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Control by light and oxygen of B875 and B850 pigment–protein complexes in *Rhodopseudomonas sphaeroides*

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Control by light and oxygen of the formation of B875 and B850 pigment–protein complexes in *Rhodopseudomonas sphaeroides* was evaluated by use of Hill plots. Kinetics of oxygen-dependent control exhibited Hill coefficients of $n = 1.06$ and $n = 0.65$ for B875 and B850 complexes, respectively. Half-maximum inhibition of B875 complexes was at 20.4% air saturation of the medium and of B850 complexes at 0.9%. Light controlled both complexes with an identical sigmoidal kinetics of $n = 2.1$.

Phototrophic bacteria Rhodopseudomonas sphaeroides *Pigment complex* *Light-dependent control* *Oxygen-dependent control*

1. INTRODUCTION

In phototrophic bacteria the formation of bacteriochlorophyll and thus the formation of the photosynthetic apparatus is controlled by light and oxygen [1]. Because of lack of more specific information it has been assumed for many years that light and oxygen act on bacteriochlorophyll synthesis along common transmitters [2]. However, in *Rhodopseudomonas sphaeroides* both external factors were active with different types of kinetics suggesting differences at least in the rate limiting steps of control mechanisms [3]. In particular, low concentrations of oxygen inhibited bacteriochlorophyll formation in a negatively cooperative fashion and high concentration in a hypobolic fashion. Light, on the other hand, exerted its influence in a positively cooperative form. Here, we demonstrate that the observed differences in the control by oxygen can be confined to differences in control of the two light-harvesting bacteriochlorophyll–protein complexes B875 and B850, respectively, while light controls both complexes with identical kinetics.

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2. MATERIALS AND METHODS

Rhodopseudomonas sphaeroides (ATCC no. 17023) was grown in continuous culture at 30°C and pH 7.0 with automatic pH and pO_2 measure and control devices (Biostat S, B. Braun, Melsungen). The pO_2 meter was calibrated with air and, therefore, relative oxygen concentrations are given as percent air saturation of the culture medium [3]. The complex growth medium R8ÄH [4] with 1.5 g malate/l and 0.5 g yeast extract/l, was added at a dilution rate of $D = 0.109\ h^{-1}$. Phototrophic conditions were established by illuminating the cultures with white incandescent light as in section 3. All determinations were performed with cultures after they had reached steady states. Bacteriochlorophyll and protein were estimated as in [5,6]. B875 and B850 bacteriochlorophyll–protein complexes were determined with cell homogenates on the basis of the corrected absorption peaks at 875 and 850 nm [7] and of molar absorption coefficients from [8].

3. RESULTS

Rhodopseudomonas sphaeroides was grown at

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different oxygen concentrations in the dark to evaluate the effect of oxygen on the formation of bacteriochlorophyll complexes. Specific cellular levels of B875 as well as B850 pigment–protein complexes were determined and plotted against the corresponding values of air saturation of the medium employed to grow the organisms (fig.1). This revealed steep increases of the amounts of both complexes per cell protein as the oxygen concentration approached zero. But while B875 pigment–protein complexes were measurable over the entire range of oxygen concentrations obtainable with air, B850 pigment–protein complexes were not detectable above 50% air saturation of the medium. Analyses of the inhibitory kinetics exerted by oxygen through Hill plots revealed a hyperbolic kinetics (n = 1.06) for the control of B875 pigment–protein complexes while the Hill coefficient (n = 0.65) for the control of B850 pigment–protein complexes indicated negative cooperativity (fig.2). Half-maximum inhibitions of B875– and B850–protein complex cellular levels were obtained at 20.4% and 0.9% air saturation, respectively. Calculation of correlation coefficients (r) for the regression lines in fig.2 revealed significance of the data on a 99% confidence level.

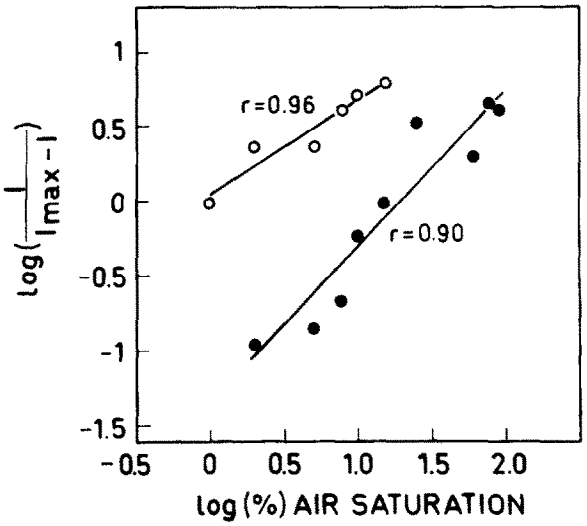

Fig.2. Hill plot of the data of fig.1: B875 pigment–protein complexes (●); B850 pigment–protein complexes (○); correlation coefficients (r).

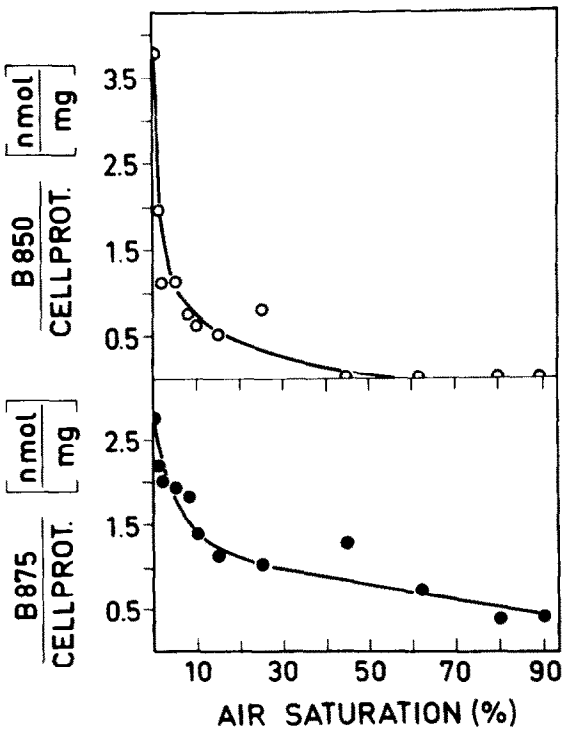

Fig.1. Steady state cellular levels of B875 and B850 pigment–protein complexes in *Rhodopseudomonas sphaeroides* growing in the dark at different oxygen concentrations of an aerated chemostat culture.

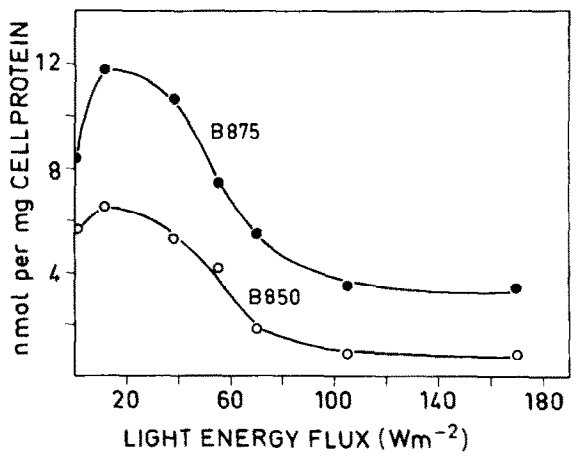

Fig.3. Steady state cellular levels of B875 and B850 pigment–protein complexes in *Rhodopseudomonas sphaeroides* growing in a chemostat at a constant oxygen concentration of 1% air saturation of the medium and different light energy fluxes.

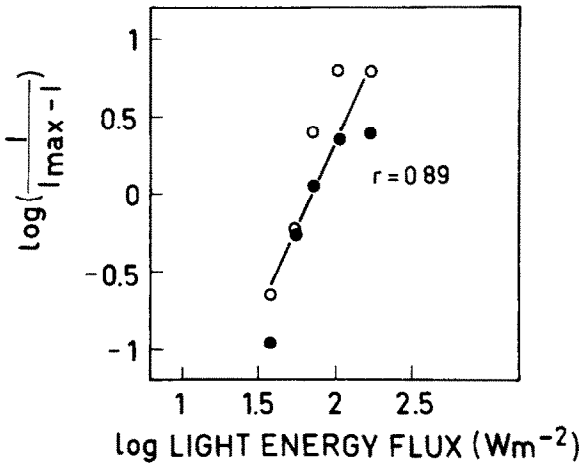

Fig.4. Hill plot of the data of fig.3. For symbols see fig.2,3.

When growing at low light energy fluxes *R. sphaeroides* increases its cellular contents of bacteriochlorophyll which is predominantly associated with B850 pigment–protein complexes while the levels of B875 pigment–protein complexes stay largely constant [9]. Therefore, investigations on the control of both of the pigment–protein complexes by light have to be performed with cells of relatively low initial bacteriochlorophyll contents. This is conveniently achieved by growing cultures at an appropriately low constant oxygen concentration and different light energy fluxes [3]. Here, oxygen of 1% air saturation allowed the simultaneous study of the control of both complexes by light. The data revealed (fig.3) that light controlled both pigment complexes in a sigmoidal fashion. Calculation of the data on the basis of the Hill equation and subsequent statistical treatment of the individual regression lines for B875–protein and B850–protein complexes, respectively, showed the significance of only one identical regression line for both complexes with a Hill coefficient of $n = 2.1$ (fig.4). The corresponding correlation coefficient (r) revealed significance of the data on a 99% confidence level.

4. DISCUSSION

According to [10] *Rhodopseudomonas sphaeroides* produces the light-harvesting B875 pigment–protein complexes in a largely constant proportion to the photochemical reaction center–pigment complex. These data indicate that the formation of the entire reaction center–B875 pigment unit, on the one hand, and, on the other, of accessory light-harvesting B850 pigment–protein complexes are controlled by oxygen through at least partially different reactions. No such differences could be registered with respect to the control of both complexes by light. Moreover, the data confirm observations of different types of kinetics underlying the control of bacteriochlorophyll synthesis by oxygen and light [3]. Investigations on the molecular basis for the observed differences of control are in progress.

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