KOR. JOUR. MICROBIOL. 7, 1~9 (1969)

클로렐라의 同調培養法에 의한 細胞分裂의 生理學的 硏究 (第1報)

——細胞의 生活史에 따른 燐酸化反應의 變化에 관하여——

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Physiological studies on cell division by the technique of synchronous culture of Chlorella (I).

—On the changes in phosphorylation of the cells during the life cycle—

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ABSTRACT

Changes in the phosphorylation of *Chlorella* cells during the life cycle in the autotrophic and mixotrophic synchronous culture were followed under the light and dark.

1. In the autotrophic culture of *Chlorella* the amounts of esterified phosphate compounds of the algal cell under the light increased during the growing period and decreased strikingly in the ripening period showing a peak at the L_1-cell stage.

2. The amount of total esterified phosphate compounds of the cell under the dark, however, decreased during the growing period and then kept fairly constant during the ripening and division periods showing the greatest activity of the oxidative phosphorylation in the early growing stage.

3. It is presumed that the energy requirement of the dividing algal cell in the autotrophic culture is fulfilled prior to the nuclear division mostly by the photosynthetic phosphorylation.

4. In the mixotrophic culture, the amount of esterified phosphate compounds of the algal cells under the light increased during the growing period and decreased during the late ripening and early division periods showing a peak in the L_2-cell stage as in the case of the phosphorylation under the dark.

5. The phosphorylation of the cell grown in the glucose medium is more active under the dark than under the light in the stages of the growing and early ripening periods.

6. It is considered that the excess glucose in the algal cell not only promotes the oxidative phosphorylation but also inhibits the photosynthetic phosphorylation of the cell.

7. It is presumed that the energy requirement of the dividing algal cell in the glucose medium is fulfilled prior to the nuclear division by the combined action of oxidative and photosynthetic phosphorylation, mostly by the oxidative phosphorylation.

This work was supported by the grant from the Ministry of Education.

INTRODUCTION

The technique of synchronous culture for the study of physiological and biochemical changes during the life cycle of *Chlorella ellipsoidea* has been developed by Tamiya *et al.*(1953, 1966), and it has now become possible to synchronize the algal growth not only at the cellular level but also at the level of nuclear development and division. It is now widely accepted that the life cycle of *Chlorella ellipsoidea* proceeds in a manner as schematically illustrated in. Fig. 1. The cycle starts from a young and small cell, referred to in the figure as the Dn-cell which, derives from the fully matured mother cell denoted by L_4. When grown under saturating light intensity with provision of CO_2 and mineral nutrients the Dn-cell grows and, after passing successively the stages of Da, D-L, L_1, and L_2, it attains the stage of L_3-cell. The L_3-cell further ripens, without requiring the light, into L_4-cell which in turn, also independently of light, divides into four Dn-cells.

Tamiya *et al.* (1961) studied the mode of nuclear division in synchronized *Chlorella* culture by the Feulgen staining and reported that the cells at the stages of Dn, D-L were mono-nuclear while the L_1-cells were either mono- or di-nuclear. All these cells could not divide when incubated in the dark. At the L_2-stage, the cells were found to be either mono-, di- or tetra-nuclear, and when incubated in the dark, some of them performed cell division giving rise to mono-nuclear Dn-cells. The L_3-cells were either di- or tetra-nuclear, and when incubated in the dark, they transformed themselves into tetra-nuclear L_4-cells, which divided in the dark into four mono-nuclear Dn-cells each.

The present study was undertaken with a view to examine in what manner the normal autotrophic life cycle of the alga is affected by mixotrophic culture adding glucose, and in what manner the energy requirements of dividing cells is fulfilled during the life cycle of the alga in both cases.

MATERIAL AND METHODS

Synchronous culture: The experimental organism used was *Chlorella ellipsoidea*, and the culture methods employed were virtually the same as those reported previously (Lee, and Kim, 1964). The precultures were run at 20–25°C in an oblong flat culture chamber of about 1 liter capacity with constant aeration of CO_2-enriched air under illumination with daylight fluorescent lamps. The synchronous culture, which was started from the stage of Dn-cell, was performed using so-called "DLD'-method" of Tamiya *et al.* (1961) and the method of obtaining the homologous population of Dn-cell was the same as those reported by Morimura(1959). The basic culture medium used was a modified Knop's solution which had the following composition per liter solut-

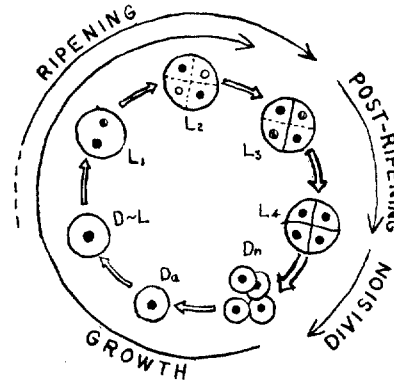

Fig. 1. Schematic representation of successive developmental stages in the normal life cycle of *Chlorella ellipsoidea*("DLD'-cycle"). The dots in the circles (cells) represent nuclei.

ion: KNO_3, 5.0 g; $MgSO_4 \cdot 7H_2O$, 2.5 g; KH_2PO_4, 1.25 g; $FeSO_4 \cdot 7H_2O$, 0.003 g and 1 ml. of Arnon's A_5 trace element solution(Arnon, 1938). In the case of mixotrophic culture, 50 mM glucose was added to the above basal medium.

Measurement of developmental stage: The process of cell development was followed by measuring the number of cells per liter using a hemocytometer, the statistical distribution of cell size by means of an ocular micrometer, and the packed volume of cells per liter using a hematocrit. At various stages during the synchronous culture some portions of the algal cells were taken out for the phosphorylation test.

Measurement of Phosphorylation: It is widely known that there are much more polyphosphates than ATP in *Chlorella* cells (Miyachi and Tamiya, 1961) and the phosphorus in polyphosphate may be further transferred into other phosphate compounds(Nihei, 1957; Lee, 1967) and that the energy of P-O-P bonds in the polyphosphate molecule is of the same magnitude as that of the energy-rich phosphaate bond (Meyerhof *et al.*, 1953; Yosida, 1955). Therefore, the phosphorylative activity of the cell is expressed by the content of total esterified phosphate compounds during a relatively short period of time in the whole cell system using ^{32}P-phosphate as the tracer. The cells, after being washed twice with M/500 K_2SO_4 solution by centrifugation, were suspended in 15 ml. of reaction medium containing 100 mM potassium phosphate and 0.333 mCi ^{32}P-phosphate per liter of tris-buffer(pH 7.4)for 15 minutes at 23–25°C under the light or under the dark. At the end of the reaction time the algal cells were harvested and after being washed twice with M/500 K_2SO_4 solution they were analyzed for the content of ^{32}P-phosphate.

Assay: To trace the distribution of ^{32}P-containing substances in the cell material, the algal cells separated from the reaction medium were fractionated into three portions. The algal cells were treated twice with cold 5% perchloric acid(PCA) and separated by centrifugation into PCA-soluble fraction and residual fraction. From the perchloric acid soluble fraction, ortho-phosphate was collected by the method of Belenblum and Chain(1938). The amounts of phosphate in each fractions were determined by measuring their radioactivity with a Gas-flow type GM counter.

RESULTS AND DISCUSSION

As a preliminary experiment to obtain the adequate reaction time in the ^{32}P-containing medium, a mixed population of the algal cells was suspended in a reaction medium under the dark. At the predetermined time intervals during the reaction period, some portions of the algal cells were taken out and analyzed for radioactivity. The results obtained were represented in Fig. 2. The amount of esterified ^{32}P in the PCA-soluble fraction of the cells was saturated almostly within 15 minutes of the reaction, although the amount of ^{32}P-phosphate in the acid-insoluble fraction

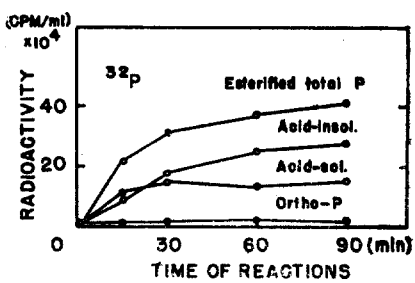

Fig. 2. Changes in the amounts of esterified ^{32}P-phosphate of *Chlorella* cells under the dark during incubation period.

increased gradually until the end of the incubation. Therefore, the cells harvested at different cell stages for the measurement of phosphorylation were suspended in the reaction medium containing ^{32}P-phosphate for 15 minutes in this experiment.

In the synchronous culture of *Chlorella* the process of cell development was followed by measuring the number of cells per liter medium, the statistical distribution of cell size and the packed volume of cells per liter. The results obtained were shown in Fig. 3. In the normal autotrophic synchronous culture of this experiment the ripening period from the stages of L_1-cell to L_4-cell, during which nuclear division has been reported to occur (Tamiya *et al.*, 1951), ranged from 20 to 28 hours of the culture, and the division period from the stage of L_4-cell to Dn-cell took 28—34 hours. In mixotrophic culture in a glucose medium, on the other hand, the division period ranged 28 to 38 hours of the culture, although the ripening period was the same as in the case of autotrophic culture. As shown in Fig. 3. the average volume of the matured cell grown in the glucose medium is by far larger and the number of cells arising from a single cell is much greater than those in the case of autotrophic culture.

In the autotrophic and mixotrophic synchronous cultures of *Chlorella*, the change in the phosphorylative activity of the cells in the course of the life cycle was measured under the light and dark. The results obtained were summarized in Table 1 and 2 respectively. As shown in Fig. 4, the amount of esterified phosphate compounds in the algal cells in the autotrophic synchronous culture under the light increased during the growing period, but decreased strikingly in the ripening period showing a peak at the L_1-cell stage, and thereafter kept almost constant at low value

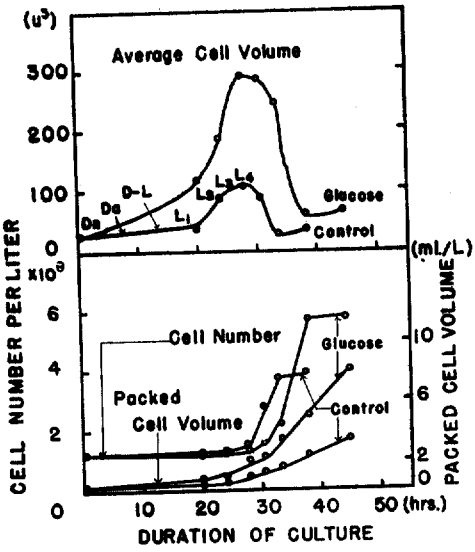

Fig. 3. The process of growth and cellular division followed by the number of cells per liter medium, the statistical distribution of cell size, and the packed volume of cells per liter medium during synchronous autotrophic and mixotrophic culture.

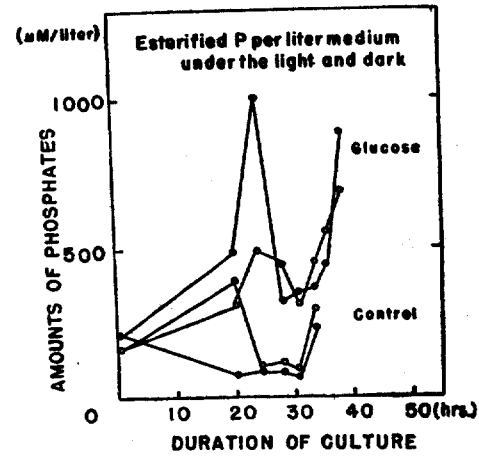

Fig 4. Changes in the amounts of esterified ^{32}P-phosphate compound of *Chlorella* cells per liter medium under the light and dark during the autotrophic and mixotrophic synchronous culture. Open circle indicates the phosphorylation under the light and solid circle, under the dark.

Table 1. Amounts of esterified ^{32}P-phosphate of *Chlorella* cells at various developmental stages during the autotrophic and mixotrophic culture for 15 minutes under the light.

Fraction	Duration of culture (hrs.)	Control			Glucose		
		CPM/L medium	μM/L medium*	μM/ml. cell*	CPM/L medium	μM/L medium*	μM/ml. cell*
PCA-soluble P	0	12,488	120	324	12,488	120	324
	20	19,824	193	1,425	31,898	318	335
	24	7,526	70	475	37,988	367	283
	26				42,587	413	207
	28	7,897	76	90	32,470	313	89
	30.5	7,762	403	336	27,817	269	71
	33.5	16,106	453	324	42,433	412	86
	35.5				41,254	399	78
	38				66,499	543	87
Ortho-P	0	6,993	67	181	6,993	67	181
	20	9,394	91	1,281	11,355	110	116
	24	3,564	35	431	9,848	95	74
	26				8,929	87	44
	28	5,200	50	59	7,695	74	21
	30.5	3,766	364	303	8,296	80	21
	33.5	3,409	330	236	8,866	86	18
	35.5				6,853	66	13
	38				12,153	117	16
PCA-soluble esterified P	0	5,495	53	143	5,405	53	143
	20	10,529	102	144	20,543	208	219
	24	3,961	35	44	28,139	272	209
	26				33,658	326	163
	28	2,697	26	31	24,775	239	68
	30.5	3,996	39	33	19,521	189	50
	33.5	12,697	123	88	33,566	326	68
	35.5				34,401	333	65
	38				54,346	526	71
PCA-insoluble P	0	11,523	111	300	11,523	111	300
	20	29,704	287	404	9,557	93	98
	24	5,794	56	70	23,077	223	172
	26				8,725	84	42
	28	8,625	84	99	22,011	213	61
	30.5	5,295	51	43	13,020	126	33
	33.5	17,383	168	120	22,078	213	44
	35.5				26,484	159	31
	38				17,849	172	23
Total esterified P	0	17,016	164	443	17,016	164	443
	20	40,233	389	548	30,100	301	317
	24	9,745	91	114	51,216	495	381
	26				12,080	410	205
	28	11,322	110	130	46,786	452	129
	30.5	9,291	90	76	32,541	315	83
	33.5	30,080	291	208	55,644	452	96
	35.5				50,885	539	112
	38				72,195	698	94

* Values were calculated from the specific radioactivities of the incubation medium and the fractionated cell components.

Table 2. Amounts of esterified ^{32}P-phosphate of *Chlorella* cells at various developmental stages during the autotrophic and mixotrophic culture for 15 minutes under the dark.

Fraction	Duration o culture (hrs.)	Control			Glucose		
		CPM/L medium	μM/L medium*	μM/ml cell*	CPM/L medium	μM/L medium*	μM/ml. cell*
PCA-soluble P	0	11,147	108	292	11,147	108	292
	20	5,041	49	69	43,786	423	446
	24	5,718	56	70	44,021	395	304
	26				30,096	291	146
	28	5,134	49	58	27,972	271	77
	30.5	5,658	55	46	22,817	220	58
	33.5	15,096	146	104	32,697	316	66
	35.5				39,732	385	76
	38				67,333	652	88
Ortho-P	0	2,814	27	73	2,814	27	73
	20	1,353	13	18	9,221	89	94
	24	2,468	24	30	14,502	109	84
	26				9,426	91	46
	28	1,038	10	12	4,434	43	12
	30.5	1,838	18	15	3,836	37	10
	33.5	2,399	23	16	5,031	49	10
	35.5				6,641	65	13
	38				19,647	190	26
PCA-soluble estrified P	0	8,333	81	219	8,333	81	219
	20	3,788	36	51	34,565	334	352
	24	3,250	32	40	29,518	286	220
	26				20,671	200	100
	28	4,096	39	46	23,588	228	65
	30.5	3,820	37	31	18,981	183	48
	33.5	12,697	123	88	27,666	267	56
	35.5				33,092	320	63
	38				47,686	462	62
PCA-insoluble P	0	14,319	138	373	14,319	138	373
	20	3,663	35	49	16,683	161	169
	24	5,594	54	68	73,893	716	551
	26				11,089	107	54
	28	4,496	43	51	9,557	92	23
	30.5	3,730	36	30	16,623	162	43
	33.5	11,555	111	79	10,157	108	23
	35.5				13,220	128	25
	38				43,923	426	58
Total esterified P	0	22,651	219	592	22.651	219	592
	20	7,450	71	100	51,258	495	521
	24	8,844	86	108	103,410	1,002	771
	26				31.760	307	154
	28	8,592	82	97	33,095	320	88
	30.5	7,550	73	61	29,604	345	91
	33.5	24,252	234	167	37,822	375	79
	35.5				46,312	448	88
	38				90,609	888	120

* Values were calculated from the specific radioactivities of the incubation medium and the fractionated cell components.

during the division period. The amount of the total esterified phosphate compounds of the cells under the dark, however, decreased during the growing period and then almost unchanged during the ripening and dividing periods showing the greatest activity of oxidative phosphorylation in the early growing stage.

It is, therefore, considered that the increase of the amount of total esterified phosphate compounds of the cells under the light during the growing period is due to the increase in photosynthetic phosphorylation of the cells in the growing period.

These results are quite in accordance with the observations that the endogenous (Sorokin *et al.*, 1957) and glucose respiration (Chin and Lee, 1965) of *Chlorella* cells is considerably active during the early growing stage, whereas it is suppressed strikingly during the division period, and that the photosynthetic activity(Nihei *et al.*, 1954) of the algal cell is the most active and the chlorophyll content (Lee and Kim, 1964) is much larger during the early growing period than the other cell stages. It is presumed, therefore, that the energy requirement of dividing algal cells in the normal autotrophic culture is fulfilled prior to the ripening period, in which nuclear division occurs, mostly by the photosynthetic phosphorylation.

In the mixotrophic culture, on the other hand, the amounts of esterified phosphate compounds of the algal cells under the light during the life cycle increased gradually throughout the growing and early ripening periods, but decreased during the late ripening and the early division periods. The change in the amounts of esterified phosphate compounds of the cells under the dark during the life cycle showed the similar tendency with that of the phosphorylation of the cells under the light, showing a peak in the L_2-cell stage. It is noteworthy, however, that the phosphorylation of the cells grown in the glucose medium is more active under the dark than under the light in the cell stages of the growing and early ripening periods. It is presumed, therefore, that the excess glucose in the cell not only promotes the oxidative phosphorylation but also inhibits the photophosphorylation in the stages of growing period, and that the light interferes in oxidative phosphorylation of the young growing cells in a glucose medium.

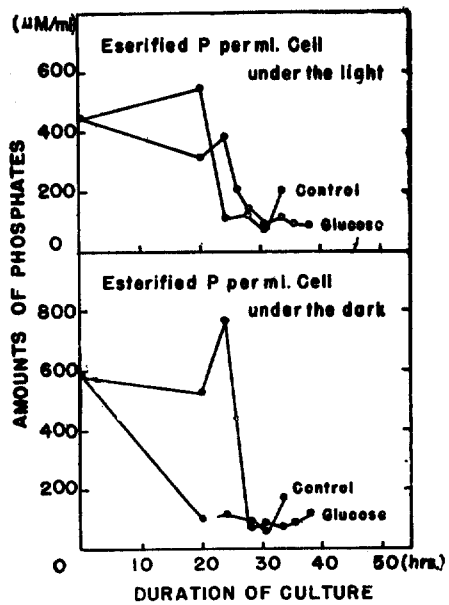

Fig. 5. Amounts of esterified ^{32}P-phosphate per milli liter packed cells of *Chlorella* under the light and dark at various developmental stages during the autotrophic and mixotrophic synchronous culture.

As represented in Fig. 5, it is also noteworthy that the phosphorylation of the cells per unit packed cell volume under the light or dark in the glucose medium decreased prominently in the ripening and division periods compared with the growing period as in the case of autotrophic synchronous culture, although the phosphorylation per unit cell

number did not decrease during the division period owing to the increase in packed cell volume. Therefore, it is considered that the energy requirement of the dividing cell in the glucose medium is fulfilled prior to the nuclear division by the combined action of photosynthetic and oxidative phosphorylation, mostly by the latter.

摘 要

클로레라를 기본배지 및 포도당배지에서 同調的으로 培養하고 배양의 중간기에 發育時期를 같이하는 一定量의 細胞集團을 수획하여 그 燐酸化反應을 ^{32}P를 追跡子로 사용하여 明暗에서 각각 測定하므로써 細胞分裂過程에서 소요되는 에너지가 生活史의 어느 時期에서 어떠한 反應으로 공급되는가 하는 것을 조사 하였다.

細胞를 기본 배지에서 배양하였을 때 光線下에서 에스테르化한 燐酸化合物의 量은 生長期에서는 增加하고 成熟期에서는 減少하여 L_1-cell stage에서 가장 높은 값을 나타내었다. 그러나 暗處에서 에스테르化한 燐酸化合物의 量은 生長期에서 부터 점차로 감소되어 核分裂이나 細胞質分裂이 이러나는 시기에서는 거의 변화하지 아니 하였고, 초기의 생장과정에서 가장 높은 酸化的 燐酸化反應을 나타내었다. 따라서 細胞가 기본배지에서 자랄 때 細胞分裂과정에서 요구되는 에너지는 核分裂 직전의 L_1-cell stage에서 주로 光化學的 燐酸化反應으로 충족되는 것으로 생각된다.

細胞가 포도당배지에서 자랄 때에는 光線下에서 에스테르化한 燐酸化合物의 量은 暗處에서의 경우와 같이 細胞의 生長期에서는 增加하고 成熟期나 分裂期에서는 減少하여 L_2-cell stage에서 가장 높은 값을 나타내었다. 그러나 포도당배지에서 자라는 세포의 燐酸化反應은 生長期 및 成熟期初에서는 光線下에서 보다도 暗處에서 더욱 컸었다. 따라서 多量의 포도당은 細胞의 酸化的 燐酸化反應을 촉진시킬 뿐만 아니라 光合成的 燐酸化反應을 抑制하는 것으로 생각된다. 또한 細胞가 포도당배지에서 자랄 때 細胞分裂과정에서 요구되는 에너지는 L_2-cell stage에서 주로 酸化的燐酸化反應에 의하여 충족되는 것으로 짐작된다.

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