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PORPHYRIN BIOSYNTHESIS IN *RHODOPSEUDOMONAS PALUSTRIS*—XII. δ-AMINOLEVULINATE SYNTHETASE SWITCH—OFF/ON REGULATION*

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Abstract—The high levels of δ-aminolevulinate synthetase (ALA-S) in *Rhodopseudomonas palustris* cells grown anaerobically in the light (Ph) decrease to those found in cells grown aerobically in the dark (A), when the former cultures were vigorously oxygenated; simultaneously bacteriochlorophyll (Bchl) synthesis abruptly halted leading to diminished steady-state specific Bchl content. When flushing oxygen was interrupted, enzymic activity increased, whether chloramphenicol was present or not in the medium; if the protein synthesis inhibitor was added when oxygenation started, ALA-S declined in the same fashion as in its absence, but thereafter reactivation of the enzyme was lower than before. Succinyl-CoA-synthetase and ALA-dehydratase activities were also measured under the conditions described, and no changes at all have been observed. The existence of different forms of ALA-S in *R. palustris* depending on growth conditions is postulated along with the formation of low molecular weight factors which can modulate ALA-S activity by binding to the enzyme; a widespread mechanism in the adaptation of micro-organisms to changes in environment. It is also proposed that this particular regulatory phenomenon, could be referred to as a switch off/on mechanism controlling ALA-S activity in *R. palustris*.

INTRODUCTION

The photosynthetic bacterium *Rhodopseudomonas palustris* can grow either anaerobically in the light developing an intracytoplasmatic membrane system and forming a variety of pigments, particularly bacteriochlorophyll (Bchl) or aerobically in the dark; under these latter conditions Bchl synthesis is repressed and the intracellular structures are absent (Viale *et al.*, 1980). Therefore synthesis of Bchl is accompanied by morphological changes involving the formation intracytoplasmic membrane system. One of the earliest responses associated to the adaptation from a photosynthetically grown culture to an aerobic atmosphere is a decrease in the activity of the first enzyme specific to porphyrin biosynthesis, δ-aminolevulinate synthetase (ALA-S). The levels of ALA-S in aerobic (A) cells are about half of those in photosynthetically (Ph) cells. This is indicating that this enzyme plays an important role in the control of Bchl synthesis. In previous papers (Viale *et al.*, 1980b, 1986) we have reported evidence on the existence of low molecular weight factors, which would modulate the activity of ALA-S in *R. palustris*. It was assumed that the enzyme could exist in two forms depending on the growing conditions, a low and a high activity form in A and Ph cells respectively. Under aerobic conditions a factor is formed which can increase basic enzyme activity when able to bind it. Under anaerobic conditions, another factor, or perhaps the same but structurally changed, would also bind ALA-S very likely at a different site, resulting in a high activity form.

It was of interest to investigate the ability of this bacterium to adapt its Bchl synthesis to shifts in oxygen tension and to determine the role, if any, of the activator of ALA-S in connection to adaptability of *R. palustris* to changing atmosphere.

MATERIALS AND METHODS

Chemicals were obtained from Sigma, BDH, Oxoid, Merck or Fluka. *R. palustris* cells, from the collection of the Microbiology and Immunology Unit, FCEN, UBA, were grown anaerobically in the light or aerobically in the dark in the medium of Cohen-Bazire *et al.* (1957) as described by Viale *et al.* (1980a). Organisms were harvested in the late exponential phase of growth. These precultures provided the start of experiments on the effects of O_2 on Bchl synthesis and ALA-S. After growing anaerobically in the light, cells were grown under a mixture of 95% oxygen–5% nitrogen (v/v), bubbling at a total gas flow rate of 1 l gas/l medium/min during 4 hr. Where indicated chloramphenicol (2 mg/l) was added to the cultures either before or after oxygenation. All systems were kept in the light since the begining of growth up to harvesting. At intervals, portions of the cell suspension were taken, suspended in buffer, washed and disrupted by sonication as already described (Viale *et al.*, 1980a). In these samples, culture density (measured as turbidity in terms of OD at 680 nm), Bchl (Schön, 1968), ALA-S (Viale *et al.*, 1980a), succinyl-CoA-synthetase (Suc-CoA-S) (Kaufman and Alivisatos, 1955), ALA-dehydratase (ALA-D) (Gibson *et al.*, 1955) and protein content (Lowry *et al.*, 1951) were measured. Enzymic activities were determined immediately after obtaining the cell-free extracts, to avoid possible activations or inactivations which might occur spontaneously with time. Enzymic units are expressed as the amount on enzyme cata-

*This paper is dedicated to Dr Luis F. Leloir on the occasion of his 80th birthday, 6th September 1986.

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Table 1. Levels of enzymic activities and Bchl in *Rp. palustris* cells grown under different atmospheric and illuminating conditions

Growing conditions	Enzymic specific activities			Bchl $\left(\frac{\text{nmol}}{\text{mg prot}}\right)$
	Suc.CoA-S	ALA-S	ALA-D	
Anaerobically in the light	320	43.1*	30.6	32
Aerobically in the dark	370	15.5*	34.3	0
Anaerobically and 4 hr oxygenation in the light	340	16.2*	28.8	22

Values reported are the average of more than ten determinations. $*P < 0.001$. Experimental conditions are indicated in the text.

lysing the formation of 1 nmol of product per hour under the standard incubation conditions, and specific activity as the number of units per mg of protein.

RESULTS AND DISCUSION

Changes in ALA-S activity and Bchl content during adaptation from anaerobic to aerobic conditions

A correlation between Bchl content and the levels of ALA-S was found, when the cells were grown under different conditions of aereation (Table 1). Highest ALA-S activity and synthesis of Bchl was obtained in cells grown anaerobically in the light; in aerobiosis in the dark, Bchl formation is nil, while ALA-S activity is lower, with a value similar to that reached by cells grown anaerobically and then oxygenated for 4 hr; correspondly Bchl concentration also diminished after oxygenation. Instead, no changes in Suc-CoA-S and ALA-D activities were observed. These findings are indicating that ALA-S should have a key role in the regulation of Bchl synthesis in *R. palustris*.

When oxygen was introduced (zero time) into a culture growing exponentially under photoheterotrophically conditions (Fig. 1) the growth rate was unaffected, but Bchl synthesis was immediately halted; it can be seen that the steady-state specific Bchl content (quantity of Bchl per mg of protein, or per unit of cell mass) diminished. At the same time ALA-S activity was abruptly inhibited to nearly half, but when oxygen gassing was stopped, enzymic activity rapidly recovered its original levels and Bchl formation was slowly resumed; growth rate continued unaltered.

To determine whether or not increase in ALA-S

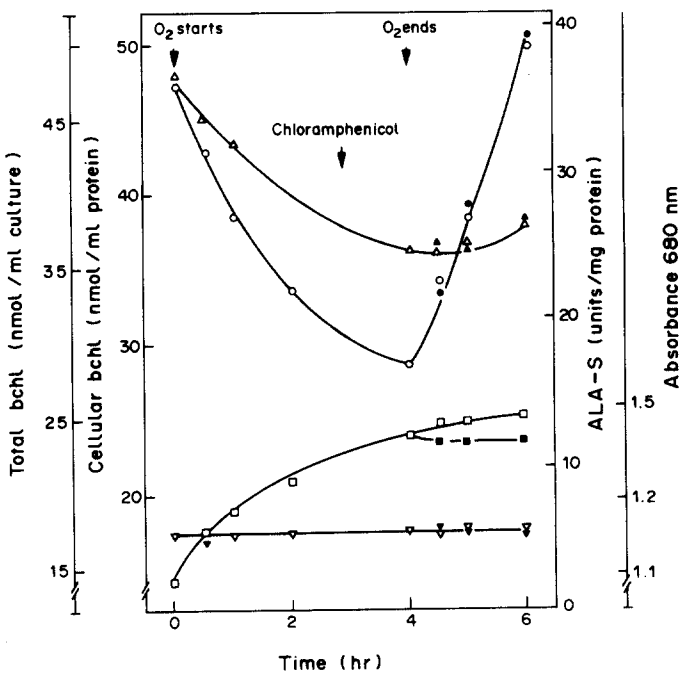

Fig. 1. Effect of oxygenation on *R. palustris* growing anaerobically in the light on ALA-S activity (○, ●), total Bchl content (▽, ▼), cellular Bchl content (△, ▲) and growth (□, ■). Cells were oxygenated (95% O_2–5% N_2) for 4 hr as inidcated by arrows. At the time oxygenation stopped, to half of the cultures chloramphenicol (2 mg/l) was added (closed symbols) while the other half without any addition (open symbols) was used as control, and both kept in the light and cultured for another 2 hr. At the times shown samples were taken for measurements as described in the text.

Table 2. "*In vitro*" effect of chloramphenicol on ALA-S activity in *Rp. palustris*

Cells growing conditions	Chloramphenicol	ALA-S specific activity
Anaerobically in the light	−	35.06
	+	36.19
Anaerobically and 4 hrs O_2 in the light	−	16.21
	+	15.30

Chloramphenicol (2 μg/ml) was added to the standard incubation mixture for measuring ALA-S in extracts of cells grown photoheterotrophically without and with 4 hr of oxygen gassing. Experimental conditions are as indicated in the text.

activity observed upon removal of oxygen from the medium, was due to the synthesis of new protein molecules, part of oxygenated cultures were supplemented with chloramphenicol. As expected its presence repressed growth; however it did not affect ALA-S reactivation or Bchl synthesis. The additon of chloramphenicol to the incubation mixture in the assay of ALA-S did not modify activity either (Table 2).

On the other hand, the activities of Suc-CoA-S and ALA-D were not altered during oxygenation or after its removal in the presence or absence of the protein synthesis inhibitor (Table 3) showing that these enzymes are not involved in the adaptation of *R. palustris* to changing environmental conditions.

The fact that reactivation of ALA-S activity and Bchl synthesis was not dependant on the presence of chloramphenicol, would indicate that this process might be caused by a metabolite that acts as an enzyme activator, which is accumulated during anaerobically growth, but which is not able of functioning or is depleted of, in aerobiosis.

Effect of chloramphenicol during oxygenation on ALA-S activity

The influence of chloramphenicol when the cultures were being oxygenated was studied (Fig. 2). As expected growth was inhibited by the inhibitor. It was found that the decay in ALA-S was the same, whether or not chloramphenicol was present during flushing of oxygen, but reactivation was lower when it had been added to the cultures since the beginning of gassing, a result different from that found when supplementing the medium with chloramphenicol after removal oxygen (Fig. 1). Moreover, if the crude extracts obtained after 4 hr of oxygenation were immediately diluted and activity measured at different intervals, ALA-S was much lower or not reactivation occurred at all. These findings are suggesting that in the spontaneous activation process of *R. palustris* ALA-S, observed when oxygenation has been interruped, would be involved more than one activator, supporting our proposal (Viale *et al.*, 1980b), and that its action, is concentration dependant, because activation was partially or completely prevented when cell suspensions (50 mg dry wt/ml) were disrupted and diluted right after.

CONCLUSIONS

It has been found that ALA-S activity of *R. palustris* is quickly diminished or enhanced in response to oxygen tension, indicating a rapid adaptation of this bacterium to changing atmosphere. Similar findings have been reported to occur in *R. sphaeroides*, although the experimental conditions were not the same (Higuchi *et al.*, 1968).

Increase in oxygen tension resulted in decreased rate of Bchl synthesis leading to diminished steady-state specific Bchl content, in agreement with Lascelles (1964) and Firsow *et al.* (1977) who demonstrated that in *R. sphaeroides*, development of the photosynthetic apparatus and Bchl formation were dependant on oxygen partial pressure in the cultures.

The increase in ALA-S activity when oxygenation stopped or its decline during gassing in the presence or absence of chloramphenicol are suggesting that the changes are not associated with protein synthesis but to the formation of some kind of factors which would

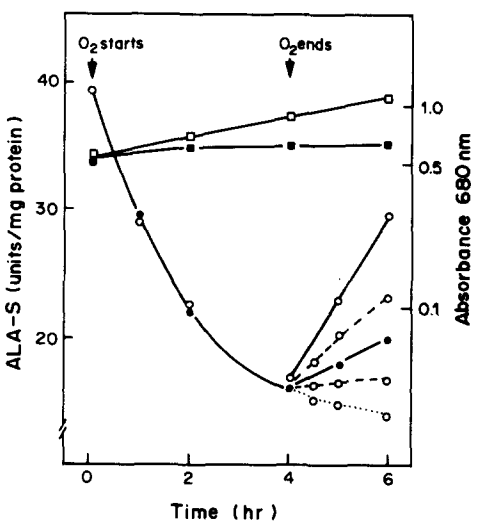

Fig. 2. Influence od chloramphenicol during oxygenation of *R. palustris* anaerobically grown in the light on ALA-S activity (○, ●) and growth (□, ■). Cells were oxygenated (95% O_2–5% N_2) for 4 hr as shown by the arrows. At zero time to half of cultures chloramphenicol (2 mg/l) was added (closed symbols). While the other half without any addition (open symbols) was used as control. After flushing oxygen was stopped, culturing continued for another 2 hr. Illumination was constant during the whole period. Samples were taken at intervals for measurements, as indicated in the text. Upon removal of oxygen from the cultures, ALA-S was determined in crude extracts obtained in the usual way, from suspensions of 50 mg dry wt/ml (Viale *et al.*, 1980a) (----) or diluted 1:2 (----) and 1:4 (. . . .) immediately after sonication.

Table 3. Effect of oxygenation of *Rp. palustris* growing anaerobically in the light on Suc.CoA-S and ALA-D activity

Treatment	Time (hr)	Specific activities	
		Suc-CoA-S	ALA-D
Oxygenation	0	227	27.5
	0.5	216	27.8
	1	214	28.0
	2	203	25.4
	4	220	28.8
Oxygenation stopped			
Without chloramphenicol	0.5	229	24.7
	1	223	25.5
	2	235	27.1
With chloramphenicol	0.5	212	19.9
	1	220	23.5
	2	237	23.4

Experimental conditions are the same as those indicated in legend to Fig. 1 and in the text.

act rapidly, modulating enzyme activity, in response to certain environmental stimuli, such as oxygen tension. However in *R. sphaeroides*, chloramphenicol had completely suppressed the induced synthesis of ALA-S (Higuchi *et al.*, 1965).

We have proposed that ALA-S of *R. palustris* would exist in two forms according to the conditions of growth (Viale *et al.*, 1980b). When cells are grown aerobically in the dark (A) a constitutive enzyme (ALA-S_c) of very low activity, and a factor (F_A) are formed; this factor, F_A, can bind the enzyme increasing its activity. Under anaerobic conditions in the light (Ph) besides the ALA-S_c, another factor (F_{Ph}) is formed, which binds the enzyme at different site, resulting in a high activity form of ALA-S, which can be activated further by binding to F_A, giving rise to a form exhibiting the highest activity detectable. Alternatively F_A and F_{Ph} could be the same low molecular weight compound, which would change, somehow, its structure under different atmospheric conditions (Viale *et al.*, 1980b).

When cells are oxygenated, the F_{Ph} factor is inactivated or partially destroyed, therefore ALA-S diminishes, in the same fashion whether chloramphenicol was present or not; paralelly under aerobiosis the formation of F_A is induced. After removal of oxygen, reactivation of ALA-S occurs by binding to F_A and F_{Ph}; however this process is less efficient when chloramphenicol had been added at the beginning of oxygenation, suggesting that formation of F_A might have been affected by the protein synthesis inhibitor, because when chloramphenicol had been supplemented to the medium at the time flushing oxygen stopped, subsequent reactivation was similar in cultures having or not the antibiotic.

In brief, it has been shown that *R. palustris* is able to promptly modify its steady-state Bchl content and the levels of activity of the pivotal enzyme ALA-S in response to changes in environmental conditions, particularly oxygen tension and light intensity. This rapid adaptation is possibly due to the existence of different forms of ALA-S, as well as factors which are able to interact with the enzyme under certain conditions, changing its activity. This particular regulatory phenomenon operated through the formation and action of low molecular weight compounds, namely F_A and F_{Ph}, could be conveniently referred to as a switch off/on mechanism of control of *R. palustris* ALA-S, which in turn would play a key role in determining the extent of synthesis of the pigmented membranous structures which serve to harvest and transduce light energy (Lascelles, 1968; Oelze and Drews, 1972).

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REFERENCES

Cohen-Bazire G., Sistron W. R. and Stanier R. Y. (1957) Kinetic studies of pigment synthesis by non-sulphur purple bacteria. *J. cell. comp. Physiol.* **49,** 25–68.

Firsow N. N. and Drews G. (1977) Differentiation of the intracytoplasmatic membrane of *Rhodopseudomonas palustris* induced by variations of oxygen partial pressure or light intensity. *Arch. Microbiol.* **115,** 299–306.

Gibson K. D., Neuberber A. and Scott J. J. (1955) The purification and properties of δ-aminolaevulic acid dehydratase. *Biochem. J.* **61,** 618–629.

Higuchi M., Goto K., Fujimoto M., Namiki O. and Kikuchi G. (1965) Effect of inhibitors of nucleic acid and protein synthesis on the induced synthesis of bacteriochlorophyll and δ-aminolevulinic acid synthetase by *Rhodopseudomonas spheroides. Biochim. biophys. Acta* **95,** 94–110.

Higuchi M., Ohba T., Sakai H., Kurashima Y. and Kikuchi G. (1968) Change of metabolic stabilty of δ-aminolevulinate synthetase in *Rhodopseudomonas spheroides* cells probably related to changes of intracellular oxidation-reduction state *J. Biochem.* **64,** 795–805.

Kaufman S. and Alivisatos S. G. A. (1955) Purification and properties of the phosphorylating enzyme from spinach. *J. biol. Chem.* **216,** 141–152.

Lascelles J. (1964) *Tetrapyrrole Biosynthesis and its Regulation*, pp. 84–111. Benjamin, New York.

Lascelles J. (1968) The bacterial photosynthetic apparatus. In *Advances in Microbial Physiology* (Edited by Rose A. H. and Wilkinson J. F.), Vol. 2, pp. 1–42. Academic Press, New York.

Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193,** 265–275.

Oelze J. and Drews G. (1972) Membranes of photosynthetic bacteria. *Biochim. biophys. Acta* **265,** 209–239.

Schön G. (1968) Der Einfluss von Amytal, 2,4-Dinitrophenol und Phenazinmethosulfat auf die Bacteriochlorophyllsynthese in anaeroben Kulturen von *Rhodospirillum rubrum*. *Arch. Mikrobiol.* **61,** 187–194.

Viale A. A., Wider E. A. and Batlle A. M. del C. (1980a) Biosintesis de bacterioclorofila en *Rhodopseudomonas palustris*. *Rev. Arg. Microbiol.* **12,** 1–9.

Viale A. A., Wider E. A. and Batlle A. M. del C. (1980b) Porphyrin biosynthesis in *Rhodopseudomonas palustris*. II. *Int. J. Biochem.* **12,** 729–733.

Viale A. A., Wider E. A. and Batlle A. M. del C. (1986) Porphyrin biosynthesis in *Rhodopseudomoas palustris*. XI: Extraction and characterization of δ-aminolevulinate synthetase. *Comp. Biochem. Physiol.* Part A. In press.