THE RELATION BETWEEN CELLULOSE SYNTHESIS AND AN UNIDENTIFIED GLUCOSE-COMPLEX IN PEA STEM SEGMENTS

H. WINTER, S. SPAANDER and P. K. WIERSEMA

Botanisch laboratorium, Universiteit, Groningen.

SUMMARY

An as yet unidentified glucose-complex is formed in the cells of 5 mm stem segments cut from the third internode at 1 mm from the tip of etiolated pea seedlings. In segments growing in solutions containing 10 μg/ml IAA and various concentrations of glucose the quantities of the unknown glucose-complex and cellulose are proportional.

Increasing the IAA concentration at a constant glucose concentration of 0.1 % causes an increase in the glucose-complex and in the synthesis of cellulose up to a concentration of 10 μg/ml. At IAA concentrations higher than 50 μg/ml cellulose synthesis decreases to the value obtained when no IAA is present, whereas the amount of the glucose-complex is increased.

Galactose inhibits cellulose synthesis by way of interfering with the glucose absorption.

In low concentrations of glucosecobalt chloride promotes sugar absorption, the formation of the glucose-complex, and cellulose synthesis. In high sugar concentrations it does not affect these processes.

The results obtained indicate a close relationship between cellulose synthesis and the formation of the glucose-complex. It is suggested that the latter is an intermediate in the synthesis of cellulose.

1. INTRODUCTION

Stem segments of *Pisum sativum* can grow in a lateral and in a longitudinal direction. Whether lateral or longitudinal growth predominates depends on the composition of the medium solution in which the stem segments are growing (BULT & VAN RAALTE 1961).

Addition of sugar and IAA to the medium solution was required in order to obtain optimal lateral growth. It could be proved that the effect of sugar and IAA on lateral growth was not due to an increase in the osmotic pressure of the cell content caused by increased absorption of sugar and subsequent rounding out of the cell (WINTER & VENEMA 1964). Nor was there any proof that the lateral growth was a result of an increase in cell number by cell division (BULT 1961, SIKKEMA 1964, unpublished results).

However, lateral growth appeared to be positively correlated with the formation of cellulose (WINTER 1966), and evidence could be provided that the amount of reducing sugars in the cells of the stem segments is a limiting factor for the synthesis of cellulose (WINTER 1967).

IAA stimulates the formation of cellulose (WINTER 1966), and it could be shown (WINTER 1967) that IAA also strongly promotes the formation of an unidentified glucose-complex from the absorbed sugar. The glucose-complex

may therefore have a function in the synthesis of cellulose. In the present publication experiments will be described that support this hypothesis.

2. MATERIAL AND METHODS

Peas (*Pisum sativum*) cv. "Alaska" were grown in pans containing water-soaked vermiculite; they were placed in a dark room at 25 °C and high humidity. Light, necessary when handling the plants, was provided by an incandescent bulb screened by a green glass. After 7 days, when the third internode had reached a length of 1–3 cm, a segment of 5.1 mm was cut from each plant at a distance of 1 mm from the tip. The segments were floated on 10 ml aerated (air-stirred) 20 mM phosphate buffer solutions (pH 6.0). After 24 hrs the segments were rinsed in de-ionised water and dried with filter paper. The increase in fresh weight was recorded, and increases in length and width were measured by projecting enlarged images of the segments on to a positive film.

Samples consisting of 25 stem segments were frozen in liquid nitrogen, ground in a mortar, and extracted with 80% ethanol on a boiling water bath. U-^{14}C-labelled glucose was used for measuring glucose uptake into the stem segments and the conversion of glucose into other metabolic products. From samples of the ethanol extracts two-directional chromatograms were prepared on Whatman 3 mm filter paper, with butylacetate: acetic acid: water (3:3:1) and pyridine: ammoniumhydroxide: isobutanol (4:2:1) as solvents. Autoradiograms of the chromatograms were prepared on Kodak No-Screen X-ray film. Radioactive spots on the chromatograms, located by reference to the autogradiographs, were cut out and counted with the aid of a liquid scintillation counter (Mark 1 Nuclear-Chicago). For measuring the amount of radioactivity in the cellulose fraction the 80% ethanol insoluble fraction was extracted with 20% KOH and the residue (α-cellulose) broken down to CO_2 in a carbon-14 glassware system (Model GW 1 Nuclear-Chicago) and transferred to an ionisation chamber. The ion current produced was measured with a Dynacon (Nuclear-Chicago model 6010) electrometer. From the electrometer readings the ^{14}C content could be calculated.

3. RESULTS

3.1. Effect of glucose concentration

By varying the sugar concentration of the medium solution the amount of glucose absorbed could also be varied. It was found that the absorption of glucose is proportional to the external glucose concentration. At the same time there is a shift in the relative amounts of labelled glucose and glucose-complex present in the cell. This means that though these compounds are positively correlated, the correlation is not rectilinear (WINTER 1967). So by varying the sugar concentration of the medium solution, the amount of cellulose formed from the absorbed glucose could be compared with the amount of glucose and glucose-complex present in the cell.

Sets of 25 stem segments were incubated in solutions containing ^{14}C-labelled glucose and IAA (10 μg/ml). The glucose concentration ranged from 0.006% to 2% (*table 1*). The IAA cencentration was kept at 10 μg/ml, the concentration necessary for obtaining optimal lateral growth and cellulose formation (WINTER 1966).

Table 1. The amount of ^{14}C-labelled glucose, glucose-complex, and cellulose, expressed as μg glucose, in the cells of 25 stem segments after an absorption period of 24 hrs in medium solutions with different glucose concentrations.

Medium solution Glucose conc.	Glucose	Glucose-complex	Cellulose	Total
0.006 % glucose	1 μg	16 μg	9 μg	26 μg
0.1 % glucose	34	200	113	347
0.5 % glucose	644	760	445	1449
1 % glucose	1932	912	719	3563
2 % glucose	4830	1185	862	6877

Each figure represents the average of 7 experiments.

Table 1 shows that the amount of glucose and glucose-complex present in the cells after an absorption period of 24 hrs increases with the external glucose concentration. This amount represents at least 90% of total radioactivity in the 80% ethanol extract. The same conclusion holds for the formation of cellulose.

However, it appears that the metabolism of the absorbed glucose cannot keep pace with the absorption process when the sugar concentration of the medium solution increases. Whereas at the lowest glucose concentration about 96% of the absorbed glucose is metabolized, no more than about 30% is metabolized at the highest concentration. This means that the amount of glucose in the cells of the stem segments increases more than the amount of glucose-complex. On the other hand it appears that the amount of ^{14}C-labelled cellulose increases rectilinearly with the amount of glucose-complex. This strongly suggests that the rate of cellulose synthesis depends on the amount of glucose-complex formed and is therefore rate limiting for the synthesis of cellulose.

3.2. Effect of galactose

ORDIN & BONNER (1957) reported that galactose specifically inhibited the formation of cellulose in *Avena* coleoptiles. An attempt was therefore made to inhibit cellulose synthesis in the pea stem segments by adding galactose to the glucose-containing medium in order to investigate whether or not the formation of the glucose-complex was affected, too.

Table 2 shows that galactose not only strongly inhibits cellulose formation but also the absorption of glucose. The percentage inhibition of these two processes does not vary significantly, and therefore the conclusion may be drawn

Table 2. The effect of galactose on cellulose synthesis in stem segments of *Pisum* during an absorption period of 24 hrs.

Medium solution	Glucose absorption		Cellulose synthesis	
	cpm	% inhibition	cpm	% inhibition
1 glucose 0.09%	73300	0	11490	0
2 glucose + mannitol 0.09%	75500	–3	11820	–3
3 glucose + galactose 0 09%	36050	51	6428	44
1 + IAA 10 μg/ml	91350	0	20478	0
2 + IAA 10 μg/ml	86000	6	18357	12
3 + IAA 10 μg/ml	60000	34	15357	26

Each figure represents the average of 7 experiments.

that the inhibiting effect of galactose on cellulose synthesis is primarily due to its effect on glucose absorption.

Table 2 also shows that IAA (10 μg/ml) stimulates both the absorption of glucose (at least at this concentration) and the formation of cellulose. However, the formation of cellulose is stimulated more than glucose absorption. This is in agreement with earlier findings (WINTER 1966).

The inhibiting effect of galactose on glucose absorption and hence on cellulose synthesis is still present when IAA is added to the medium solution, but the percentual inhibition is much smaller.

3.3. Effect of cobalt chloride

MILLER (1954) found that $CoCl_2$, when added to solutions containing sugar and IAA, greatly increased the elongation of etiolated pea stem segments.

In the light of the evidence that under certain experimental conditions (BULT & VAN RAALTE 1961) growth in length competes with growth in width, an explanation for the effect of Co might be an inhibition of lateral growth and hence of cellulose synthesis by way of inhibiting the formation of the unidentified glucose-complex.

However, no definite indication could be obtained that $CoCl_2$ actually interfered with cellulose synthesis. Miller's observation that $CoCl_2$ did not

Table 3. The effect of $CoCl_2$ on the absorption of glucose (80% ethanol extract) by *Pisum sativum*.

Medium solution	$-$ $CoCl_2$	$+$ $CoCl_2$ 8. 10^{-5}M	% stimulation
Glucose ^{14}C 0.003%	9428 cpm	18100 cpm	+192
Glucose ^{14}C 0.003% + IAA (10 μg/ml)	11200	29050	+259
Glucose 1% + IAA (10 μg/ml)	8010	7885	– 2
Glucose 2% + IAA (10 μg/ml)	5066	4820	– 5

Each figure represents the average of 6 experiments.

stimulate the absorption of glucose when supplied to the medium solution in a concentration of 2% was confirmed (*table 3*). The same conclusion holds for a glucose concentration of 1%.

On the other hand, $CoCl_2$ strongly stimulated the absorption of glucose from solutions containing a very low glucose concentration.

3.4. Effect of IAA concentration

IAA strongly stimulates the formation of the glucose-complex (WINTER 1967), so varying the IAA concentration of the medium solution provided another means of varying the amount of glucose and glucose-complex in the cell independently.

In the next experiment the sugar concentration in the medium solution was kept at 0.1%, whereas the auxin concentration was varied from 0.1 μg/ml to 100 μg/ml.

Table 4. The effect of IAA on the synthesis of the glucose-complex and on cellulose.

IAA concentration Medium solution	Glucose absorbed	Glucose not metabolized	Glucose-complex	Cellulose
0 μg/ml	210 μg	55 μg	42 μg	50 μg
+0.1 μg	205	49	39	48
+ 1 μg	195	27	48	55
+ 10 μg	182	14	71	65
+ 50 μg	161	10	85	57
+100 μg	145	5	92	39

Each figure represents the average of 8 experiments.

The results are presented in *table 4*, showing that IAA inhibits glucose absorption. At IAA concentrations of 50 and 100 μg/ml the decrease in absorption rate is significant ($p = < 0.01$). This means that an effect of IAA on the metabolism of the absorbed glucose is obscured by its effect on the absorption process. In order to obtain a true picture, the effect of IAA on glucose absorption is eliminated by expressing all data obtained as a percentage of the glucose absorption (*fig. 1*).

Fig. 1 shows, firstly, that with increasing IAA concentrations the amount of glucose absorbed and not metabolized during the absorption period of 24 hrs decreases strongly.

Secondly, it shows that starting at a concentration of 0.1 μg/ml IAA the amount of glucose-complex formed from the absorbed glucose increases with the IAA concentration. Up to a concentration of 10 μg/ml IAA the same holds for the synthesis of cellulose. However, the stimulating effect of IAA on cellulose synthesis does not exist at concentrations of 100 μg/ml, whereas the formation of the glucose-complex is still enhanced.

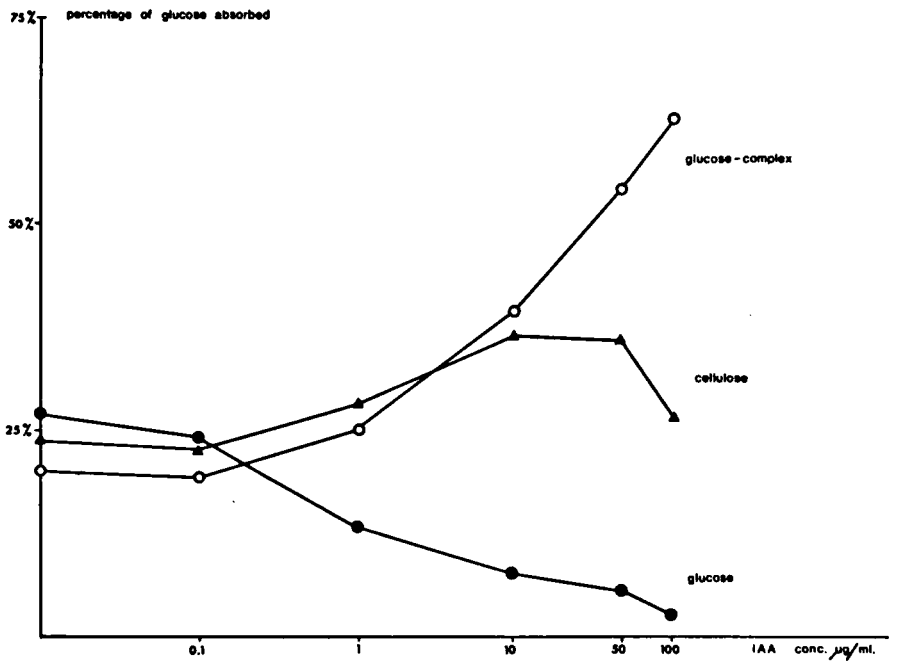

Fig. 1. The effect of IAA on glucose metabolism. ●—● glucose not metabolized o—o glucose-complex ▲—▲cellulose.

4. DISCUSSION

As shown by *table 1*, the increase in rate of cellulose synthesis bears the same relationship to the amount of glucose absorbed as the increase in synthesis of the glucose-complex.

WINTER (1967) showed that IAA (10 µg/ml) stimulates both the formation of the glucose-complex and the formation of cellulose. This means that if the glucose-complex is not a precursor of cellulose, IAA would stimulate two different enzyme systems competing for the glucose. This possibility can not be ruled out. However, it is hard to imagine that at all glucose concentrations used both enzyme systems, when operating independently, compete with equal efficiency. It is to be expected that with increasing internal glucose concentrations the enzyme systems involved obtain optimal operation at different glucose concentrations. Therefore, a change in the concentration will very likely result in a different change in the formation rate of the two compounds, at least when operating in a rate-limiting glucose range.

Only if the glucose-complex is a precursor of cellulose a linear relationship in formation rate may be expected.

Table 2 showed that, at least in pea epicotyles, the interference of galactose

with cellulose synthesis must be ascribed to inhibition of glucose absorption by galactose. Therefore, a specific influence of galactose on cellulose synthesis is not present here.

Table 2 also shows that the effect of galactose on glucose absorption is less when IAA is added to the medium solution. This might be due to the fact that IAA promotes the metabolism of the absorbed glucose. As a result the concentration of the absorbed glucose decreases and this might positively effect glucose absorption.

When subtracting the IAA stimulated part of the glucose absorption and cellulose synthesis from the amounts actually found when galactose is present in the medium solution, the inhibition of glucose absorption about equals the inhibition found when no IAA is added to the medium solution.

Table 3 showed that $CoCl_2$ stimulated the absorption of glucose from solutions containing a very low glucose concentration. CARLIER *et al.* (1967) studied the effect of auxins and cobalt on cell wall synthesis in Mung bean tissue. They found that a combination of NAA and $CoCl_2$ strongly favoured the incorporation of uronic acids and pentoses in the cell wall. In the light of the results obtained with the absorption of glucose by pea stem segments, it might be possible to ascribe the increased incorporation of glucuronic acid in the cell wall to an enhanced absorption of glucuronic acid by the Mung bean tissue.

Fig. 1 shows that IAA, starting at a concentration of 0,1 μg/ml, stimulates the formation of the glucose-complex. The same holds for the formation of cellulose up to a concentration of 10 μg/ml. Again the interpretation might be that the glucose-complex is a precursor in the synthesis of cellulose. At IAA concentrations of 100 μg/ml at least the same amount of glucose-complex is present in the cell (*table 4*), whereas the amount of cellulose formed is strongly reduced.

One explanation might be that the enzyme system involved in the formation of the glucose-complex tolerates high concentrations, whereas the same concentration might be poisonous for the enzyme systems involved in glucose absorption and the transfer of the glucose from the glucose-complex to cellulose.

The stimulation of the formation of the glucose-complex at high IAA concentrations (100 μg/ml) might be partly due to an accumulation of the glucose-complex as a result of an inhibition of cellulose synthesis. It is therefore concluded that the results obtained so far corroborate the idea that the glucose-complex is a kind of precursor for the cellulose. The function, however, is not clear.

COLVIN (1959) could detect a glucose-complex, possibly a glucolipid, which is formed by the bacterium *Acetobacter xylinum*. This bacterium forms extracellular cellulose. His suggestion is that this glucolipid, which passes into the medium solution, is required to mediate between U.D.P.G. and the polymerisation reaction. The lipid-bound activated glucose may be required for facilitating the transport of the polar glucose across the plasma membrane. Then an enzymatic transfer of the glucose portion of the molecule to the growing cellulose microfibril would follow and the lipid fraction would probably be recycled.

COLVIN (1961) and KHAN & COLVIN (1961) could also detect a glucolipid in

green plants, though to a much more limited extent than for the bacterium *Acetobacter xylinum*. Our unpublished data indicate that the glucose-complex is a glucolipid. This glucolipid, however, is not identical with the glucolipid detected by Colvin in the bacterium *Acetobacter xylinum*.

ACKNOWLEDGEMENTS

The authors wish to thank Prof. Dr. M. H. van Raalte and Dr. R. J. Helder for their critical reading of the manuscript and Miss S. Barker for the correction of the English text.

REFERENCES

BULT, P. & M. H. VAN RAALTE (1961): The effect of auxin and sucrose on growth and form of pea stem sections. *Acta Bot. Neerl.* **10**: 451–459.

CARLIER, A., C. VAN HOVE & J. VAN ASSCHE (1967): Influence of auxins and cobalt on respiration pathways, growth and cell wall synthesis in mung bean tissue. *Seventh Int. Congr. Biochem. Abstr. Tokyo.* **J**: 438.

COLVIN, J. R. (1959): Synthesis of cellulose in ethanol extracts of Acetobacter xylinum. *Nature (Lond.)* **183**: 1135–1136.

— (1961): Synthesis of cellulose from ethanol-soluble precursors in green plants. *Can. J. Biochem. Physiol.* **39**: 1921–1926.

KHAN, A. W. & J. R. COLVIN (1961): Isolation of the precursor of bacterial cellulose. *J. Polymer Sci.* **51**: 1–9.

MILLER, C. D. (1954): The influence of cobalt and sugars upon the elongation of etiolated pea stem segments. *Plant Physiol.* **29**: 79–82.

ORDIN, L. & J. BONNER (1957): Effect of galactose on growth and metabolism of Avena coleoptile sections. *Plant Physiol.* **32**: 212–215.

WINTER, H. (1966): Effect of auxin and sugar on cell wall synthesis and growth of pea stem segments. *Proc. Kon. Ned. Akad. Wetensch. Amsterdam* **C69**: 64–72.

— (1967): The effect of IAA on sugar metabolism by growing pea stem segments. *Proc. Kon. Ned. Akad. Wetensch. Amsterdam* **C70**: 294–301.

— & T. H. VENEMA (1965): Sucrose uptake by pea stem segments and its effect on growth. *Acta Bot. Neerl.* **14**: 366–371.