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Synthesis and Turnover of Cell-Wall Polysaccharides and Starch in Photosynthetic Soybean Suspension Cultures[1]

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Soybean (*Glycine max* [L.] Merr.) suspension cultures grown under photoautotrophic and photomixotrophic (1% sucrose) culture conditions were used in $^{14}CO_2$ pulse-chase experiments to follow cell-wall polysaccharide and starch biosynthesis and turnover. Following a 30-min pulse with $^{14}CO_2$, about one-fourth of the ^{14}C of the photoautotrophic cells was incorporated into the cell wall; this increased to about 80% during a 96-h chase in unlabeled CO_2. Cells early in the cell culture cycle (3 d) incorporated more ^{14}C per sample and also exhibited greater turnover of the pectin and hemicellulose fractions as shown by loss of ^{14}C during the 96-h chase than did 10- and 16-d cells. When the chase occurred in the dark, less ^{14}C was incorporated into the cell wall because of the cessation of growth and higher respiratory loss. The dark effect was much less pronounced with the photomixotrophic cells. Even though the cell starch levels were much lower than in leaves, high ^{14}C incorporation was found during the pulse, especially in older cells. The label was largely lost during the chase, indicating that starch is involved in the short-term storage of photosynthate. Thus, these easily labeled and manipulated photosynthetic cells demonstrated extensive turnover of the cell-wall pectin and hemicellulose fractions and starch during the normal growth process.

The plant cell-wall compartment can have high metabolic activity and may be engaged in important events related to plant cell function and development, including control of growth and morphogenesis, cell-cell recognition, disease resistance, and signaling (Bolwell, 1993; Cosgrove, 1993). The alterations found in the cell-wall composition during the cell growth cycle and those found under different biotic and abiotic influences represent the observable chemical basis for the control of growth and differentiation in plants. At present there is a lack of information about how the composition of the plant cell wall may change during normal cell growth and how these changes are controlled.

The composition of cell walls of heterotrophic (grown with sugar as the carbon and energy source) cell cultures has been found to be influenced greatly by the culture conditions and carbon sources (Nevins et al., 1967; Blaschek and Franz, 1983; Iraki et al., 1989a, 1989b, 1989c) and the stage of the culture cycle (Takeuchi and Komamine, 1978; Asamizu et al., 1983).

The processes of biosynthesis and degradation of cell-wall components and their alteration under the influence of different factors have also been studied with heterotrophic cell-suspension cultures in pulse-chase experiments. In these studies, the cells were first cultured in the presence of radioactive sugars for a certain period (pulse) and then in the absence of the labeled substrate (chase). It has been shown that the noncellulosic components of the cell wall undergo the degradation or interconversion associated with growth in several different species, including *Vinca rosea* (Takeuchi and Komamine, 1980a, 1980b; Takeuchi et al., 1980), proso millet (Gibeaut and Carpita, 1991), and rose cells (Edelmann and Fry, 1992).

Although exogenous sugars can be natural substrates for heterotrophic tissues, their transport, metabolism, and fate could be different from that of endogenous substrates. For example, differences in postphotosynthetic metabolism have been shown for assimilates formed in different plant parts by Chetverikova et al. (1991) and Chikov et al. (1993). Saturation of endogenous substrate pools for cellulose synthesis by exposure of wheat leaves to increased CO_2 levels inhibited the uptake of exogenous Glc used for cellulose formation, which indicates that photosynthates and exogenous Glc can compete for utilization in cellulose synthesis (Tarchevskii et al., 1984). The addition of Glc to ripening banana fruit led to major changes in carbohydrate metabolism, including increases in the size of the hexose-monophosphate pools, and in starch synthesis and inhibition of starch breakdown (Hill and apRees, 1995). Likewise, the sugars can affect their own metabolism, since sugars that are the substrates of hexokinase can cause repression of this enzyme at low concentrations (1–10 mM) (Jang and Sheen, 1994). Thus, the use of exogenous ^{14}C-labeled sugars for cell-wall studies could possibly give misleading results.

We propose the use of another system for cell-wall metabolism studies, photoautotrophic suspension cultures, which utilize CO_2 and light as the sole carbon and energy sources, respectively. This system can allow the observa-

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Abbreviations: ECP, extracellular polysaccharides; SB-M, mixotrophic soybean line; SB-P, photoautotrophic soybean line.

tion of long-term synthesis and hydrolysis of cell-wall components during the cell cycle and under different culture conditions using $^{14}CO_2$ fixed by the natural process of photosynthesis. The *Glycine max* (soybean) SB-P and SB-M cell lines (Horn et al., 1983) were used in the studies presented here. These cell lines consist of finely divided suspensions with relatively high chlorophyll and Rubisco levels and low PEP carboxylase activity, making them similar to leaves in these characteristics (Roeske et al., 1989; Goldstein and Widholm, 1990) and unlike many photoautotrophic cultures that have low chlorophyll and Rubisco and high PEP carboxylase (reviewed by Widholm, 1992). There are many other photoautotrophic cultures available that could also be used in such studies (Widholm, 1992).

Photosynthetic cell-suspension cultures have a number of advantages for cell-wall metabolism studies, including the direct fixation of $^{14}CO_2$ and its metabolism by normal processes. The culture conditions can be easily manipulated so that the effects of exogenous substrates and other environmental changes on the cells and photosynthesis can be readily determined. The cultures are a convenient and reproducible source of relatively homogeneous cells and cell walls. Thus, photosynthetic cell-suspension cultures appear to provide a unique possibility for the study of cell-wall dynamics and its role in plant cell metabolism.

The goal of this study was to characterize cell-wall polysaccharide synthesis and turnover during the cell culture growth cycle and under different light conditions in pulse-chase experiments with $^{14}CO_2$ fixed by photoautotrophic and mixotrophic soybean cells. Some initial information from these studies was reported recently (Lozovaya and Widholm, 1995).

MATERIALS AND METHODS

Plant Material

The *Glycine max* (L.) Merr. cv Corsoy soybean suspension culture (Horn et al., 1983) was grown photoautotrophically (SB-P) with a 5% CO_2 atmosphere in MIN liquid medium plus 0.1 mg L^{-1} thiamine-HCl (Goldstein and Widholm, 1990) or mixotrophically (SB-M) with 1% Suc in the same medium (KN1) with ambient CO_2. Cell suspensions were grown under continuous fluorescent light (about 250 μE m^{-2} s^{-1}) at 28 to 30°C and were subcultured every 21 d. Cultures were used in the experiments 2 to 3, 10, and 16 d after inoculation, which corresponded to the time of cell division, expansion, and stationary phases, respectively.

Pulse Labeling with $^{14}CO_2$

Usually a 30-min pulse was carried out in a closed system of about 1.5 L with a circulating air pump using 1% CO_2 with $^{14}CO_2$ produced from $NaH^{14}CO_3$ (2.09 GBq $mmol^{-1}$) by acidification. The flasks were incubated under lights on a gyratory shaker under sterile conditions. The atmosphere in the closed system was circulated through a 1 M KOH trap before the pulse to remove CO_2 and after the pulse to remove all $^{14}CO_2$. The chase was conducted with 5% CO_2 for the SB-P cells and ambient CO_2 for the SB-M cells. Individual flasks or aliquots were harvested over time during the chase for analysis. During the chase the atmosphere of each flask was bubbled through 1 M KOH to collect the respired $^{14}CO_2$. The flasks were wrapped in aluminum foil for the dark treatments.

Cell samples, usually 25 mL, were harvested by vacuum filtration on a filter paper disc, rinsed with fresh medium, frozen in liquid N_2, and freeze-dried. The cell dry weights per 25-mL samples at time 0 were about 80 mg for 3-d cells, 200 to 250 mg for 10- and 16-d photoautotrophic cells, 180 mg for 3-d cells, and 280 to 320 mg for 10- and 16-d mixotrophic cells. The results reported are the means of two samples for each time, and similar results were obtained in two or more similar experiments.

Continuous and Pulse Labeling with ^{14}C-Glc

A total of 0.259 MBq of D-Glc ([U-^{14}C], 9.259 Bq $mmol^{-1}$) was added to SB-M cells grown for 5 d in 25 mL of KN1 medium. For the continuous labeling experiment, the cells were incubated for 30 min (time 0), 4 h, and 24 h before samples were removed for analysis. For the pulse-chase experiment, the cells were filtered and rinsed after the 30-min pulse and were then incubated in fresh KN1 for the chase periods of 4 and 24 h before analysis. The experiment was performed once with two replicates.

Fractionation of Material

The freeze-dried cells or fully expanded leaves of field-grown plants were weighed. Lyophilized plant material was first homogenized in ice-cold 50 mM Tes(KOH) buffer with a mortar and pestle and sonicated for 2 to 3 min with a high-intensity ultrasonic processor (Sonics and Materials, Danbury, CT) with the output control at 8 and the duty cycle pulser at 40%. Cell disruption was monitored by light microscopy. The cell walls were purified by washing and centrifugation with 0.2 M KPO_4 buffer (pH 7.0) (4 times), water (3 times), chloroform:methanol (1:1, v/v) at 45° for 30 min (2 times), acetone (2 times), methanol (1 time), and water (2 times). Starch was extracted by treatment of the pellet with α-amylase from porcine pancreas2 (600 units) twice for 20 h (Carpita and Kanabus, 1987). Glc was measured in the combined supernatants as described by Dubois et al. (1956), or the radioactivity of each fraction was determined. Material remaining after extraction was stained with I-KI and checked by microscopy to ensure that no undigested grains remained. The insoluble material was washed twice with water and then suspended in water and freeze-dried. Cell-wall polysaccharide fractions were obtained as described by Nishitani and Masuda (1982). Pectins were extracted with 0.1 M oxalic acid at 100°C (boiling water bath) for 1 h with stirring every 10 to 15 min (two times). Hemicellulose was extracted with 24% KOH supplemented with 3 mg mL^{-1} $NaBH_4$ for 24 h at 25°C (two times), and cellulose was recovered after boiling the pellet for 1 h in acetic-nitric reagent (Updegraff, 1969) or KOH-insoluble material was analyzed (if indicated). The ^{14}C in each sample obtained from tissues labeled with $^{14}CO_2$ was determined using scintillation fluid (Bio-SafeII, Research Products International, Mount Prospect, IL) with a scintil-

lation spectrometer (TRI-CARB460, Hewlett-Packard). Each ^{14}C-labeled cell sample taken was fractionated, and two separate samples of the unlabeled cell-wall material were analyzed with three replicates. The results of a typical experiment are presented.

Monosaccharide Analysis

Pectins and hemicellulose were hydrolyzed by 2 N trifluoroacetic acid at 121°C for 1.5 h, and alditol acetates were prepared (Englyst and Cummings, 1984) and analyzed by GLC with myo-inositol as the internal standard (3-m × 2-mm glass column packed with Chrom WAW80–1000 mesh, coated with 3% OV275; Chrompack, Middelburg, The Netherlands).

The total uronic acid and neutral sugar contents were determined colorimetrically with automated *m*-hydroxydiphenyl (Thibault, 1979) and orcinol/sulfuric (Tollier and Robin, 1979) tests, respectively. All analyses were performed two or three times with two replicates.

RESULTS

Cell Culture Growth

The growth patterns of the SB-P and SB-M cells as measured by dry weight are shown in Figure 1. These cells grew slower than heterotrophically grown cells, but the dry weight increased to 3 times the inoculum by 16 d. Like most cell-suspension cultures, the growth can be generally divided into three phases, cell division (d 1–4), cell expansion (d 5–10), and stationary (after d 10). The mitotic index of SB-M cells was high for about 4 d after transfer to fresh medium and then decreased to about 0 by d 6 (N. Tikhenko, unpublished data).

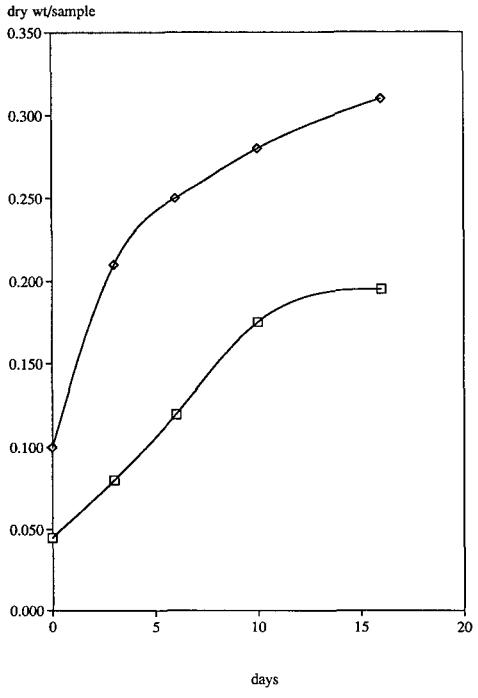

Figure 1. Growth of SB-P and SB-M suspension cultures as measured by dry weight (wt) per flask (25 mL of medium). □, SB-P; ◇, SB-M.

Cell-Wall Composition

The cell walls of the SB-P cells and young soybean leaves were composed of about equal proportions of ammonium oxalate-extractable pectins, KOH-extractable hemicellulose, and KOH-insoluble cellulose, whereas the SB-M cell walls contained more hemicellulose and less cellulose (Table I). This comparison shows that even a low level of sugar in the medium (1% Suc) altered the proportion of the cell-wall fractions. The SB-P cell and leaf wall polysaccharide ratio is similar to those usually found for dicots (Fry, 1988). The hemicellulose content increased in both SB-P and SB-M cells during the growth cycle, whereas the cellulose content decreased, especially in the SB-P cells. Similar changes in the 24% KOH-soluble and -insoluble fractions were described by Takeuchi and Komamine (1978) with heterotrophic *V. rosea* suspension cultures. In proso millet cells, the hemicellulose proportion also increased during the culture period, whereas the pectin and strong acid-resistant cellulose fractions remained constant (Carpita et al., 1985). However, the pectin and cellulose fractions made up a smaller proportion of the cell wall because of an increase in hemicellulose, which is characteristic of cereal cell walls.

The sugar composition of the cell-wall pectins, hemicellulose, and soluble polymers secreted by cells into the medium (ECP) at the stationary phase of the growth cycle in SB-P and SB-M cultures are shown in Table II. Glc is by far the most abundant neutral sugar in the pectin fraction of photoautotrophic cells, whereas pectins prepared from mixotrophic cells contain much less Glc. On the other hand, the proportion of uronic acids is considerably higher in the pectins of mixotrophic cells than in photoautotrophic cells. There was no difference in the percentage of Ara, the predominant sugar in the hemicellulose fraction, between photoautotrophic and mixotrophic cells, but proportions of Glc and Gal, also found in large amounts, were different. The uronic acid content was lower in the mixotrophic cell hemicellulose fraction. It should be noted that the level of Glc in the ammonium oxalate-extracted fraction of SB-P cells was lower in another experiment (9.3%), which can be explained by incomplete digestion of starch in the experiment presented in Table II, although it was still much higher than in SB-M cells (2.7%). The proportions of other sugars and uronic acids in the cell-wall fractions analyzed were very similar in different experiments. The sugar composition of the ECP in the culture medium was similar to that of the cell-wall polymers, with the exception of rhamnose, which was missing. The predominant sugars in the medium of both photoautotrophic and mixotrophic cells were Gal and Ara. The presence of arabinogalactans in various cultured cells and media has been shown in many studies (reviewed by Clarke et al., 1979). Arabinogalactans have been found as polysaccharides free from associated proteins and also in association with proteins. The type II arabinogalactan proteins are well-known secretory molecules of cell-suspension cultures (as reviewed by Fincher et

Table I. *Cell-wall composition of SB-P and SB-M cells at different times during the cell growth cycle and of fully expanded soybean leaves*

Material	Days after Inoculation	Percentage of Total Cell-Wall Polysaccharide[a]		
		Pectin	KOH soluble	KOH insoluble
SB-P	2	30.0 ± 0.5	29.7 ± 2.8	40.3 ± 4.1
SB-P	10	27.5 ± 0.3	33.1 ± 1.7	39.4 ± 1.9
SB-P	16	29.1 ± 0.3	46.1 ± 3.3	24.8 ± 2.5
SB-M	2	36.9 ± 2.4	44.3 ± 3.5	18.8 ± 2.1
SB-M	10	32.8 ± 1.2	47.0 ± 3.2	20.2 ± 3.7
SB-M	16	33.1 ± 1.9	51.0 ± 1.9	15.9 ± 1.3
Leaves		35.2 ± 2.8	32.1 ± 3.1	32.7 ± 2.3

[a] Values represent weight percent of cell-wall polysaccharides recovered (75–82% of the original wall material was recovered) with three replications and SE shown.

al., 1983). The possibility was considered by Takeuchi and Komanine (1980b) that arabinogalactan in the cell wall is degraded and secreted into the medium to form a part of the ECP. Another origin was proposed by Gibeaut and Carpita (1991) for arabinogalactan proteins, i.e. from the buffer-soluble pool distinct from cell-wall metabolism. The functional role of arabinogalactan or arabinogalactan proteins remains poorly understood.

The photoautotrophic cell culture medium contained a 10-fold higher proportion of uronic acids than that of the mixotrophs, although in both cases the percentage of uronic acid in the medium was much lower than that of the wall polysaccharide fractions. These results show that there are differences in the cell-wall and extracellular polymer compositions of photoautotrophically and mixotrophically cultured cells.

$^{14}CO_2$ Pulse-Chase Experiments

When $^{14}CO_2$ was incorporated by soybean cells of different ages during a 30-min pulse, between 25 and 28% of the label incorporated was found in the cell wall (compare data in Table III and Figs. 2 and 3). During a 96-h chase in unlabeled CO_2, the percentage of the total $^{14}CO_2$ in the cells found in the cell wall increased greatly. These results show that the cell wall is the major final product in these cells, since about 80% of the ^{14}C accumulated in this compartment. This proportion of total radioactivity incorporated into the cell wall was about the same in young dividing soybean cells and in stationary phase cells. When the chase was in the dark the soybean cell walls accumulated a lower proportion of the ^{14}C remaining in the cells (46–67%) than when in the light. This decreased cell-wall incorporation under dark conditions was probably due to the cessation of growth, which occurred in the dark probably because of the lack of energy. The cells remained viable, however, as shown by viability staining. The differences in the amount of ^{14}C lost between the cells incubated in the light and those incubated in the dark can be accounted for by increased respiratory loss in the dark (Lozovaya and Widholm, 1995).

The $^{14}CO_2$ pulse-chase results obtained with SB-P cells of different culture stages are given in Figures 2 and 3 on a per culture flask basis. The 3-d cells incorporated much higher levels of ^{14}C into the cell-wall components than did the 10- and 16-d cells. The hemicellulose fraction was the most highly labeled, especially in the dividing cells (3 d). During the 96-h chase, the pectin fraction of the 3- and 10-d cells lost a large proportion of the label (about 40%), whereas the 3-d cell hemicellulose fraction accumulated less ^{14}C than the acetic-nitric glucan and cellulose fractions. This indicates that there was a turnover in these wall components (pectin and hemicellulose) during growth. This turnover was lower in older cells, because in 16-d cells the pectin counts were stable and the hemicellulose counts approximately doubled. The other two fractions, acetic-nitric glucan and cellulose, accumulated ^{14}C by 2-fold or more during the chase, indicating lessened or no turnover in all cases.

Table II. *Monosaccharide composition of pectins, hemicellulose, and ECP in photoautotrophic SB-P and mixotrophic SB-M suspension-cultured soybean cells at stationary phase of the cell growth cycle*

The data of one experiment with two replicates are presented. The deviation does not exceed 3%.

Cell Wall Fraction	Cell Line	Monosaccharide						
		Fuc	Rhamnose	Ara	Gal	Glc	Xyl + Man	Uronic acid
		mol %						
Pectins	SB-P	0.8	2.0	3.2	3.8	23.4	0.9	65.8
	SB-M	0.3	2.9	2.8	2.9	3.5	0.6	87.1
Hemicellulose	SB-P	1.5	4.2	23.7	11.2	15.0	9.7	34.7
	SB-M	1.1	0.8	23.6	7.7	31.6	8.7	26.4
ECP	SB-P	0.5	–[a]	26.1	34.2	11.3	5.4	22.9
	SB-M	3.8	–	24.2	45.1	18.3	8.7	2.3

[a] –, Too low to measure.

Table III. *Label incorporation into SB-P cells and their cell walls during a 30-min pulse with 1% $^{14}CO_2$ and following a 96-h chase with 5% CO_2 in light or darkness on a per flask basis*

Results are percentages of time 0 ^{14}C remaining in the cells with percentages of total cell ^{14}C in the cell wall in parentheses.

Days after Inoculation	Time 0	After 96-h Chase	
		Light	Dark
3	100 (25.0)	68.0 (81.8)	42.0 (46.2)
16	100 (28.3)	65.0 (80.0)	39.0 (66.7)

If the chase period occurred in the dark, most cell-wall fractions had lower ^{14}C levels than in the light (Fig. 3). The decreased accumulation in the 10- and 16-d cells could apparently have been due to the loss of the source of energy and carbon leading to decreased growth; therefore, less carbon would have been used for cell-wall synthesis.

When SB-M (mixotrophic growth in 1% Suc) cells were used in the $^{14}CO_2$ pulse-chase experiments, the hemicellulose fraction was most highly labeled after the pulse (Table IV). During the chase, the ^{14}C of the pectin and hemicellulose fractions decreased as a proportion of the cell wall total, whereas the acetic-nitric glucan and cellulose fractions increased. These changes were greatly decreased in 16-d cells, however. The differences in labeling pattern in cells incubated in the light or dark during the chase were very small with SB-M cells. This was probably due to the presence of 1% Suc in the medium, which could have served as a carbon and energy source. However, the results obtained during the chase in the light were very similar to those found with the photoautotrophic SB-P cells, in which the changes were greater in the 3- and 10-d cells (compare the data of Table IV and Figs. 2 and 3).

Starch Labeling

When the amount of ^{14}C found in the starch fraction was measured in the pulse-chase experiments, an amount equal to 60% of the total cell-wall radioactivity of the 16-d SB-P cells was in starch immediately after the pulse, whereas the amounts were 39 and 17% in 10- and 3-d cells, respectively (Table V). After the 96-h chase, the ^{14}C in starch decreased to very low levels in both light and dark conditions in the SB-P cells.

There was even higher incorporation of ^{14}C into the starch fraction of SB-M cells after the 30-min pulse, with amounts even greater than the total that was incorporated into the cell wall in 16-d cells (Table V). This high incorporation was most probably due to the presence of the additional carbon source in the medium, which could

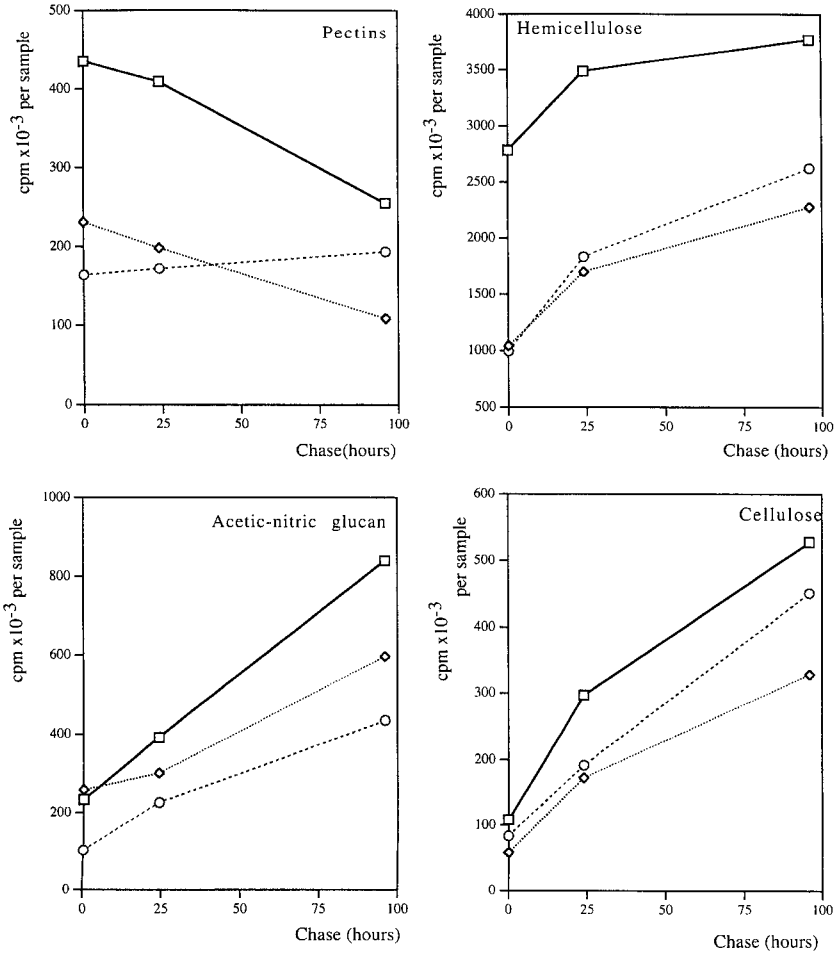

Figure 2. Amount of ^{14}C in cell-wall fractions per flask of SB-P cells following a 30-min pulse with $^{14}CO_2$ (time 0) and then chased in a 5% CO_2 atmosphere in the light. SB-P cells were used 3, 10, and 16 d after inoculation. □, 3 d; ◇, 10 d; ○, 16 d.

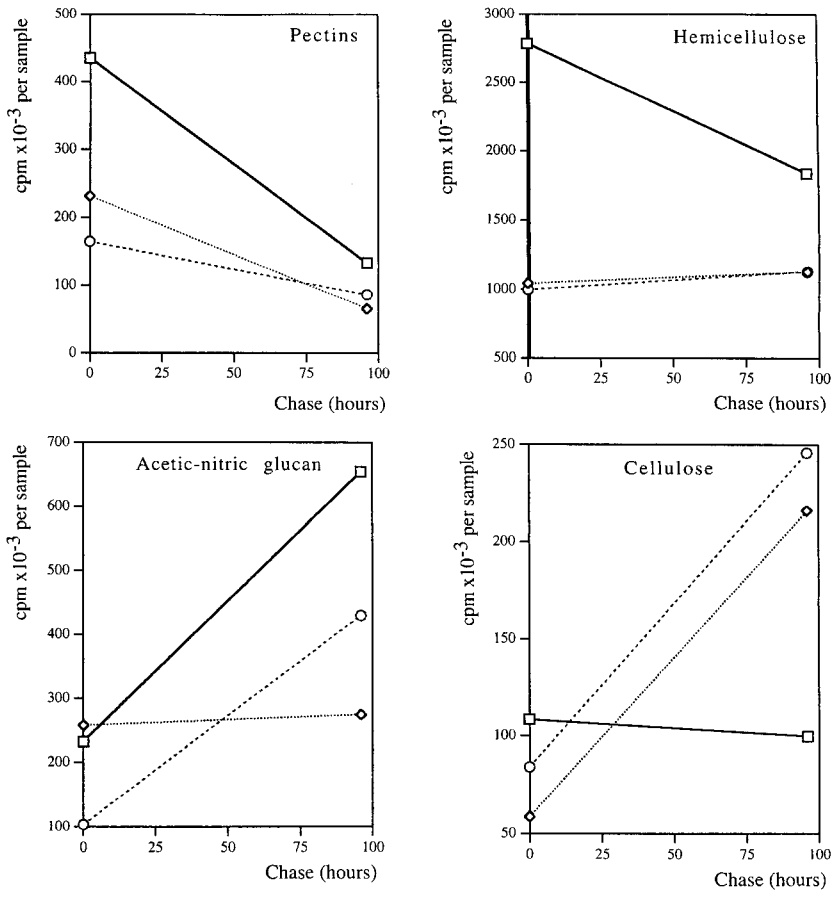

Figure 3. Amount of ^{14}C in cell-wall fractions per flask of SB-P cells following a 30-min pulse with $^{14}CO_2$ (time 0) and then chased in a 5% CO_2 atmosphere in the darkness. SB-P cells were used 3, 10, and 16 d after inoculation. □, 3 d; ◇, 10 d; ○, 16 d.

have resulted in an increased level of endogenous sugars in cells and/or chloroplasts, which could have stimulated carbohydrate synthesis and/or decreased degradation. As with the SB-P cells, however, the label was lost from the SB-M cell starch fraction during the chase, except in the case of the 16-d cells, in which an amount equal to 29% of the ^{14}C found in the cell wall at this time remained. We found that the α-amylase-extractable material accounted for only about 1% of the SB-P cell dry weight and about 2% of the SB-M cell dry weight of 10-d-old cells, whereas in soybean leaves this was 6 to 8%. Similar data were reported previously for SB-P cells (Martin et al., 1984) and for soybean leaves (Martin et al., 1984; Carpita and Kanabus, 1987). Comparison of the data concerning label incorporation into the starch fraction and the actual starch content indicates that there was rapid turnover of starch in the SB-P and SB-M cells. Starch content of heterotrophically grown cells was much higher (5–14% of the cell dry weight, Carpita et al., 1985; Carpita and Kanabus, 1987), which also shows that there was a significant difference in carbohydrate metabolism between heterotrophic and photoautotrophic and mixotrophic cultured cells.

[^{14}C]Glc Pulse-Chase Experiment

We also compared label incorporation from [^{14}C]Glc into the cell-wall polysaccharide fractions from SB-M (5 d) cells when [^{14}C]Glc was continuously present in the medium during 24 h of incubation and when [^{14}C]Glc was pulsed for 30 min and the cells were then incubated in culture medium with 1% Suc (Fig. 4). Radioactivity in pectins and hemicelluloses increased as a function of time somewhat more when the first approach (continuous labeling) was used, whereas the increase of cellulose (KOH-insoluble material) radioactivity was about the same. After the 24-h chase, the ^{14}C in pectin decreased from the 4-h level, which again indicates turnover of the pectin fraction. The ^{14}C in the hemicellulose fraction leveled off but did not decline during the chase; therefore, turnover was not clearly evident.

DISCUSSION

These photosynthetic suspension cells can be proposed as an additional or alternative experimental system to heterotrophic suspension-cultured cells for studies of cell-wall turnover. The use of $^{14}CO_2$ as a substrate allows one to avoid the possible alteration of cell carbohydrate metabolism by exogenous sugars that would serve as radiolabeled substrates in experiments with heterotrophic cultures.

Studies have shown that the nature of the medium in which heterotrophically cultured cells are grown markedly affects the cell-wall polysaccharide composition (Nevins et al., 1967; Asamizu et al., 1983; Iraki et al., 1989b, 1989c; Shedletzky et al., 1992, among others). For example, when

Table IV. *Effect of culture age and light on the ^{14}C cell-wall polysaccharide proportion in SB-M during a 96-h chase in ambient CO_2 with 1% Suc in the medium following a 30-min pulse of $^{14}CO_2$ (1%) in the light*

Days after Inoculation	Light Condition during the Chase	Time 0				96-h Chase			
		Pectin	Hemicellulose	Acetic-nitric glucan	Cellulose	Pectin	Hemicellulose	Acetic-nitric glucan	Cellulose
		cpm × 10^{-3} in sample (% of cell-wall polysaccharide radioactivity)							
3	Light	158 ± 13 (10.3)	1057 ± 53 (69.1)	217 ± 27 (14.2)	98 ± 5 (6.4)	101 ± 6 (3.4)	1792 ± 329 (60.5)	560 ± 13 (18.9)	510 ± 59 (17.2)
	Dark					61 ± 8 (3.1)	1164 ± 219 (59.2)	380 ± 76 (19.3)	362 ± 39 (18.4)
10	Light	102 ± 12 (7.0)	917 ± 56 (62.8)	324 ± 45 (22.2)	137 ± 25 (9.4)	63 ± 4 (1.7)	1851 ± 173 (50.6)	1187 ± 8 (32.2)	940 ± 60 (25.5)
	Dark					54 ± 3 (3.2)	818 ± 40 (48.4)	535 ± 47 (31.7)	280 ± 60 (16.6)
16	Light	260 ± 11 (16.2)	944 ± 65 (58.8)	273 ± 37 (17.1)	129 ± 9 (8.0)	277 ± 27 (8.6)	1885 ± 115 (58.6)	637 ± 37 (19.8)	415 ± 25 (12.9)
	Dark					55 ± 10 (3.4)	969 ± 70 (59.5)	391 ± 34 (24.0)	213 ± 14 (13.1)

sycamore cell-suspension cultures were grown with a variety of sugars, the quantity of both total cell wall and sugar composition varied greatly (Nevins et al., 1967). Gal feeding led to high levels of Gal in the walls, whereas Ara was clearly the dominant sugar with the other carbon sources. The relative Glc concentration was increased in maltose- and Glc-containing media.

Thus, since photoautotrophic cells are able to grow in minimal medium with CO_2 as a sole carbon source, they offer a convenient model to study cell-wall metabolism related to development and adaptation to different environmental conditions.

Our results clearly show that counts are lost from the soybean cell-wall pectin and hemicellulose fractions in many cases during the chase period. Cell-wall polysaccharide turnover during the growth cycle has also been studied in a *V. rosea* heterotrophic suspension culture with the pulse-chase approach by Takeuchi and Komamine (1980a, 1980b) and Takeuchi et al. (1980) using [^{14}C]Glc (50 mM). In this case, the cell wall did not accumulate much label during the 36-h chase, and the radioactivity of pectins and cellulose remained almost constant. However, the KOH-extractable hemicelluloses showed a marked decline in radioactivity during the chase in both 3-d and 5-d cultured cells. Radioactivity in ECP (the soluble polymers secreted by cell-suspension cultures) increased up to 36 h during incubation with unlabeled Glc. These heterotrophic suspension cells show label distribution during the chase that is quite different from that observed in our photoautotrophic system.

For the pulse-chase experiments, a 5% CO_2 chase was used for the SB-P cells and an ambient CO_2 chase was used for the SB-M cells, since these levels of CO_2 reflect the conditions under which the cells are normally grown. It is possible, however, that the culture of cells under different CO_2 levels might alter photosynthetic performance. We showed previously (Lozovaya and Widholm, 1995) that an increase in the $^{14}CO_2$ concentration in the system during the pulse led to an increase in the rate of cell-wall and starch formation. The increase in the cell-wall polysaccharide level seems to be possible when cells are saturated with assimilates, since excess amounts of osmotically active substrates (sugars) are withdrawn and cell walls capable of resisting increased turgor pressure are produced by the increased synthesis. The possibility of starch accumulation might be limited because it can lead to the mechanical injury of chloroplasts. The effects of different CO_2 levels on the metabolism during the chase have not been determined, however.

The decrease of wall matrix radioactivity we observed could be partially connected with liberation of some polysaccharides into the culture medium that were not tightly bound to the cell wall, as shown with heterotrophic cell-suspension cultures (Fry, 1988). Pectin polysaccharides are found to be sloughed into the medium of cultured cells in response to growth-promoting treatments with GA_3 (Fry, 1980; Morvan, 1982). Postphotosynthetic changes of matrix polysaccharide radioactivity in our experiments could also be due to the cell-wall reorganization during development, turnover, and modification of specific polymers after their deposition into the cell wall. According to a popular hypothesis of wall extension in growing cells, enzymatic loosening of the wall takes place in conjunction with matrix polysaccharide turnover (Fry, 1988). In dicots, xyloglucan cleavage is considered to be involved in wall loosening of growing cells (Fry et al., 1992). Marked differences in the chemical structure and organization of pectins were found as a result of the adaptation of tobacco cells to NaCl (Iraki et al., 1989b, 1989c). It was also shown in this study that much of the pectin in the adapted cells is retained by the wall throughout the culture period, whereas in unadapted cells it is released into the medium. Pectin and hemicellulose breakdown may also result in the formation of oligosaccharins (Albersheim and Darvill, 1985; Aldington and Fry, 1993) or monosaccharides that could be reutilized. In all of these cases, the pectin and hemicellulose fraction polymers would be degraded and released into the medium.

Table V. *The distribution of ^{14}C in starch during pulse-chase experiments with SB-P and SB-M cells as a percentage of that in the cell wall*

The SB-P data were reported previously (Lozovaya and Widholm, 1995).

Cell Line	Days after Inoculation	Chase Time and Light Conditions		
		Time 0	96 h of light	96 h of dark
		% of cell-wall radioactivity at the time		
SB-P	3	16.7	2.6	1.3
SB-P	10	39.2	6.2	1.4
SB-P	16	60.5	4.2	1.3
SB-M	3	35.6	3.9	1.2
SB-M	10	92.0	3.5	3.8
SB-M	16	113.9	29.4	2.8

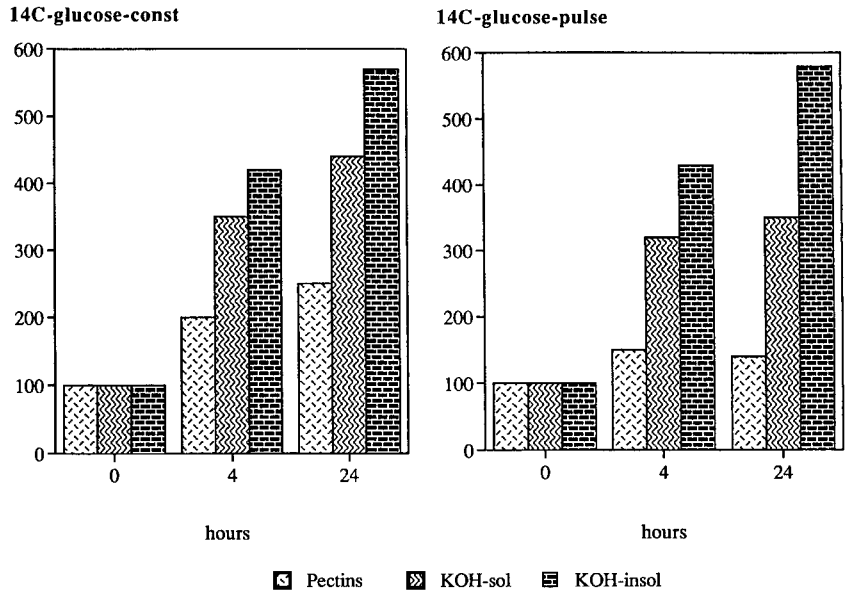

Figure 4. Incorporation of ^{14}C into the cell-wall polysaccharides of SB-M cells incubated for 30 min with [^{14}C]Glc (time 0) and then continuously with [^{14}C]Glc (left) or chased in fresh KN1 medium with 1% Suc after rinsing (right). The time 0 ^{14}C content after the pulse is set to 100% for each fraction.

We observed a gradual increase in the culture medium radioactivity (up to 5–11% of the cell ^{14}C content) during the 96-h chase in our experiments (Lozovaya and Widholm, 1995), which supports the possibility of enzymic degradation of the matrix polysaccharides. As a whole, the constituents of the ECP secreted by SB-P cells (Table II) were similar to those reported for other suspension cultures, with the high proportion of Ara, Gal, and uronic acid (Takeuchi and Komamine, 1980b; Blaschek and Franz, 1983; Fry, 1988). The uronic acid content of the SB-P and SB-M cell ECP was lower than that of the cell-wall pectin and hemicellulose fractions, indicating that the turnover noted in these fractions was not due to direct release of typical components. However, the recycling of sugars cannot be excluded, since hydrolytic enzymes, including exopolygalacturonase (Konno et al., 1989), accumulate in the medium during growth.

The role of individual components and their precursors in cell-wall turnover is now under investigation using GLC to determine the proportion of monosaccharide radioactivity and mass in the wall matrix polysaccharide fractions of photoautotrophic cells pulsed with $^{14}CO_2$ after different chase periods. As a result, changes in cell-wall composition may be found that can be correlated with certain events of the cell growth cycle. The cause of the alterations, such as changes in specific hydrolytic enzyme activities and other mechanisms that might affect these components, will also be studied.

The cell-wall-labeling results from photosynthetically fixed ^{14}C-labeled precursors in our pulse-chase experiments with photoautotrophic soybean cells has shown that (a) the bulk of the photosynthates are incorporated into cell-wall polysaccharides throughout the cell growth cycle of the photoautotrophic cells; (b) the rate of biosynthesis and breakdown of cell-wall polysaccharides is the highest in young cells; (c) the pectin and alkali-soluble hemicellulose breakdown rates are highest during the cell division phase; and (d) the incorporation of $^{14}CO_2$ into starch during the $^{14}CO_2$ pulse is much higher in stationary phase cells than in growing cells, but the starch is predominately metabolized during a 96-h chase, so it is apparently a transient storage form.

The data obtained show that these photoautotrophic suspension cultures can be a convenient new model to study some largely unknown aspects of plant physiology and biochemistry, such as the fate of photosynthates in post-photosynthetic synthesis and the degradation and modification of cell-wall polymers under normal and modified environmental conditions (including stress and pathogenesis). This system should also allow the study of the relationship between photosynthesis and cell growth in regard to cell-wall component accumulation, which could lead to strategies for increasing growth and biomass production.

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