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Effect of Light on Several Metabolites of Carbohydrate Metabolism in *Phycomyces blakesleeanus*

JAVIER RUA, LEANDRO B. RODRIGUEZ-APARICIO, FÉLIX BUSTO, AND JOAQUÍN SOLER*

Departamento de Bioquímica y Biología Molecular, Universidad de León, 24007 León, Spain

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The concentrations of all metabolites studied, except fructose 1,6-bisphosphate from wild-type *Phycomyces blakesleeanus*, were light dependent. This photoregulation appears to be independent of the *mad* gene product(s) and also independent of carotene biosynthesis regulation. However, the photoregulation of glyceraldehyde 3-phosphate, 2-phosphoglycerate, and phosphoenolpyruvate may be assigned to these *mad* and *car* S gene products.

The ability to respond to environmental changes by adaptative corrections to physiological processes is a very important characteristic of any living system. For widely different organisms, a change of illumination conditions supplies most of the essential information about the surrounding medium.

Phycomyces blakesleeanus shows responses to different stimuli, including visible light. Responses to light include changes in metabolism, changes in growth, and positive phototropism (1, 5, 23). Known light-dependent metabolic variations in *P. blakesleeanus* include enhanced carotene formation (11, 17), an increase in pyruvate carboxylase activity (24), a decrease in asparagine metabolism (25), and a decrease in cellular ornithine decarboxylase activity (19); chitin synthetase activity is also stimulated by light in vitro and in vivo (16), and light-dependent alcohol dehydrogenase activity decreases (10). Metabolic changes associated with light as a condition of growth have been reported for several other fungi (13, 22, 26).

Little is known about the effect of light on carbohydrate metabolism in *P. blakesleeanus*. We have previously reported that for three pyridine-linked dehydrogenases of carbohydrate metabolism of *P. blakesleeanus*, i.e., glucose 6-phosphate dehydrogenase, lactate dehydrogenase, and malate dehydrogenase, the enzyme activity values are affected by light (D. De Arriaga, J. Soler, F. Teixido, and E. G. Galarraga, Abstr. *Phycomyces* Meet. 1982, p. 31).

This paper reports the effect of light on the levels of several metabolic intermediates of carbohydrate metabolism from mycelia of wild-type strain NRRL 1555 (−) and of *car* and *mad* mutants [C 115 (−) and C 111 (−)] of *P. blakesleeanus*. Mutant C 115 (−) [*car* S42 *mad* 107 (−)] is abnormal in photocarotenogenesis response and is superyellow, and mutant C 111 (−) [*mad* B 103 (−)] shows abnormal phototropism (20).

The *Phycomyces* strains were kindly provided by A. P. Eslava, Department of Genetics, University of Salamanca, Spain. The details for liquid minimal medium and the general method of cultivation were previously described (8). The cultures were incubated in a New Brunswick Scientific Co. Psychrotherm G-27 orbital shaker at 200 rpm at 20 to 22°C in absolute darkness or under continuous white light by exposure of the culture under a battery of eight white fluorescent tubes (Norelco; model F 24 T 12/CW/MO; 32 W, 50 cm) with an intensity of 1 ± 0.1 W m^{-2}. The mycelia were obtained by filtration at 48 h of growth at the upper part of the exponential growth phase.

The mycelia were cut into pieces and suspended in distilled water (1:7 [wt/vol]). The suspensions were homogenized in an MSK cell disruptor (B. Braun Instruments) for two cycles of 30 s. After homogenization, the extracts were centrifuged at 20,000 × *g* for 25 min (4°C), and the pellets were discarded. The supernatants were brought to 0.5 M in $HClO_4$ by the slow addition of 6% $HClO_4$ to saturation with constant stirring at ice bath temperature, and the pellets were removed by centrifugation at 12,000 × *g* for 20 min at 4°C. A fraction of each supernatant (pH 5 to 6) was reserved for the determination of oxalacetate. Later, the supernatants were neutralized to pH 7.0 to 7.6 by the addition of 2 N KOH. In all cases, after neutralization, the extracts were filtered to remove the $KClO_4$ formed.

The determinations of the levels of the metabolites studied were performed with the fresh mycelial extracts as described above by spectrophotometric assays with a recording spectrometer (Beckman Instruments, Inc.; model 35) equipped with a temperature control unit, except in the case of oxalacetate, which was determined by a fluorimetric assay.

Glucose 6-phosphate and fructose 6-phosphate were determined in a 0.4 M triethanolamine buffer (pH 7.6; 25°C) as described by Lang and Michal (18) by measuring the increase in A_{340} associated with NADPH (ε = 4,963 M^{-1} cm^{-1}) formation. Fructose 1,6-bisphosphate, dihydroxyacetone phosphate, and glyceraldehyde 3-phosphate were determined in a 0.4 M triethanolamine buffer (pH 7.6; 25°C) as described by Michal and Beutler (21) by measuring the decrease in A_{340} associated with NADH oxidation.

2-Phosphoglycerate, phosphoenolpyruvate, and pyruvate were determined in 0.5 M triethanolamine buffer (pH 7.6)–5 mM EDTA (25°C) as described by Czok and Lamprecht (6) by measuring the decrease in A_{340} associated with NADH (ε = 4,526 M^{-1} cm^{-1}) oxidation.

L-(+)-Lactate and L-(+)-malate were assayed in a hydrazine-glycine buffer (pH 9; 0.4 M hydrazine, 0.5 M glycine; 37°C) as described by Gutmann and Wahlefeld (14, 15) by measuring the increase in A_{340} associated with NADH (ε = 4,055 M^{-1} cm^{-1}) formation. Acetyl coenzyme A was assayed in a 0.5 M Tris hydrochloride buffer (pH 8.1; 25°C) as described by Decker (9) by measuring the increase in A_{340}

* Corresponding author.

TABLE 1. Glycolytic intermediate levels from mycelia of light- or dark-grown cultures of several *Phycomyces* strains

Metabolic intermediate	Growth condition[a]	Concn for *P. blakesleeanus* strain[b]:		
		NRRL 1555 (−)	C 111 (−)	C 115 (−)
Glucose 6-phosphate	l	4.00 ± 1.00[c]	4.46 ± 0.51[c]	5.48 ± 0.22[c]
	d	5.57 ± 0.40	2.54 ± 0.75	3.15 ± 1.04
Fructose 6-phosphate	l	1.00 ± 0.33[c]	0.69 ± 0.15	1.12 ± 0.24[c]
	d	0.46 ± 0.22	0.63 ± 0.15	0.56 ± 0.12
Fructose 1,6-bisphosphate	l	0.26 ± 0.06	0.23 ± 0.04	0.34 ± 0.10[c]
	d	0.20 ± 0.02	0.23 ± 0.17	0.96 ± 0.27
Glyceraldehyde 3-phosphate	l	0.32 ± 0.05[c]	0.11 ± 0.02	0.19 ± 0.06
	d	0.22 ± 0.02	0.10 ± 0.04	0.28 ± 0.07
Dihydroxyacetone phosphate	l	1.47 ± 0.14[c]	0.56 ± 0.11	1.01 ± 0.14[c]
	d	0.43 ± 0.06	0.37 ± 0.11	0.50 ± 0.07
2-Phosphoglycerate	l	0.30 ± 0.07[c]	0.31 ± 0.10	0.29 ± 0.04
	d	0.82 ± 0.23	0.39 ± 0.10	0.25 ± 0.11
Phosphoenolpyruvate	l	0.32 ± 0.07[c]	0.48 ± 0.05	0.25 ± 0.06
	d	0.88 ± 0.05	0.51 ± 0.05	0.24 ± 0.04
Pyruvate	l	4.73 ± 1.20[c]	2.65 ± 0.62[c]	4.79 ± 1.06
	d	11.20 ± 3.09	1.57 ± 0.22	4.42 ± 0.28
Lactate	l	59.62 ± 13.7[c]	85.58 ± 14.64[c]	82.87 ± 7.56[c]
	d	38.35 ± 5.35	32.60 ± 1.05	43.76 ± 7.02

[a] l, Light; d, darkness.
[b] The concentrations are expressed in micromoles per gram (dry weight) ± standard deviation.
[c] By Student's *t* test, deviation of light intermediate level from dark was significant. $P < 0.05$.

associated with NADH (ε = 4,955 M^{-1} cm^{-1}) formation. Citrate was assayed in a triethanolamine-$ZnCl_2$ buffer (pH 7.6; 0.1 M triethanolamine, 0.2 mM $ZnCl_2$; 25°C) as described by Dagley (7) by measuring the decrease in A_{340} associated with NADH (ε = 4,319 M^{-1} cm^{-1}) oxidation.

L-Alanine was assayed in a Tris-hydrazine buffer (pH 10; 40 mM Tris, 1 M hydrazine; 25°C) as described by Williamson (28) by measuring the increase in A_{340} associated with NADH (ε = 2,904 M^{-1} cm^{-1}) formation.

Oxalacetate was assayed by the fluorimetric method of Goldberg and Passonneau (12) at 340 and 465 nm for excitation and emission wavelengths, respectively (4). The assay was done in a digital spectrofluorimeter (Shimadzu Scientific Instruments, Inc.; model RF-510) by measuring the fluorescence of NADH.

The determination of the concentration of metabolites was performed by the endpoint method (2), and the metabolite content was quantitated in micromoles of metabolite per gram (dry weight) of mycelia. All values listed in the tables are the averages of at least four tests, given with the standard deviation. Statistical significance was calculated by Student's *t* test.

Table 1 summarizes the levels of glycolytic metabolites, glucose 6-phosphate, fructose 6-phosphate, fructose 1,6-bisphosphate, glyceraldehyde 3-phosphate, dihydroxyacetone phosphate, 2-phosphoglycerate, phosphoenolpyruvate, pyruvate, and lactate for the three strains of *P. blakesleeanus* studied.

Lactate showed the highest value of all metabolites in cultures grown in light and in darkness for all *Phycomyces* strains tested; the glyceraldehyde 3-phosphate level was the lowest in all experimental conditions. Glucose 6-phosphate and pyruvate were also present in higher concentrations, although they reached values only of between 3 and 30% of the lactate values.

The C 111 (−) mutant showed the response to light with respect to the levels of the glycolytic intermediates assayed. The concentrations of glucose 6-phosphate, pyruvate, and lactate were the only ones affected by light; they were higher for mycelia grown in light. Cultivation in light or darkness had no influence on the concentration of the other metabolites.

For the wild-type strain NRRL 1555 (−), fructose 6-phosphate, glyceraldehyde 3-phosphate, dihydroxyacetone phosphate, and lactate accumulated in cultures grown in light, whereas glucose 6-phosphate, 2-phosphoglycerate, phosphoenolpyruvate, and pyruvate accumulated in cultures grown in darkness. The concentration of fructose 1,6-bisphosphate appeared to be light independent. On the other hand, for the C 115 (−) mutant, the levels of glucose 6-phosphate, fructose 6-phosphate, dihydroxyacetone phosphate, and lactate were higher in light than in darkness, whereas fructose 1,6-bisphosphate accumulated when the cultures were grown in the dark. None of the other metabolites was affected by light.

Cultures exposed to light had concentrations of 2-phosphoglycerate and phosphoenolpyruvate similar to those of cultures grown in the dark for both mutants C 111 (−) and C 115 (−).

Table 2 shows the concentrations of metabolites assayed as related to pyruvate metabolism. L-Malate and citrate are present in the highest concentrations for each strain tested in cultures grown in light or in darkness. Oxalacetate concentration was the lowest in all cases, being 10^2-fold lower than L-malate concentration. The metabolites accumulated in light in *Phycomyces* wild type were acetyl coenzyme A, oxalacetate, and citrate, whereas L-alanine and L-malate were present in higher concentrations in dark-grown cultures. All metabolites described from this strain were light dependent. For the C 111 (−) strain, only oxalacetate in light-grown cultures and citrate in dark-grown ones accumulated.

For the C 115 (−) strain, L-alanine and citrate were higher for cultures grown in light, whereas acetyl coenzyme A and L-malate were higher for cultures grown in darkness. Oxalacetate concentrations showed no change according to light or dark culture conditions.

It is generally assumed that the study of the changes in concentration provides a major understanding of the light regulation of the enzymes and of the light perception-

TABLE 2. Levels of metabolites related to pyruvate metabolism from mycelia of light- or dark-grown cultures of several *Phycomyces* strains

Metabolic intermediate	Growth condition[a]	Concn for *P. blakesleeanus* strain[b]:		
		NRRL 1555 (−)	C 111 (−)	C 115 (−)
L-Alanine	l	1.10 ± 0.39[c]	3.51 ± 0.89	3.33 ± 0.33[c]
	d	1.98 ± 0.27	4.03 ± 1.13	1.60 ± 0.35
Acetyl coenzyme A	l	2.34 ± 0.48[c]	1.69 ± 0.22	0.62 ± 0.20[c]
	d	1.35 ± 0.37	1.48 ± 0.30	1.01 ± 0.11
Oxalacetate	l	0.26 ± 0.09[c]	0.48 ± 0.10[c]	0.22 ± 0.06
	d	0.12 ± 0.03	0.15 ± 0.03	0.21 ± 0.08
Citrate	l	6.24 ± 0.13[c]	6.06 ± 0.71[c]	11.82 ± 1.08[c]
	d	3.25 ± 1.22	7.79 ± 0.67	4.00 ± 0.43
L-Malate	l	12.84 ± 2.64[c]	22.44 ± 4.61	16.53 ± 0.64[c]
	d	19.88 ± 1.04	20.38 ± 1.80	25.78 ± 2.41

[a] l, Light; d, darkness.
[b] The concentrations are expressed in micromoles per gram (dry weight) ± standard deviation.
[c] By Student's *t* test, deviation of light intermediate level from dark was significant. $P < 0.05$.

metabolic response sequence. It should be noted that the concentration of each metabolite may be controlled by more than one enzyme activity from any of several metabolic pathways, interacting or not.

From the concentrations of chosen metabolites, we can see that all except fructose 1,6-bisphosphate were photoregulated in *Phycomyces* wild type. On the other hand, and from the results obtained with the *Phycomyces* mutants, we cannot exclude the possibility that some aspects of this photoregulation are related to the *mad* and *car* S gene product(s).

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