INHIBITION OF ORGANOGENESIS BY STARCH IN TISSUE CULTURES OF GENETIC TUMOROUS CONDITION

by

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Introduction

The mono-, di-, and polysaccharides as carbon sources can be utilized by plant tumor tissue cultures. Utilization of polysaccharides is less effective than that of their mono- or disaccharid components (Gautheret 1959). It has been established that the growth of tumor tissue cultures (of *Vinca* crown-gall, virus tumor of *Rumex*, genetic tumor of F_1 hybrid of *Nicotiana glauca*×*N. langsdorffii*) was moderate on culture media which contained starch (Hildebrandt et Riker 1949, 1953, Nickell et Burkholder 1950).

According to earlier results buds and shoots are spontaneously produced by tissue cultures of genetic tumorous condition but root formation never occurs (Kovács 1967). Failure of root formation can not be explained by a lack or disturbance of growth hormon production. It has genetic reasons (Kovács 1969).

I have observed that starch can suppress organogenesis of tumorous tissue cultures (Kovács 1968). In the present work results on the inhibitory effect of starch on organ formation, nucleic acid and protein content are presented.

Materials and methods

An twenty-two-week-old tissue culture clone of tumor forming F_1 hybrid of *Nicotiana glauca* (4n)×*N. langsdorffii* (2n) was used. Tissues were prepared as described previously (Kovács 1967). Nucleic acids and proteins were separated by the method of Schmidt and Thannhauser (Paech et Tracey 1955).

The amount of DNA was determined by ultraviolet absorption and Dische's diphenylamine test (Dische 1930, Paech et Tracey 1955). Quantitative estimation of RNA was performed by ultraviolet absorption and the orcinol method (Paech et Tracey 1955). The protein content of the tissues was measured by method of Lowry et al. (1951).

The carbohydrate content of the culture medium was one per cent. The starch was washed four times with 96% ethanol and water before use.

Tissues were cultured in 100 ml flasks. When flasks of other sizes were used appropriate controls were included. (Rate of organ formation is independent of flask size.)

Results

The growth and organization of cultures were compared to tissues which grown on the medium containing sucrose. These cultures were used as a control.

The growth (on the basis of fresh weight) of four-week-old tissue cultures was vigorous on the medium containing sucrose. These cultures had an intensive bud and shoot formation (Table I.). Tissue cultures of genetic tumorous condition consist of shoots an unorganized tissue (cf. Kovács 1967). The growth of tissue cultures was very slow in the presence of one per cent of starch. During four weeks these cultures scarcely grew as compared to their initial weight (380 mg). After 8—12 weeks the growth of tissue cultures approached the appropriate fresh weight value (about 60 per cent of control) (Table I.). These tissue cultures did not form buds and shoots on culture media containing starch. In the presence of starch only undifferentiated callus was produced by tissues without buds and shoots. Consequently, starch inhibited the bud and shoot formation of tumorous tissue cultures.

When the unorganized calli growing in the presence of starch were placed in sucrose-containing culture media again, buds could form in four weeks. After six weeks these tissues produced new shoots. According to these experiments the inhibitory effect of starch on shoot formation could be compensated by sucrose. Growth and organ formation of tissue cultures growing in the presence of both starch and sucrose behaved like control tissues (Table I.).

No iodine-reaction of blue color was given by cut surfaces of tissue cultures growing in presence of starch. Consequently, the tumorous tissues decompose the starch. Certain decomposition products of starch with a lower molecular weight may inhibit organ formation of the cultures.

Dextran is not a suitable carbon source for the growth of tumorous tissue cultures although it is also composed of glucose molecules just as starch. The tissue cultures did not grow on media containing dextran.

During subculture these tissues became brownish in color and then perished. Dextran might have a toxic effect.

The tumorous tissue cultures don't perish in the absence of carbon source (sucrose or starch). The growth of the tissues is minimal but shoot formation is not inhibited. (Table I.) These experiments show that these tissues survive despite a lack of exogenous carbon source, that is they have endogenous reserve of carbohydrate.

Glucose and maltose are possible decomposition products of starch, thus the effect of these sugars on the tissues was studied. In the presence of glucose the growth of the tissue cultures was similar to the control (sucrose medium). The rate of bud and shoot formation was the same as in the control (Table I.).

The difference in growth rate of cultures growing on maltose or sucrose was minimal. The bud and shoot formation of tissues on the medium containing maltose was similar to that of the control (Table I.).

Table I.

Effect of different carbohydrates on the growth and organ formation of tissue cultures of genetic tumorous condition

Carbohydrates 1%	Age of tissues in week	Fresh weight in mg ($\bar{x} \pm s_{\bar{x}}$)	Per cent of growth	Bud and shoot formation
Glucose	4	6365 ± 328	97.3	yes
Maltose	4	5870 ± 281	89.7	yes
Sucrose	4	6538 ± 338	100	yes
Starch	4	502 ± 31	7.6	no
	12	3824 ± 268	58.4	no
Starch plus sucrose	4	6425 ± 366	98.8	yes
Dextran	4	necrotic	–	necrotic
Sucrose*	3	987 ± 79	100	yes
None*	3	85 ± 5	8.6	yes

* These cultures were grown in 25 ml flasks.

These experiments suggest that glucose and maltose may not be responsible for the effect of starch. It is possible that the action of starch is based on the effect of its other decomposition products or reduced energy supply.

In the next step nucleic acid and protein contents of the tissues were studied. It was established that RNA and protein contents of shoot-parts were lower than those of the unorganized callus of the control culture (on sucrose). DNA, RNA and protein contents were expressed on the basis of fresh weight. The difference in DNA content was insignificant between shoot and callus of control cultures (Table II.).

Table II,

Effect of starch on DNA, RNA, protein and dry matter content of tumorous tissue cultures

Carbon source	Character of tissues	Content in mg/g fresh weight				RNA / DNA	Protein / DNA	Protein / RNA
		DNA	RNA	Protein	Dry matter			
Sucrose	Shoot	0.183	0.985	2.840	31.10	5.38	15.51	2.88
	Unorganized callus	0.185	2.334	6.715	67.06	12.62	36.29	2.87
Sucrose	Mean of shoot and callus	0.184	1.660	4.777	49.08	9.00	25.90	2.87
Starch	Unorganized	0.133	1.050	4.050	52.30	7.86	30.33	3.86

The means are significant at a level of 5 per cent.

Dry matter content of unorganized callus was about two times higher than that of the shoots of control tissues (Table 2).

In the unorganized callus-parts the ratios of RNA to DNA and protein to DNA were higher than those in shoot-parts. This suggests an increased RNA and protein synthesis in the callus-part of control tissues (Table II.). It is interesting to note that the ratio of protein to RNA is the same in the both shoot- and callus-parts. It seems that protein synthesis in shoots and calli depends on RNA to the same extent.

Since organization of tissue cultures is missing on culture media containing starch, whole tissue were used. Thus, the data obtained on media containing starch were compared with mean values shoot and callus tissues of the control cultures.

Conspicuous reduction of DNA and RNA content was observed in tissue cultures growing in the presence of starch. Protein and dry matter content of these tissues scarcely diminished compared to the mean values characteristic of shoot plus callus tissues of control. This clearly shows that chiefly DNA and RNA synthesis is reduced in the presence of starch. This is supported by the protein to RNA ratio which is higher with tissues grown on starch than with those grown on sucrose (Table II). The change in the protein to RNA ratio is brought about by a lower RNA content of these tissues.

Values of RNA and protein content of tissues growing on starch approach those characteristic of the shoots of the control. The value for dry matter content approaches the mean of shoot and callus (Table II).

The data of the present experiments clearly show that RNA and especially DNA synthesis is inhibited by the presence of starch.

Discussion

Earlier Kastens and Meester-Manger Cats (1960) and van Lith-Vroom et al. (1960) have reported that growth and structure of tissue cultures changed on media which contained starch. The inhibitory effect of starch on organogenesis were first pointed out by the author's experiments (Kovács 1968).

The present experiments clearly show that in the presence of starch the growth of tumorous tissue cultures is very slow and their organogenesis is inhibited. The inhibitory effect of starch could be reverted by the addition of sucrose. It is a question whether incomplete energy supply would result in an inhibition of bud production on culture media containing starch.

It is possible that nucleic acid and protein synthesis is inhibited by incomplete energy supply. Thus, inhibition of nucleic acid and protein synthesis leads to a failure of organ formation. This is supported by earlier work of the author. It has been pointed out that chloramphenicol, acridine orange, ethionine inhibited bud initiation of tumorous tissue cultures. That is, specific proteins are required for organ formation in these cultures (and probably also tumor formation (Kovács 1970). Data of Table II confirm the above assumption because they show a reduction in nucleic acid and protein content of tissues growing on starch-media. Consequently, the absence of essential proteins or nucleic acids

for organogenesis results in an inhibition of bud formation. This agrees with my earlier results indicating that derepression of genetic activity is responsible for spontaneous organ formation in tumorous tissue cultures (Kovács 1967, 1968).

According to Table I there is shoot development in the absence of carbon source (although 3-week-old cultures were studied only). This suggests that inhibition of bud formation on media containing starch results not only from a lack of carbon source but also from inhibitory effects of starch decomposition products. Certain components of these products may influence the genetic regulation of organ formation, directly or indirectly.

Hurel-Py (1950) have pointed out toxic effects of polysaccharides. In the present experiments a toxic effect of dextran could be observed. Others have reported that certain mono- and disaccharides are toxic for the growth of roots and of tissue cultures (Faludi et Parádi 1964, Van Lith-Vroom et al. 1960).

Mono- and disaccharides can influence morphogenesis of plant tissues. Fern prothallia can develop either antheridia or archegonia or they may be filamentous depending on the concentration of glucose (Hurel-Py 1950). Gautheret (1940) has experienced that, appropriate concentration combinations of maltose and saccharose, levulose, glucose or galactose stimulate bud formation in *Ulmus* tissue cultures.

Tissue cultures of genetic tumorous condition could be characterized by increased spontaneous shoot formation (Kovács 1968, 1969). This shoot formation was inhibited by starch in the present experiments, that is, the tumorous growth and features were inhibited by starch. This means that the presence of starch can inhibit tumor forming reactions. Consequently, starch has an antitumor activity. The above statements are supported by experiments of Nakahara et al. (1967) and Chihara et al. (1969). They have found an antitumor activity of polysaccharide fractions of different plants with respect to sarcoma 180.

Summary

The inhibitory effect of starch on the growth and organogenesis of genetically tumorous tissues was studied. The spontaneous organogenesis of tissues can be suppressed by starch. Growth and organogenesis in tissue cultures were similar in the presence of glucose, maltose, sucrose and sucrose plus starch, the amount of RNA, protein and especially DNA was reduced in tissue cultures which were growing in the presence of starch. Incomplete energy supply may lead to a reduction of nucleic acid and protein synthesis. Suppression of organogenesis in tissues resulted in the disappearance of essential nucleic acid and protein components. It is possible that decomposition products of starch influence organogenesis and nucleic acid and protein synthesis of tumorous tissues. Starch has an antitumor activity.

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