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Photosynthetic Products of Division Synchronized Cultures of *Euglena*[1]

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ABSTRACT

Rates and products of photosynthetic $^{14}CO_2$ fixation by division synchronized cultures of *Euglena gracilis* strain Z were determined over the cycle. Rate of $^{14}CO_2$ fixation doubled in a continuous manner throughout the light phase followed by a slight reduction of photosynthetic capacity in the dark phase. Greater ^{14}C incorporation into the nucleic acid-polysaccharide fraction occurred with mature cells. Products of $^{14}CO_2$ fixation varied markedly over the cycle: although with mature cells ^{14}C-labeled sucrose was not detected, with dividing cells this was the main sugar labeled; in young cells ^{14}C maltose was formed. Cells removed at end of dark phase accumulated ^{14}C in glycolate, whereas at other stages over the cycle less ^{14}C was present in glycolate, and this was accompanied by a rapid incorporation of ^{14}C into glycine and serine. Glycerate was an early and major product of photosynthesis with cells at the mature stage of the cycle.

Changes in ribulose 1,5-diphosphate carboxylase activity paralleled changes in photosynthetic rate, but activity was not great enough to account for the observed rates of CO_2 fixation at most stages of the division cycle investigated.

When grown phototrophically on an appropriate light-dark regime, cultures of *Euglena gracilis* divide synchronously, an approximate doubling of cell number occurring in each dark period within certain limits of cell concentration (5, 8, 11). These cultures have been used for the study of patterns of biosynthesis during the cell cycle by determining some of the major cell constituents: proteins, polysaccharides, phosphorus, pigments, and nucleic acids, using samples of the culture withdrawn at intervals over the cycle (6, 10, 12). A doubling of these constituents occurred over characteristic parts of the cycle. In phototrophic synchronously dividing cultures of *Euglena* the flow of carbon along different anabolic routes at different stages of the cycle must partly be a manifestation of the control of the path of carbon in the early products of photosynthesis. We have investigated this problem by determining products of $^{14}CO_2$ fixation at various stages over the cell division cycle.

MATERIALS AND METHODS

Growth, Synchronization Regime, and Sampling of Culture. Axenic cultures of *E. gracilis* Klebs strain Z were grown in 2-liter Pyrex vessels equipped with a glass stirrer and siphoning device, and air at a rate of 7 l/hr was bubbled through the culture from a sintered disc at the bottom of the vessel after first passing through a sterile cotton plug and membrane filter. An inline reservoir of fresh medium (autotrophic medium of Cramer and Myers [9]) was used to dilute the culture, enabling cell number to be maintained within desired limits. The culture vessel was kept at 25 C, and illumination from two opposite sides by banks of fluorescent tubes (Osram white) gave a light intensity of 3500 lux at the surface of the culture vessel. Cell counts were made with a Coulter counter Model B. The culture vessel was aseptically inoculated to give an initial concentration of about 5×10^3 cells/ml. The culture was grown on a 14-hr light followed by a 10-hr dark cycle, cell divisions being confined to the dark period when a doubling of cell number occurred in each division burst between a cell density of 10^4 and 10^5 cells/ml. Figure 1 shows part of a typical growth curve and the points at which aliquots of a culture or whole culture were removed. The samples are termed t_3, t_{12}, t_{17}, and t_{23}, referring to the hour of sampling after commencement of the 24-hr cycle in which they were harvested.

Photosynthetic Incorporation of $^{14}CO_2$. For cell fractionation experiments, 300 ml of culture were withdrawn, centrifuged at 2000*g* for 5 min, washed once, and resuspended in growth medium, the final volume being noted to determine by how much the suspension had been concentrated. Warburg flasks containing 2 ml of *Euglena* suspension in the main compartment and 40 μmoles of $NaH^{14}CO_3$ containing 4 μc in the side arm, were allowed to equilibrate for 5 min at a light intensity of 3500 lux at 25 C. After tipping in the bicarbonate, flasks were removed at intervals, 1 ml of their contents being pipetted into ice-cold centrifuge tubes, cells rapidly centrifuged, and the supernatant fluid decanted (fraction A). The cells were killed by placing the centrifuge tubes in a boiling water bath for 2 min. Four further cell fractions were prepared; water soluble compounds (fraction B), 80% (v/v) ethanol soluble compounds (fraction C), a polysaccharide-nucleic acid fraction (fraction D), and a cell residue suspended in 50% ethanol, according to the procedure of Syrett *et al.* (23). Radioactivity was determined in the various fractions by plating aliquots on glass discs and counting at infinite thinness in a Nuclear-Chicago gas flow counter, Model D.47. In short term photosynthesis experiments, 1500 ml of culture were withdrawn, washed as previously, and resuspended in four parts distilled water plus one part growth medium to give 20-ml culture. Experiments were carried out using an apparatus similar to that described by Syrett *et al.* (23). The center compartment contained 20 ml of *Euglena* suspension. Following equilibration for 10 min at 25 C and 12,000 lux, 10 μmoles of $NaH^{14}CO_3$ containing 50 μc were added, and at intervals 1.80-ml samples were removed into absolute ethanol at − 40 C. ^{14}C-Labeled compounds were

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extracted and separated by chromatography using the techniques of Bassham and Calvin (1). Radioactivity was determined using a Nuclear-Chicago Actigraph 11 gas-flow chromatogram scanner.

Ribulose 1,5-diP carboxylase was assayed by the method of Jakoby *et al.* (14). Cells were harvested by centrifugation, washed and resuspended in 50 mM tris-HCl buffer, pH 7.5, and disrupted by treatment with three 15-sec bursts of ultrasonic waves (M.S.E. ultrasonic disintegrator 1.5 amp). After centrifugation at 5000*g* for 10 min, the supernatant was used in enzyme assays. Protein was determined by the method of Lowry *et al.* (17).

Reagents. Sodium bicarbonate-^{14}C was obtained from the Radiochemical Centre, Amersham, Bucks. L-Tyrosine, D-ribulose 1,5-diP, D(−)-3-P-glyceric acid, D-glucose-6-P, and uridine-5-diphosphoglucose were obtained from Sigma.

RESULTS

Incorporation of ^{14}C into Cell Fractions over the Cycle. The total rate of $^{14}CO_2$ fixation by 12-hr cells, harvested towards the end of the light phase, was approximately double that of 3-hr cells (Table I). The photosynthetic capacity of dividing cells was somewhat greater than at 12 hr, and this capacity was slightly reduced towards the end of the dark phase. The differences in distribution of total photosynthetically fixed ^{14}C among cell fractions over the cycle were considerable (Table I), but little difference was recorded between the percentage of the total activity in a given cell fraction from cells harvested at different stages over the cycle. An exception was the percentage of the total label incorporated into the polysaccharide-nucleic acid fraction, which increased at the 12-hr stage of the cycle.

Table I. *Photosynthetic $^{14}CO_2$ Incorporation into Cell Fractions of Euglena over the Division Cycle*

Incorporation into fractions is expressed as nc/100 ml of culture and as a percentage of total incorporation per cell fraction. Fraction A is supernatant, B is water soluble compounds, C is lipid compounds, D is a nucleic acid-polysaccharide fraction, and E is cell residue.

Cell Sample	Cell Fraction	5 min		10 min		20 min	
		nc/100 ml	%	nc/100 ml	%	nc/100 ml	%
hr							
	A	3.0	14.3	5.9	18.70	10.5	23.3
	B	14.5	69.9	21.9	69.30	29.6	65.7
3	C	2.8	13.3	2.3	6.7	3.2	7.1
	D	0.7	3.3	1.5	4.7	1.8	4.0
	E	0.2		0.2		0.2	
	Total	21.0		31.6		45.1	
	A	4.4	16.8	5.6	13.0	12.3	13.6
	B	11.3	42.7	22.6	52.3	46.3	49.5
12	C	4.0	15.1	3.4	7.9	5.8	6.2
	D	5.4	20.7	8.8	20.4	22.9	24.4
	E	1.2	4.7	2.8	6.4	5.9	6.3
	Total	26.3		43.2		93.2	
	A	9.5	16.9	13.9	16.6	26.5	22.1
	B	34.8	61.8	61.6	73.4	82.8	69.1
17	C	7.0	12.4	3.5	4.2	4.5	3.8
	D	4.3	7.6	4.5	5.4	6.0	5.0
	E	0.7	1.2	0.5	0.6	0.2	0.2
	Total	56.3		84.0		120.0	
	A	6.9	15.0	11.5	17.7	26.5	23.5
	B	33.0	72.0	45.7	70.1	70.6	62.6
23	C	2.7	5.9	4.0	6.1	10.5	9.3
	D	3.0	6.5	3.7	5.7	5.0	4.4
	E	0.2	0.4	0.2	0.3	0.2	
	Total	45.8		65.1		112.8	

Table II. *Photosynthetic Incorporation of ^{14}C-Bicarbonate (dpm) into Ethanol-soluble Compounds by E. gracilis from the t_3 Stage of the Synchronous Cell Cycle*

Compound	Time of Sampling						
	7 sec	15 sec	30 sec	60 sec	120 sec	600 sec	1200 sec
Glycerate 3-P	440	970	970	2860	4400	5010	5490
Other phosphates	3300	7810	9750	21840	59100	142520	159500
Glycolate	210	260	740	1640	1150	3740	4360
Glycerate	210	200	230		1400	4300	2020
Other organic acids		290	600	1250	6070	31830	53520
Glycine + serine	380	1180	1490	4570	24800	55210	45270
Other amino acids		380	1100	3530	9470	36210	73240
Sucrose						8290	15250
Maltose					3700	1030	4820
Unidentified and origin	450	590	2820	1250	5210	28640	87670

Table III. *Photosynthetic Incorporation of ^{14}C-Bicarbonate (dpm) into Ethanol-soluble Compounds by E. gracilis from the t_{12} Stage of the Synchronous Cell Cycle*

Compound	Time of Sampling						
	7 sec	15 sec	30 sec	60 sec	120 sec	600 sec	1200 sec
Glycerate 3-P	380	4900	21540	35870	17040	14370	11160
Other phosphates	7680	49220	150960	287190	334140	179870	77760
Glycolate					1950	2010	350
Glycerate	5340	29330	12030	10790	1380	3050	1900
Other organic acids			1100	7420	14210	22090	16920
Glycine + serine	1870	19920	22330	31530	39810	39100	15990
Other amino acids	780	7120	13630	26470	35670	44410	34700
Sucrose							
Maltose				5910	11730	21500	22940
Unidentified compounds plus origin	170	1740	5060	3140	58570	39550	25400

Products of $^{14}CO_2$ Fixation in Division Synchronized *Eu-*

Table IV. *Photosynthetic Incorporation of ^{14}C-Bicarbonate (dpm) into Ethanol-soluble Compounds by E. gracilis from the t_{17} Stage of the Synchronous Cell Cycle*

Compound	Time of Sampling						
	7 sec	5 sec	30 sec	60 sec	120 sec	600 sec	1200 sec
Glycerate 3-P	3900	1100	23380	19050	15170	13180	16940
Other phosphates	39350	88100	182290	270670	344410	220010	122490
Glycolate			1580	3130	4070	4710	2440
Glycerate	1080	1620	2770	2190	2750	1500	
Other organic acids	630	1060	2600	9840	13000	14010	9840
Glycine + serine	2000	3210	10590	20100	34870	38240	17970
Other amino acids	1300	3110	7300	18870	26310	42430	41370
Sucrose			4570	20030	43120	94400	115120
Maltose					7220		
Unidentified compounds plus origin	1830	2520	1940	5760	9760	64900	213590

glena. Certain overlying patterns and several striking differences at the molecular labeling level were revealed (Tables II, III, IV, and V). The rate of incorporation of ^{14}C into phosphate esters, organic acids, and amino acids was linear with time in 3-hr cells. The same result was obtained with 23-hr cells, except that the rate of incorporation was twice that for 3-hr cells, showing that the photosynthetic capacity of the culture to incorporate ^{14}C into these groups of metabolites had doubled over the cycle. With 12-hr and 17-hr cells there was a more rapid initial ^{14}C incorporation reaching a peak at about 120 sec followed by a decrease, reflecting an increased turnover and rate of carbon flow through phosphate esters, organic acids, and amino acids at the cell division stage.

Major differences were observed in the labeling of glycolate and glycerate. With 3-hr cells ^{14}C-glycolate was detected after 7 sec, and ^{14}C incorporation into glycolate increased throughout the experiment as it did with 23-hr cells, except that total ^{14}C present in glycolate was greatly increased (Table II and V). With 12-hr cells glycolate was not detected until after 120 sec, and thereafter incorporation of ^{14}C into glycolate increased only slightly, a similar result being obtained with 17-hr cells (Tables III and IV). Rapid incorporation of ^{14}C into glycerate occurred in 12-hr cells, accounting for 33% of the total radioactivity present on the chromatograms after 7 sec (Table III). The labeling of glycine and serine was inversely related to the labeling of glycolate, for when little ^{14}C was present in glycolate, more ^{14}C was present in glycine and serine. With 12-hr cells ^{14}C-glycolate was not detected until 120 sec, while ^{14}C was present in glycine and serine from 7 sec and rapidly increased over the first 120 sec (Table III).

The incorporation of ^{14}C into sucrose and maltose varied markedly in the synchronous culture. Labeled sucrose could not be detected using 12-hr cells (Table III), but a peak of incorporation into sucrose was observed with 17-hr cells (Table IV). Similarly, 23-hr cells also incorporated label into sucrose although the amount was somewhat less than with 17-hr cells. In contrast, greatest ^{14}C incorporation into maltose occurred with 12-hr and 23-hr cells (Tables III and V).

The reproducibility of short term labeling experiments was confirmed by repeating experiments using 3-hr and 17-hr cells, when the same results were obtained. Several significant differences in the kinetics of short term product labeling were observed between 3-hr and 17-hr cells, and it was possible that the result with 17-hr cells arose from the 3-hr dark incubation rather than culture age. When 3-hr-old cells were dark incubated for 3 hr before determining the products of $^{14}CO_2$ fixation, the result differed from that with 17-hr cells, so experiments carried out with cells from the dark phase of the cycle reflected the photosynthetic capacity of the culture in that phase of the cycle rather than dark incubation.

Ribulose 1,5-diP Carboxylase Activity and CO_2 Fixation over the Cycle. Ribulose 1,5-diP carboxylase was assayed throughout the cycle in order to determine whether levels of activity could support the observed photosynthetic rate. Preliminary experiments to determine the effect of the method of cell disruption on the extraction and activity of the enzyme indicated that maximal activity in cell-free extracts was obtained by ultrasonic disruption. Activity increased from 3 to

Table V. *Photosynthetic Incorporation of ^{14}C-Bicarbonate (dpm) into Ethanol-soluble Compounds by E. gracilis from the t_{23} Stage of the Synchronous Cell Cycle*

Compound	Time of Sampling						
	7 sec	15 sec	30 sec	60 sec	120 sec	600 sec	1200 sec
Glycerate 3-P	1430	2740	3560	3860	10340	12430	29530
Other phosphates	5650	12290	15990	55620	135680	364600	235100
Glycolate	540	1490	3070	590	11650	21550	33190
Glycerate	230	670	490	370	1350	2180	3070
Other organic acids			2330	12940	13590	69850	65500
Glycine + serine		1230	4090	11570	34290	98690	119560
Other amino acids			1400	5800	23000	167360	157940
Sucrose				690	5480	43750	86240
Maltose			1350	2790		9460	4170
Unidentified compounds plus origin	370	740	1070	910	2000	48420	101170

Table VI. *Photosynthetic Rate and Ribulose 1,5-diP Carboxylase Activity in Euglena over the Division Cycle*

Cell Sample	Rate of Photosynthesis	Ribulose 1,5-diP Carboxylase	Specific Activity of Ribulose 1,5-diP Carboxylase
	μmoles CO_2 fixed/100 ml culture·hr		*μmoles CO fixed/mg protein·hr*
Light phase			
t_3	0.41	0.47	1.52
t_{12}			
Dark phase			
t_{17}	0.96	0.78	1.78
t_{23}	0.88	0.66	1.60

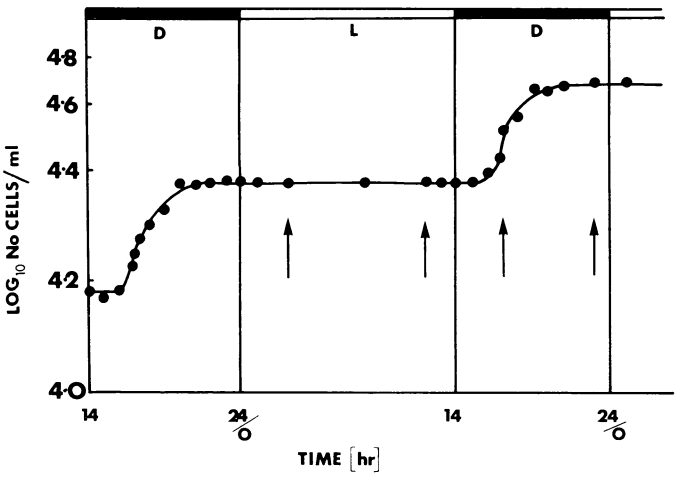

FIG. 1. Synchronous increase in cell number in cultures of *Euglena gracilis* Klebs Z. ↑: Represents times at which cultures were sampled; D: dark; L: light.

12 hr in the light phase, showing an approximate doubling of activity per volume of culture during the period of photosynthesis. After a further increase up to the 17-hr stage in the dark phase, activity declined slightly towards the end of the cycle (Table V). The specific activity of ribulose 1,5-diP carboxylase showed only slight variation over the cycle (Table VI), indicating that the rate of enzyme synthesis approximated to that of total protein synthesis during the light phase.

DISCUSSION

The present investigation is concerned with the ability of *Euglena* cells at different stages of development to carry out photosynthesis. Cells harvested at 3 hr in the light phase (Fig. 1) are "young cells," and at 12 hr, near the end of the growth period in the light phase, are "old cells." The culture removed at 17 hr represents dividing cells and that removed at 23 hr are new daughter cells still in the dark phase. The rates of total ^{14}C incorporation by the four types of cells show that the rate of photosynthetic $^{14}CO_2$ fixation doubles in a continuous manner throughout the light phase followed by a slight reduction of photosynthetic capacity in the dark phase.

Photosynthetic rate may be related to events occurring in the light phase of the synchronous culture, as experiments were carried out in the same medium, at the same temperature and light intensity as that of the culture. The light intensity used (3500 lux) is saturating for photosynthesis in *Euglena* (7). Changes in ribulose 1,5-diP carboxylase activity over the cycle paralleled changes in photosynthetic rate, but activity of the enzyme was not great enough to satisfy the rates of carbon dioxide fixation at all of the cell stages investigated (Table V). The possibility that ribulose 1,5-diP carboxylase activity was lost during cell disruption or during the assay *in vitro* must be considered. Also, Jensen and Bassham (15) have provided evidence for a light activation of the enzyme in isolated spinach chloroplasts, which may be an important factor in the *in vitro* assay. The apparent insufficiency of the enzyme to support observed rates of photosynthesis in *E. gracilis* var. *bacillaris* has been reported by Latzko and Gibbs (16); however, the involvement of ribulose 1,5-diP carboxylase in photosynthesis was demonstrated by their observation of a 27-fold increase in activity during the greening of bleached cells.

Edmunds (10) reported that DNA synthesis occurred in a step-wise manner during the cycle; the DNA content of the average cell remaining constant for the first 8 to 9 hr of the light phase, after which a doubling in DNA content occurred. In the present experiments, using growth conditions and synchronization regime similar to that of Edmunds (10), the percentage of total ^{14}C fixed incorporated into the nucleic acid-polysaccharide fraction was greater in 12- and 17-hr cells (Table I), showing that products of photosynthesis are probably diverted to nucleic acid synthesis at this stage of the cycle.

Marked differences in the labeling of individual compounds (Tables II to V) shows that the flow of photosynthetically fixed carbon is controlled along various pathways to different extents over the cycle; a finding in general agreement with those of Stange *et al.* (22). In particular, the amount of ^{14}C present in glycolate and glycerate shows great variation over the cycle. Although algae were previously thought to be incapable of metabolizing glycolate (13), it has been shown that several algae contain an enzyme that oxidizes glycolate to glyoxylate (18, 20, 24), and the activity of this enzyme varies with the growth conditions in *Chlamydomonas* (20) and *Euglena* (3). Label present in glycolate may result from different rates of glycolate formation and oxidation at various stages of the cell cycle. Glycolate production in synchronized *Scenedesmus* is maximal at the time of cell division or immediately afterwards (21). Many enzymes are synthesized discontinuously in the cell cycle of synchronous cultures (19), and the labeling of glycolate in synchronous cultures of *Euglena gracilis* and different rates of glycolate excretion with other algae over the cell cycle may reflect variations in the activity of enzymes of glycolate metabolism. Investigations into the activities of enzymes of the glycolate pathway over the synchronous cycle in *Euglena* support this conclusion (4).

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