements also has been observed with the so-called "normal" tobacco callus strain (see especially Fig. 3 of ref. 9).

Witham (15) has shown that high concentrations of the auxin 2,4-D permit growth of soybean callus on medium lacking cytokinin, suggesting that 2,4-D acts as a cytokinin, as well, at these levels. In view of the present results and assuming that soybean callus normally produces cytokinin at rates less than adequate for growth on the specified medium with 28 μM IAA (8), it is possible that high auxin concentrations, particularly of metabolically more stable auxins such as 2,4-D, might permit growth to occur in the absence of exogenous cytokinin.

Transformation to auxin-autotrophic growth occurs sporadically among tissue pieces cultured on 2.5 μM and 5 μM i^6Ade and therefore must depend on some property not shared by all cells of the cytokinin-autotrophic stock cultures. Jordan (4, 5) and Syono and Furuya (13) have described strains of tobacco tissue that also can be subcultured through repeated passages on media with high cytokinin concentrations and no auxin. In all of these cases and in this report, the presence of a growing shoot axis was not essential nor did the effect involve a "mutational," *i.e.* a permanently acquired, change in the tissue's ability to synthesize auxin. Jordan (5), on the basis of bioassays estimated an IAA concentration of approximately 2×10^{-3} μmol/kg of tissue in his cultures. Syono and Furuya (13) have provided rigorous chemical proof that IAA was one of the auxins produced in tobacco callus cultured on high cytokinin medium.

Auxin-autotrophic growth of tobacco callus can be induced by short term, 2-week, treatment with auxin (14). This transformation is also shown to be reversible in that a single, 6-week passage on medium with auxin restores the auxin requirement.

The clear distinction between two effects of cytokinin on the auxin requirement of tobacco callus tissue suggests that cytokinins are acting at more than one cellular site. In studies utilizing cytokinin-dependent tobacco callus, Linsmaier-Bednar and Skoog (7) found that much higher concentrations of cytokinin were required for growth in the absence of thiamine than in its presence. The finding that certain substituted pyrrolo(2,3-*d*) pyrimidines inhibit i^6Ade-induced growth of tobacco callus but enhance the effect of high i^6Ade concentrations in promoting bud formation similarly has been considered in terms of cytokinin action at more than one site (12).

Acknowledgments—I thank F. Skoog for advice and assistance in the preparation of this manuscript. Synthetic i^6Ade, prepared by S. M. Hecht, was kindly furnished by N. J. Leonard, School of Chemical Sciences, University of Illinois, Urbana, Illinois.

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