A CONVENIENT METHOD FOR PRODUCTION OF ^{11}C-LABELLED GLUCOSE

E. Ehrin, E. Westman, S.-O. Nilsson and J.L.G. Nilsson[x],
Karolinska Pharmacy, Fack, S-104 01 Stockholm 60, Sweden,
L. Widén and T. Greitz, Departments of Clinical Neurophysiology and Neuroradiology, Karolinska Hospital, S-104 01 Stockholm 60, Sweden,
C.-M. Larsson and J.-E. Tillberg, Institute of Botany, University of Stockholm, S-106 91 Stockholm, Sweden and
P. Malmborg, Department of Physical Biology, Gustaf Werner Institute, University of Uppsala, Box 531, S-751 21 Uppsala, Sweden

SUMMARY

A convenient photosynthetic method for production of ^{11}C-labelled glucose has been designed using the green alga Scenedesmus obtusiusculus Chod. The organism converts $H^{11}CO_3^-$ to glucose in 50-70% yield. The total time needed for the synthesis and isolation of the glucose is 25-30 minutes.

Key Words: ^{11}C-glucose, photosynthesis, algae

INTRODUCTION

Glucose labelled with carbon-14 has been produced by photosynthesis for many years. The positron-emitting isotope carbon-11 has also been used for labelling glucose by Lifton and Welch[1]. A modified procedure was later published by Goulding and Palmer[2]. Both these methods used

---

[x] To whom correspondence should be addressed.

0362-4803/80/0317-0453$01.00

Received May 23, 1979

leaves from Swiss chard or Broad bean which were illuminated in an athmosphere containing $^{11}CO_2$, whereupon the sugars formed were extracted and purified. The photosynthesis using $^{11}CO_2$ has recently been extensively studied by Fares et al.[3].

Carbon-11 labelled glucose was of interest to us for determination of local glucose metabolism in the brain using the positron camera[4] which recently has been installed at the Karolinska Hospital in Stockholm. For the photosynthesis of ^{11}C-glucose we decided to use green algae instead of higher plants since the algae have several advantages. This has allowed us to design a convenient and rapid method for production of ^{11}C-labelled glucose.

MATERIALS, METHODS AND RESULTS

Radionuclide production

$^{11}CO_2$ was produced at the Tandem Accelerator Laboratory, Uppsala, using the gas target system developed by the radionuclide production group of the Gustaf Werner Institute [$^{14}N(p,\alpha)^{11}C$ reaction; 10 - 12 MeV protons]. A stream of nitrogen was passed through the gas target at a rate of approximately 1 ml/sec. The gas was then bubbled through 10 ml of aqueous KOH 0.1 M which efficiently trapped the radioactive CO_2.

Cultivation of the algae

Synchronized cultures of the uni-cellular green alga Scenedesmus obtusiusculus Chod. were grown as described by Kylin et al.[5]. The cultures were kept at 30°C and maintained at a growth cycle of 15 h of light and 9 h of darkness. Air containing 2,5% CO_2 was continously bubbled through the cultures at a flow rate of 10 l/h and flash. Rapid growth takes place during the light period and cell division occurs during the dark period.

The cells were harvested after 10 h of illumination which was immediately before the onset of starch formation in the synchronized culture[6]. It can therefore be assumed that a rapid glucose formation then took place in the organisms.

Photosynthesis

The cells were centrifuged, washed with distilled water and resuspended in a buffer of morpholinoethanesulphonic acid (20 mM), $CaCl_2$(0,1 mM) and $MgCl_2$(1 mM) adjusted to pH 5,5 using KOH 1 M to give a cell density of approximately 10^8 cells/ml. The cell suspension (approximately 35 ml was

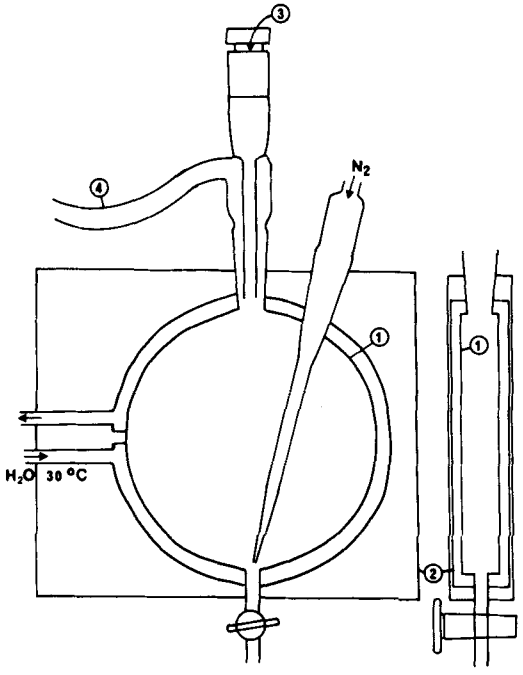

Figure 1. Temperature controlled reaction vessel for photosynthesis.
1. Glass reaction vessel. 2. Lucite shield for temperature control.
3. Introduction of $H^{11}CO_3^-$ solution. 4. Soda lime.

transferred to the temperature controlled reaction vessel (Fig. 1) where it was illuminated for about 45 min. while a stream of N_2 was passed through the suspension. During this period intracellular pools of reducible substances were consumed so that minimal substrate competition occurred when the labelled HCO_3^- -solution was added. The temperature was kept at 30°C and light was given from a 250 W Osram HWL-lamp at 25 cm distance, giving 15 000 to 20 000 lux at the surface of the reaction vessel.

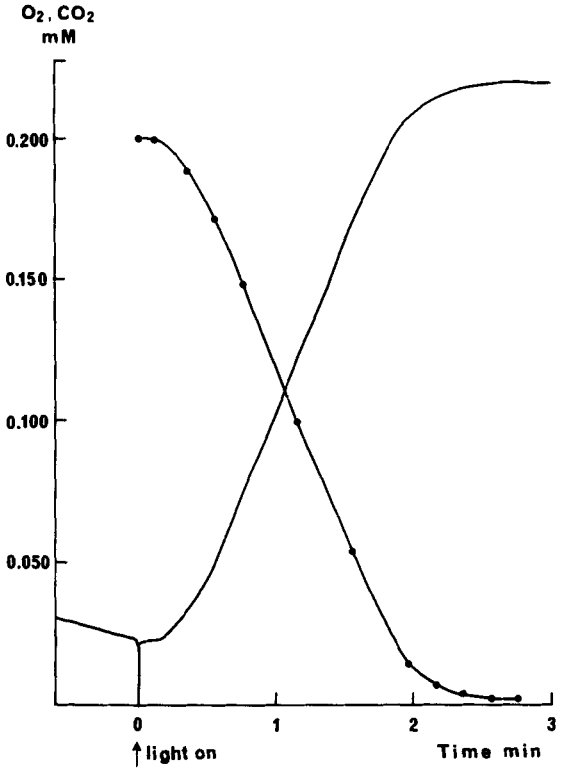

Figure 2. Time-course of photosynthesis in Scenedesmus. The figure shows the polarographic recording of O_2 evolution (———) and the calculated HCO_3^- consumption (●———●). The algae were sampled after 45 min preillumination in the reaction vessel and immediately transferred to the oxygen electrode chamber. Conditions otherwise as stated in the text.

The photosynthetic reactions were started by adding the alkaline solution of $H^{11}CO_3^-$ and unlabelled $KHCO_3$ to a final concentration of 0.2 mM. The suspension was then illuminated for another 3 min. Experiments with $H^{14}CO_3^-$ showed that this short time of photosynthesis gave a maximum yield of radioactive glucose. This observation was confirmed in an experiment where the photosynthetic evolution of O_2 was monitored (Fig. 2). The measurement was performed in a 3 ml temperature-controlled cuvette equipped with a Clarke-type oxygen electrode. The O_2 evolution ceased after about 2.5 min when almost all HCO_3^- added was consumed (the stochiometric relation between O_2 evolution and HCO_3^- consumption is 1:1). The algae showed a high rate of photosynthesis, about 300 /umoles CO_2 x (mg chlorophyll x h)$^{-1}$. Thus, with this technique a very rapid and almost complete incorporation of HCO_3^- into carbohydrates was achieved.

Isolation of glucose

After the illumination period, the cells were centrifuged and the supernatant discarded. Hydrochloric acid 1.5 M (4 ml) was added and the suspension was boiled for 4 min. in the centrifuge tube, equipped with a soda lime trap to collect any radioactivity that might be released in this step. After cooling, chloroform (10 ml) was added and the tube was stoppered and shaken vigorously for one minute using a mechanical shaker. The mixture was then passed through a glass sinter filter (G 3) into a separatory funnel and the filter was washed with water. Traces of chloroform were removed by boiling the water extract for 1 min. while a stream of N_2 was passed through. The acidic solution was then poured into a 20 ml syringe equipped with a Millipore filter (0,22 /um) and containing a basic ion exchange resin (Amberlite IRA-47) to neutralize the solution. The solution (5-7 ml) was then filtered into a sterile injection vial and the ion exchange resin was finally washed with 2 ml of water which also was filtered into the vial.

Radiochemical analysis

Due to the short half-life of ^{11}C (20,3 min.) radiochemical analysis of the product presents a problem. The analysis was therefore carried out using ^{14}C instead of ^{11}C-labelled material. Otherwise, the experiments were identical.

Table 1 Thin layer chromatography on silica gel or cellulose[a].

	R_F-values		
Sugars	System 1[a]	System 2[a]	System 3[a]
Glucose-6-phosphate	0.11	0.7	0
Raffinose	b	b	0.1
Saccharose	b	b	0.28
Lactose	0.12	0.43	0.13
Galactose	0.29	0.54	0.30
Glucose	0.30	0.56	0.39
Sorbose	0.32	0.63	0.49
Mannose	0.34	0.59	0.45
Fructose	0.36	0.61	0.42
Xylose	0.44	0.68	0.64
Rhamnose	0.58	0.77	0.75
Glyceraldehyde	0.60[c]	0.83	

a) Chromatography systems and spray reagents according to Stahl[7].
System 1: Cellulose plate developed in formic acid:ethylmethylketone: t-butanol:water 25:30:30:15. Sprayed with aniline phthalate.
System 2: Cellulose plate developed in ethylacetate:pyridine:water 2:1:1. Sprayed with aniline
System 3: Silica gel G buffered with sodium acetate 0.02 M developed in acetone:water 9:1. Sprayed with aniline:diphenylamine:phosphoric acid.
b) Not visible when the plates were sprayed with aniline
c) Tailing

The analysis was performed using thin layer chromatography as shown in Table 1. The radioactive glucose solution was chromatographed with glucose as carrier as described[8]. Scanning of the thin layer plate using a LB 2723 Berthold Dünnschicht-scanner showed only one peak corresponding to the glucose spot. Glucose and fructose were analyzed separately[9,10] yielding 75 µg/ml of glucose and 33 µg/ml of fructose in the sterile solution.

DISCUSSION

The use of algae instead of higher plants for ^{11}C-glucose production has several advantages:

1. The algae can easily and rapidly be cultivated in the laboratory in large quantities.
2. The cells can be synchronized and thus have very reproducible properties from one experiment to another.
3. The organism is aquatic and the labelled carbon dioxide can be supplied as a bicarbonate solution. This is of importance if the radiochemistry cannot be performed adjacent to the $^{11}CO_2$-producing cyclotron.
4. Incorporation of radioactive material is rapid and the radiochemical yields are high.

The cultivation of the algae and the photosynthesis were performed at conditions that have been optimized in other experiments[5]. The subsequent isolation of the glucose is analogous to the methods previously used[1,2], with some modification. After the illumination and centrifugation of the algae 50-80% of the added radioactivity was found in the cells. The cells were then directly boiled with aqueous HCl thus omitting the ethanol extraction that has previously been used. This was found to give a better yield of the final product and it also shortens the work-up

procedure. As shown by the analysis of the product, the saccharose and sugar phosphates that might have been present in the cells were also hydrolyzed by this treatment.

The acid treatment dissolved all the radioactivity present in the algae and less than 1% could be detected in the cell debris after acid treatment and chloroform extraction. Chloroform was found to be advantageous over several other solvents tested for the extraction. It removed all coloured material from the acid solution accompanied by only 1-2% of the radioactivity.

After removal of the chloroform, the acidic aqueous solution was neutralized using a basic ion exchange resin instead of adding base in order to keep the salt content of the solution low.

In the analysis of the sample, the radioactive material was co-chromatographed with glucose as a carrier and compared with 12 different sugars or sugar derivatives.

The chromatographic systems used do not differentiate between glucose, fructose and mannose. For that reason the final solution was analyzed for glucose and fructose[9,10] and these two sugars were found to be present in a ratio of approximately 2:1. Mannose has not been reported to be present in any substantial amount in the alga used. Consequently, the radioactive compounds of the solution are most likely only glucose and fructose.

The complete procedure from the introduction of the radioactivity into the algae suspension to the final sterile injection solution was carried out in 25-30 min. The practical yield of radioactive sugar was 50-70% based on the amount of $H^{11}CO_3^-$ introduced.

REFERENCES

1. Lifton J.F. and Welch M.J. - Radiat. Res. 45:35 (1971).

2. Goulding R.W. and Palmer A.J. - Int. J. Appl. Radiat. Isot. 24:7 (1973).

3. Fares Y., De Michele D.W., Goeschl J.D. and Baltaskonis D.A. - Int. J. Appl. Radiat. Isot. 29:431 (1978).

4. Bohm C., Eriksson L., Bergström M., Litton J., Sundman R. and Singh M. I.E.E.E. Trans. Ns 25:624 (1978).

5. Kylin A., Sundberg I. and Tillberg J.E. Physiol. Plant. 27:376 (1972).

6. Sundberg I. and Nilshammar-Holmvall M.Z. Pflanzenphysiol. 76:270 (1975).

7. Stahl E. Dünnschichts-Chromatographie, Springer-Verlag, Berlin 1967.

8. Sheppard G. The radiochromatography of labelled compounds. Review 14. The Radiochemical Centre, Amersham Engl. p. 12-13.

9. Hawk P.B., Oser B.L. and Summerson W.H. Practical physiological Chemistry 13th ed., Blakiston, New York, 1954.

10. Hjelm M. Scand. J. Clin. Lab. Invest. 18, Suppl. 92:85 (1966).