Metabolism of myo-Inositol and Growth in Various Sugars of Suspension-cultured Tobacco Cells

S. Harran* and D.B. Dickinson
Departments of Botany and Horticulture, University of Illinois, Urbana, IL 61801, USA

Abstract. Tobacco (Nicotiana tabacum L.) cells were cultured in a liquid medium which contained sucrose as a source of carbon and energy. Various cell-wall constituents and wall precursors (L-arabinose, D-xylose, D-galactose, D-mannose, D-glucuronate, myo-inositol) were added to cells growing in this medium to by-pass possible rate-limiting steps in the relevant metabolic pathways. None of these compounds stimulated growth as measured by increase in fresh weight; myo-inositol did cause a slight increase and L-arabinose a decrease in dry weight accumulation compared to controls grown on sucrose only. Although myo-inositol was not needed for rapid growth, tracer level amounts of \([2-^3H]\)myo-inositol were rapidly absorbed and metabolized. Label was incorporated into the uronide and pentose residues of cell walls and exocellular polysaccharide.

Key words: Cell wall — myo-Inositol — Nicotiana — Polysaccharides (cell wall) — Tissue culture.

Introduction

Cultured tobacco cells are widely used for studies of growth and metabolism. There is recent information on the sugar composition of the cell walls of suspension-cultured tobacco cells and the exocellular polysaccharides produced by these cells (Halmer and Thorpe, 1976; Kato and Noguchi, 1976; Kato et al., 1977). Exocellular polymeric substances are commonly produced by cultured plant cells. Such exocellular materials resemble primary cell walls in their chemical composition and polysaccharide structure (Albersheim et al., 1973; Becker et al., 1964; Moore, 1973). Hence, these substances are thought to be a portion of the cell wall that has escaped into the medium.

The biosynthetic pathways which lead to the cell-wall and exocellular polysaccharides of tobacco cells are not well understood. In particular, it is not clear whether the inositol oxidation pathway (Loewus, 1974; Loewus et al., 1973) is operative in cultured tobacco cells the walls and exocellular polysaccharides of which are rich in pentoses and uronides. Cell-wall pentoses and uronides apparently do arise via the inositol oxidation pathway in cultured cells of Acer (Roberts and Loewus, 1966; Verma et al., 1976) but not of Fraxinus (Jung et al., 1972).

The growth-limiting steps in the pathways leading to wall polysaccharides of cultured callus cells are also not well understood. Growth of a tobacco and an ash cell line on sucrose medium was stimulated by inositol (Jung et al., 1972; Linsmaier and Skoog, 1964). Other tobacco cell lines were grown without added myo-inositol (Filner, 1965; Kato and Noguchi, 1976). Cell-wall hexoses, pentoses and uronides are normally produced from glucose. Several of these wall constituents or their precursors can be incorporated into wall polysaccharides (Roberts et al., 1968, 1971; Roberts and Loewus, 1966) even though not all of the compounds can support growth in the absence of sucrose (Nevins et al., 1967; Nickell and Maretzki, 1970; Smith and Stone, 1973; Verma and Dougall, 1977).

In the present research, tobacco callus was grown on a sucrose-containing medium. Selected cell-wall precursors (myo-inositol, D-glucuronic acid) and various cell-wall sugars were added to determine whether any growth-limiting steps could be by-passed by these compounds. The metabolism of labeled myo-inositol was also studied.
Material and Methods

Plant Material

Suspension-cultured cells of tobacco (Nicotiana tabacum L., cv. Xanthi) were used. The callus culture was established from petiole tissue which was cultured on agar medium for 2 months and then transferred to a liquid medium 2 months before beginning the experiments reported below.

The culture medium of Murashige and Skoog (1962) was used except that kinetin was omitted and 2,4-dichlorophenoxyacetic acid (0.4 mg/ml; Widholm, 1977) was substituted for indoleacetic acid. The FeEDTA solution was prepared according to Jacobson (1951). The cultures were easily pipetted and contained no large clumps of cells. Small clumps consisted of cells connected in chains. The absence of bacterial contamination was verified by plating portions of the tobacco cell suspension on nutrient agar. No dead tobacco cells were observed when drops of culture were stained with phenosafranin and several hundred cells inspected under a microscope.

Reagents

Glass-distilled water was used for preparing all reagents and plant growth media. The nutrient agar used for checking on bacterial contamination and the bacto-agar used in the plant growth medium were from the Difco Labs. (Detroit, Mich., USA). The [2-3H]myo-inositol was from New England Nuclear (Boston, Mass., USA) (17.4 Ci/mmol; lot No. 938-017), and its radiochemical purity was checked with paper chromatography. After descending development with 88% formic acid:2-butanol:95% ethanol:water (15:30:40:15, v/v), there was a single radioactive peak which corresponded to authentic myo-inositol and contained 98+% of the radioactivity placed on the origin. Other chemicals were reagent grade or else the best quality available commercially.

Studies of Cell Growth

Suspension-cultured cells in the log phase of growth were transferred into replicate Erlenmeyer flasks which contained the 9 ml medium, 1 ml stock sugar solution, and 0.5 ml cells (Table 1). These sugars and glucuronate were added to the sterile flasks in a 60°C forced-air oven.

The cells were routinely subcultured every 7-10 d and were grown at 28°C and 150 rpm in an incubator-shaker (Model G-24; New Brunswick Co., New Brunswick, N.J., USA). The cultures were transferred to a liquid medium 2 months before beginning the experiments reported below. The culture medium of Murashige and Skoog (1962) was used except that kinetin was omitted and 2,4-dichlorophenoxyacetic acid (0.4 mg/ml; Widholm, 1977) was substituted for indoleacetic acid. The FeEDTA solution was prepared according to Jacobson (1951). The cells were routinely subcultured every 7-10 d and were grown at 28°C and 150 rpm in an incubator-shaker (Model G-24; New Brunswick Co., New Brunswick, N.J., USA). The cultures were transferred to a liquid medium 2 months before beginning the experiments reported below.

The cells were homogenized, the fluid was clarified by centrifugation (10 min at 15,000 x g) and the pellet was rinsed 5 times with 5-ml portions of 80% ethanol.

Lipids were extracted by homogenizing each pellet in 1.9 ml of methanol: chloroform: H2O, 2:1:0.8, v/v (Bligh and Dyer, 1959), clarifying the fluid by centrifugation (10 min, 15,000 x g) and rinsing the pellet with four additional 1.9 ml portions of the same solution. Enough chloroform and water were added to the combined extracts to give a final ratio of 2:1.2 methanol:chloroform: H2O. Complete separation of the chloroform and methanol phases was ensured by 5 min centrifugation at ca. 900 x g.

Exocellular polysaccharides were recovered from the radioactive culture medium. The medium from each sample was made up to 35 ml and filtered through Whatman No. 1 paper. Then 95% ethanol was added to a 15-ml portion of the filtrate to give a final concentration of 80% ethanol. The samples were stored at 3°C overnight and the precipitate collected by centrifugation. The pellet was rinsed 5 times with 5-ml portions of 80% ethanol and 4 times with 1.9 ml portions of methanol: chloroform: H2O (2:1:0.8).

The polysaccharides in the pellets were hydrolyzed using methods adapted from other workers (Jones and Albersheim, 1962; Roberts and Loewus, 1966). The entire pellet that contained exocellular polysaccharide and 10 mg of the cell-wall pellet (representing about one-third of the total wall pellet) were subjected to acid hydrolysis with 0.2 N trifluoroacetic acid for 1 h at 121°C. The samples were dried and then treated for 24 h at 37°C with 0.2% (w/v) pectinase (Pectinol R-10; Rohm and Haas, Philadelphia, Pa., USA) which contained 0.1% (w/v) Na2EDTA. Materials having a low molecular weight were extracted with 80% ethanol and fractionated by descending chromatography on Whatman No. 1 paper. The solvent (designated A) was ethyl acetate:pyridine: H2O (17.4:30:40:15, v/v). The polysaccharide materials were developed on 5.1-cm tracks with standards placed on both sides of each track. After development, the radioactive tracks were cut out and analyzed as described below. The standards were visualized with silver nitrate (Hais and Macek, 1963). The same solvent was used in earlier studies of cell-wall sugars (Roberts and Loewus, 1966). Preliminary experiments indicated that this solvent could satisfactorily separate the three major classes of cell-wall carbohydrates (pentose, hexose, uronic acids).

Small residues remained after extraction of the hydrolysates with ethanol. These residues were transferred to filter paper and saved for determination of radioactivity as described below.

Measurement of Radioactivity

Paper chromatograms were cut into 1-cm segments. These segments and the insoluble radioactive fractions were burned in an oxidizer (model 306; Packard Instrument Co., Downers Grove, Ill., USA) which dispensed the labeled sample mixed with scintillation fluid (Monophase 40; Packard Co.). Other radioactive extracts were placed directly in scintillation bottles, and all were counted at about 25% efficiency in a Packard Model 3375 scintillation counter equipped with external standardization.