BIOSYNTHESIS OF ^{14}C -LABELLED ADENINE AND GUANINE BY CHLORELLA ELLIPSOIDAE

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In the preceding paper (1), the author reported on the separation of uniformly ^{14}C -labelled amino acids from *Chlorella ellipsoidae* cultivated with ^{14}C -labelled carbon dioxide as the sole carbon source. The present paper describes the preparation of ^{14}C -adenine and guanine from the same source. The nucleic acids fraction (the radioactivity of which an equivalent to 15 % of Chlorella cell) was hydrolysed with acid and then the resulted bases were separated columnchromatography by ion exchange resin. The acid hydrolyzates were adsorbed on Dowex-1 (used as formate form) and eluted by formic acid, followed by formic acid-ammonium formate solution of various concentrations (2). The vases in the initial eluate were further run in Dowex-50 (used as chloride form) and eluted with hydrochloric acid (3). The results of two experiments were as follows. From 63×10^5 c.p.m. (2.2 %) of guanine were obtained in one experiment. In the other experiment, 23.6×10^5 c.p.m. (14 %) of adenine and guanine was obtained from 170×10^5 c.p.m.

PROCEDURE

1. Materials

The nucleic acid fraction was obtained from chlorella cell suspension after a modification of the method of W.C. Schneider (Table 1).

2. Acid hydrolysis of nucleic acid fraction

The fraction was acidified with hydrochloric acid, the final concentration of the acid being one normal, extracted with ether in order to remove trichloroacetic acid contamination which entered in the preceding process, and heated to 100° for one hour. After hydrolysis, the solution was dried over sodium hydroxide in vacuo. The dried matter was dissolved in a small volume of water and the insoluble material (fumin etc.) was removed by centrifugation and washed with water. The supernatant (about 5-7 ml) was used in the following experiment.

3. Paper electrophoresis of the hydrolysates

One tenth ml of the above hydrolysates was placed on a filter paper strip (2 cm in width) and electrophoresed in a medium of 5 % acetic acid for

2 or 4 hours. The current used was 1 mA/cm. The localizations of the active matters were detected by radioautography, being undetectable by the adsorption of ultra violet light because of their small amounts. The radioactivities were observed on the original spot and in the fraction moved to anode. The acidic component was extracted and studied paper electrophoretically but not in detail (photograph 1).

Table 1. Fractionation of algae components

4. Separation by paper chromatography

The acid hydrolysates and the component moved to anode in the electrophoresis were separated by two-dimensional paper chromatography. The solvents used were isopropanol-hydrochloric acid (41 ml of concentrated hydrochloric acid is added to 70 ml of isopropanol and filled up with water to 250 ml) and ammoniacal butanol (5 % of 28 % aqueous ammonia was added to 86 % (vol/vol) aq. *n*-butanol (4). The spots corresponding to the *R_f* values of adenine, guanine and nucleotides were detected in the chromatogram. More-

over, an unidentified spot was detected near the front of iso-propanol-hydrochloric acid and several strong radioactive spots were detected near the starting point when ammoniacal butanol was used as solvent (photograph 2).

5. Separation by ion exchange column chromatography

It has been usually reported that the free purine bases are readily formed during acid hydrolysis of both types of nucleic acids, whereas the pyrimidine bases remain for the most part as mononucleotides in the case of PNA or as nucleotide diphosphates in the case of DNA. Accordingly, the author intended to separate roughly the bases from nucleotides followed by the

Fig. 1 A. Separation of the purine bases and nucleotides by anion exchange.

Exchanger: Dowex-1-($\times 8$) formate 200-400 mesh $7\text{ cm} \times 0.64\text{ cm}^2$
 Solution: Formic acid, ammonium formate as shown.
 Sorbed material: Acid hydrolysate of nucleic acid fraction (sample 1) adjusted to pH 8.0 with 3% NH_4OH .

fractionation of each component. The hydrolysates were neutralized to pH 8 with ammonia, adsorbed on Dowex-1 (formate form) and eluted with formic acid and formic-ammonium formate solution successively. As the first eluates with 0.02 N formic acid contained bases, it was again adsorbed on Dowex-50 (chloride form) and eluted successively with hydrochloric acid.

(a) Preparation of resin column and elution procedure

(1) Separation of nucleotides: Fifteen ml of Dowex-1 ($\times 8$, 200-400 mesh) was suspended in water, the fine powder was removed by decantation, put into a glass tube (diameter 0.9 cm) to 7.5 cm, height, washed with 3 N HCl, NaOH and formic acid successively and finally washed 1N formic acid until the absorption of 260-280 $\text{m}\mu$ of the influent and effluent solution were equal. Then the column was washed with water, and the hydrolyzates run in the column. The flow rate was 0.7-1.0 ml/min. and the effluent was collected every 1-4 ml. The eluting solutions were 0.02, 0.05 and 0.15 N formic acid

Fig. 1 B. Separation of purine bases by cation exchange in an acid system.

Exchanger: Dowex-50-H+200 mesh $0.64 \text{ cm}^2 \times 7.5 \text{ cm}$

Solution: 2N HCl 1 ml/min

Sorbed material: F5 dried matter in Fig. 1-B in 2 ml 2N-HCl

Fig. 2. Separation of the purine bases and nucleotides by anion Exchange.

Exchanger: Same as in Fig. 1-A

Solution: " "

Sorbed material: Acid hydrolysate of nucleic acid fraction (sample 2)

and mixture of 0.05 N ammonium formate and 0.01 N formic acid successively.

(2) Separation of bases: Dowex-50 ($\times 8$, 200 mesh) was put into glass tube (diameter: 0.9 ml) to 7 cm height, washed with 3 N NaOH and 5 N HCl successively and finally with 3N HCl until the absorption of 260–280 $m\mu$ diminished. The fraction which was eluted with 0.02 N formic acid from Dowex-1 column, was collected, dried and dissolved in 5 ml of 2 N HCl and charged on the Dowex-50 column. The column was eluted with 2*n* HCl and the eluate was collected every 4 ml. The flow rate was 0.7–1.0 ml/min.

(b) Measurement of radioactivity and absorption at 260–280 $m\mu$ of the effluents

One drop of each effluent was put on a steel dish, dried by infrared lamp and its radioactivity (c.p.m.) was measured by a Geiger-Müller counter. The 260–280 $m\mu$ ultraviolet absorption of the high radioactive fractions were measured by a Beckman-type spectrophotometer. The separation diagram obtained from the both measurements was shown in Fig. 1A, 1B and 2.

Fig. 3 A. Absorption spectrum of Fraction No. 15 (guanine fraction)

Solution: 0.1 N HCl

Fig. 3 B. Absorption spectrum of Fraction No. 16 (adenine fraction)

Solution: 0.02 N HCl

6. Identification of each fraction

The higher radioactive fraction was dried, dissolved in a small volume of water, subjected to one-dimensional paper chromatography and *R_f* values of active components were determined by radioautography (photograph 3). The ultraviolet absorption spectra of the fractions corresponding to adenine and guanine were identified with those of the authentic specimens. (Fig. 3A and 3B)

7. Experimental results

Radioactivity of each fraction obtained by means of the above method was as follows: In experimental, number 1 to 11 fractions were obtained

by Dowex-1 column chromatography. As the total radioactivity was 63.5×10 c.p.m., the process of elution was complete. As No. 5 fraction contained the bases it was chromatographed by Dowex-50 (chloride form), and fractionated into five parts. The sum of activities of fractions No. 15 and No. 16 were adenine (2.2 %) and guanine (1.6 %) respectively. In experiment 2, fraction No. 2 contained a mixture of bases corresponding to 14.6 % of that of the input materials. (table 2)

Table 2. Radioactivity of each fraction

Experiment 1. starting material 63.8×10^5 c.p.m.		Experiment 2 starting material $168.0.6 \times 10^5$ c.p.m.	
Fraction No. 1	7.17×10^5	Fraction No. 1	1250×10^5
2	38.8	2	276
3	5.68	3	53
4	0.525	4	19
5	8.0	5	10
6	1.38	6	4
7	1.01	7	4
8	1.86	8	3
9	0.81	9	10
10	1.54	10	80
11	2.24	11	11
12	0.58		
13	1.06		
14	0.39		
15	1.39		
16	0.71		

DISCUSSION

In the above experiments, ^{14}C -labelled adenine and guanine could be separated in fairly pure form from the nucleic acid fraction of *Chlorella ellipsoides*. The nucleotides could not be isolated in pure state. The material used in this experiment was fractionated by the modified W. C. Schneider's method, i.e., the extraction with cold trichloroacetic acid was omitted following the extraction with alcohol. Therefore the nucleic acid fraction was probably contaminated by the many acid soluble substances and the separation thereafter became difficult. It seems to be necessary to remove the acid soluble substances with cold trichloroacetic acid or with some other procedures before further treatments.

SUMMARY

Uniformly ^{14}C -labelled adenine and guanine were isolated in fairly pure form from the nucleic acid fraction obtained at the same time when ^{14}C -

Photograph 1.

- A. The radioautogram of nucleic acid hydrolysate separated by paper electrophoresis.
medium: 5 % $\text{HAc} \cdot \text{H}_2\text{O}$ solution electrophoresed for 2 hours.

- B. The radioautogram of acidic fraction of nucleic acid hydrolysate separated by paper electrophoresis.
medium: 5 % $\text{HAc} \cdot \text{H}_2\text{O}$ solution electrophoresed for 4 hours.

Photograph 2.

- The radioautogram of nucleic acid hydrolysate separated by 2 dimensional paper chromatography
solvent: *n*-butanol ammonia and iso-propanal-HCl

Photograph 3.

- A. The radioautogram of each fraction of nucleic acid hydrolysate separated by ion exchange resin chromatography
solvent: iso-propanal-HCl

- B. The radioautogram of each fraction of nucleic acid hydrolysate separated by ion exchange resin chromatography
solvent: *n*-butanol-HCl

labelled amino acids were isolated from the protein fraction of *chlorella ellipsoidae*.

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