Arrhenius Plots Indicate Localization of Photosynthetic and Respiratory Electron Transport in Different Membrane Regions of *Anabaena*

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Z. Naturforsch. **36 c,** 1036–1040 (1981); received July 27, 1981

Photosynthesis, Respiration, Blue-Green Algae, Arrhenius Plots, Phase Transition Temperatures

Photosynthetic and respiratory electron-transport reactions by the blue-green alga, *Anabaena variabilis*, exhibit a strict temperature dependence *in vivo* as well as in the cell-free system. The ratio of respiratory oxygen uptake in the dark and oxygen evolution in the light is high after growth at low temperatures (20 °C) and low after growth under optimum temperatures (28 °C).

Respiration and photosynthesis show different break temperatures in the Arrhenius plots. Increase of growth temperature yields higher break points for photosynthetic or respiratory electron transport as well. These data are taken as evidence that photosynthetic and respiratory electron transport chains are embedded in different membrane areas.

Introduction

A regulation of respiration by photosynthesis was assumed, since Kok (1949) had claimed a light-induced inhibition of respiration [1]. Of particular interest is the interaction of both processes in blue-green algae, since these organisms have no intracellular compartmentation for complex redox reactions. The localization of respiratory electron transport is still unknown. It may operate in the cytoplasmatic membrane [2], the thylakoid membrane [3], or in both membrane systems.

Generally, membrane-bound electron transport reactions exhibit a significant temperature dependence: The straight lines of Arrhenius plots are broken by one or more discontinuities [4]. This is indicative of an apparent change of activation energy and can be discussed in terms of a phase-transition process within the membrane, as has been particularly worked out from measurements of photosynthetic [4, 5, 11] and mitochondrial [4] electron transport. The temperature dependence of respiration of the blue-green alga has not been examined so far.

In this communication, the temperature profiles of both photosynthetic and respiratory electron transport are documented and discussed with regard to the possible localization of the respiratory chain.

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0341-0382/81/1100-1036 $ 01.00/0

Materials and Methods

The facultatively heterotrophic blue-green alga, *Anabaena variabilis* Kütz. (ATCC 29413), was grown axenically under N_2-fixing conditions in an inorganic medium [7] at different temperatures which were exactly controlled (± 0.25 °C) in a growth apparatus built by Kniese-Edwards, Marburg (Germany). The cultures were illuminated by fluorescent light (Osram M-L, 40 W/30-1, warm, white) with an intensity of 12.5 W/m². Each culture vessel contained 200 ml; cultures were gassed with air enriched with 1.5% CO_2 (v/v). *Anabaena* was cultured with appropriate dilutions for at least six generations at a certain temperature before doing the assays.

Inoculum was 0.8 to 1.2 µl packed cell volume/ml of algae suspension. The cells were harvested at the end of the logarithmic phase, which was reached after 3 to 6 days dependent on the growth temperature used. Samples were removed from the culture vessels, temperature-adjusted in a water-bath, then immediately transferred into the reaction chamber of a Clark-type electrode [8]. Care was taken not to exceed a time gap of 1 to 3 min between removal from the culture and measurement. The temperature was controlled by a device incorporated in the electrode reaction vessel allowing to keep the temperature within ± 0.2 °C.

Electron-transport reactions of the cell-free system were assayed with membranes isolated as follows: Algae cultivated for three days were harvested

(4 min, 2000 × *g*) and incubated for 1 h at 34 °C with 5 mg of lysozyme per mg chlorophyll in a medium containing 10 mM 2(N-morpholino)-ethane sulfonic acid, MES, adjusted with NaOH pH 6.9; 5 mM Na-K-phosphate; 10 mM $MgCl_2$; 500 mM sorbitol; 2% bovine serum albumin (incubation medium, after [9]). After lysozyme treatment, followed by a centrifugation step (90 sec, 300 × *g*), the spheroplasts obtained were resuspended in the incubation medium, and subsequently washed and shocked twice with reaction medium (10 mM N-2-hydroxyethyl-piperazine-N′-sulfonic acid, HEPES, adjusted with KOH, pH 7.8; 6 mM Na-K-phosphate; 10 mM $MgCl_2$). Two centrifugation steps, 5 min at 2000 × *g* each, were included. Thereafter, the material was stored in ice with incubation medium including 0.5% bovine serum albumin. The membranes retained full photosynthetic and respiratory activity for at least 5 h.

Oxygen exchange under light-saturating conditions and in the dark was measured in reaction medium. Chlorophyll concentration was 20 µg/ml throughout. Chlorophyll was determined according to [9].

Results

1. Rates of photosynthesis and respiration

Respiration of blue-green algae is rather low as compared to bacteria [10]. Respiratory oxygen uptake reaches only a few percent of photosynthetic oxygen evolution. Table I demonstrates the rates of oxygen uptake and evolution as referred to cell mass (packed cell volume, pcv) for different growth temperatures. Chlorophyll is not a reliable reference, because pigment content changes with growth temperature; at low temperature, the ratio of chlorophyll to pcv decreases. Cells of *Anacystis nidulans*, free of chlorophyll and thylakoids, have been obtained with markedly lower temperatures, *e.g.*, under non-growing conditions [2]. Autotrophic cultures of *Anabaena variabilis* grow slowly, but substantially at temperatures higher than 17 °C. Independent of growth temperature, photosynthesis and respiration increased with the assay temperature. Depending on growth temperature, both processes exhibited an optimum varying between 27 and 29 °C.

At low temperatures (20 °C), respiration increased up to 40% of the photosynthetic rate. Cultures growing at optimum temperature showed little respiratory activity as compared to photosynthesis. Blue-green algae certainly are rather thermophilic organisms, but they grow well in temperate waters with temperatures around 20 °C. Furthermore, it is conceivable that blue-green algae adapted to aquatic environments with low light intensities do not always prefer saturated-light conditions as applied during our measurement. So, we assume that at least in temperate climates the ratio of respiration to photosynthesis is rather high (Table I, last column). Regulation of respiration by light appears to be an important factor in the energy budget of the cell.

2. Break points in the Arrhenius plots of intact-cell activities

In Fig. 1, the temperature dependence of respiration and photosynthesis is shown. In parallel experi-

Table I. Rates of apparent photosynthesis and respiration of *Anabaena variabilis* grown at different temperatures.

Growth temp. [°C]	Chlorophyll / pcv [µg/µl]	Assay temp. [°C]	Respiration µmol O_2 / ml pcv × h	Photosynth. µmol O_2 / ml pcv × h	O_2 taken up / O_2 evolved × 100
20	1.1	20	57	163	35.0
		28	86	145	35.1
		35	120	342	35.1
28	2.4	20	76	590	12.9
		28	113	1119	10.1
		35	160	1427	11.2
35	2.1	20	33	227	14.5
		28	49	412	12.0
		35	62	536	11.6

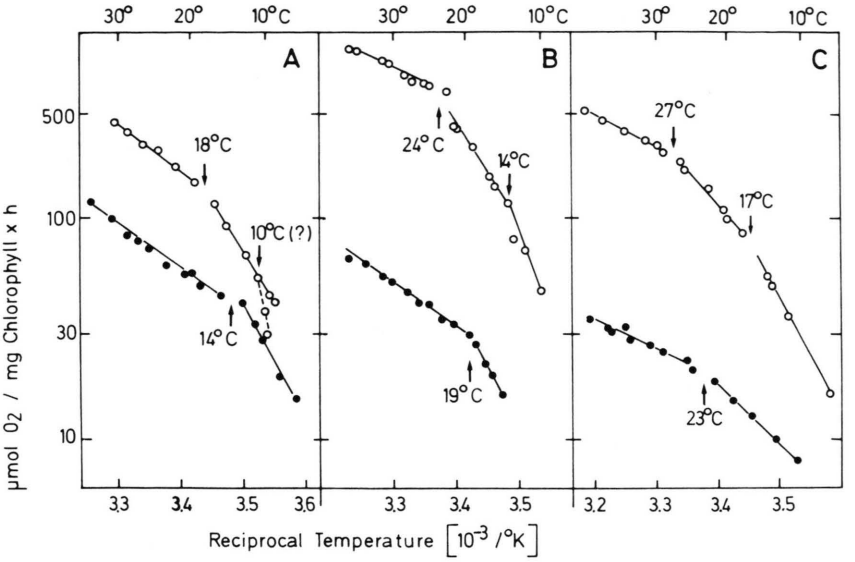

Fig. 1, A–B. Arrhenius plots of photosynthetic oxygen evolution (○–○) and respiratory oxygen uptake(●–●) measured with intact *Anabaena variabilis* after growth at different temperatures: A = 20 °C; B = 28 °C; C = 35 °C. The figures represent average values from 10 to 15 measurements with 4 to 6 different cultures.

ments somewhat different rates had been observed. However, the break points were determined with an error of ± 1 °C. Data below 10 °C were difficult to obtain due to very low reaction rates. Therefore, the break point around 10 °C in Fig. 1A could not be determined exactly.

The Arrhenius plots for photosynthetic oxygen evolution (upper curves) exhibited two break points separated by a temperature range of about 10 °C for all three growth temperatures used. The data for respiratory oxygen uptake (Fig. 1, lower curves), however, showed just one discontinuity in all cases. This break was between the two break points obtained for photosynthetic reactions at each growth temperature. The temperature for all break points determined depended on growth temperature, and the points shifted to higher values after cultivating *Anabaena variabilis* at higher temperatures. A similar behaviour has been reported for photosynthetic electron transport of isolated chloroplasts [11] and *Synechococcus* cells [12].

3. *Temperature profiles of cell-free systems*

The following *in-vitro* experiments were done to exclude the possibility of the breaks reported above being caused by enzymatic reactions of, *e.g.*, the carbohydrate pathways. The growth temperature of *Anabaena* was 28 °C. Oxygen uptake of isolated membranes in the dark was proved to be both KCN-sensitive (80% inhibition with 1 mM KCN) and catalase-insensitive, indicative of true respiration. Electron transport with N,N,N′,N′-tetramethyl-*p*-phenylenediamine (TMPD) and sodium ascorbate as donor for the respiratory chain showed a break point near 19 °C in the Arrhenius plot of Fig. 2. The light-induced, DCMU-sensitive oxygen uptake exhibited two discontinuities near 13 °C and 24 °C*. These findings are quite in accordance with our finding for whole cells as seen in Fig. 1B. Light-induced oxygen uptake by our isolated membrane material measured without any addition of co-factors is quite high (about 20 µmol O_2 per mg chlorophyll × h, assayed at 23 °C) as compared to isolated eucaryotic chloroplasts. However, the rate is lower than that of dark respiration in the presence of TMPD as electron donor (Fig. 2, lower curve). This was similar to our findings in *Nostoc muscorum* (E. Stürzl, unpubl. results).

The Hill reaction (H_2O → potassium ferricyanide) had a distinct break point at 13 °C. Above 28 °C,

* G. A. Peschek, Vienna, found temperature break points different for photosynthetic and respiratory electron transport using isolated membranes from *Anacystis nidulans* (pers. communication).

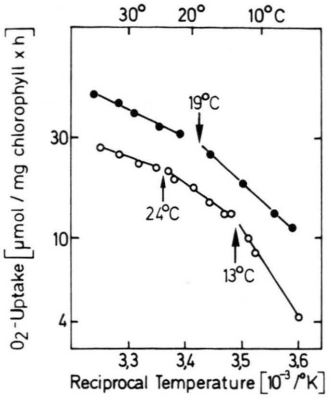

Fig. 2. Arrhenius plot of oxygen uptake in the dark, in the presence of TMPD/Asc. (●—●), and light-induced oxygen uptake without any addition of donor or acceptor (○—○). Measurement was performed with isolated membranes of *Anabaena variabilis*. The data given are means from three experiments. TMPD: 0.1 mM; Na-ascorbate: 0.5 mM.

the data scattered. Thus a second break point could not be exactly determined (data not shown). The photosystem-I reaction with TMPD/ascorbate as electron donor couple and methylviologen/O_2 as acceptor exhibited a straight line up to 28 or 29 °C, but bent off at higher temperatures without a clear discontinuity. In the temperature range between 5 °C and 28 °C, the photosystem-I reaction was hardly affected by temperature and exhibited no discontinuity (data not shown).

Discussion

Our data with intact cells and isolated thylakoids as well (Figs. 1, 2) clearly show two break points in the Arrhenius plot of photosynthetic activity. Two points were also found for *Synechococcus* [5, 12].

In *Anabaena*, the break points of the upper temperature region were located closer to the growth temperature than reported for other blue-greens. For *Synechococcus lividus* grown at 55 °C, a break point at 43 °C was reported for the higher temperature region. Cultivation of *Synechococcus* at lower temperatures did not influence the break point in the lower temperature region, whereas the break point in the higher temperature region was shifted to lower temperature [12]. Irrespective of growth temperature in *Anacystis nidulans*, the break point in the higher temperature region is 16 to 17 degrees below the growth temperature [13]. Chloroplasts isolated from *Spinacia oleracea* exhibit a discontinuity between 15 and 20 °C, which is quite close to the growth temperature. A second break at lower temperatures could not be measured [11, 14].

The break points of respiration processes in various organisms are different and below the growth temperature. In sheep-liver mitochondria the break point has been reported to be 10 to 12 degrees below the growth temperature [15]. In *Bacillus caldotenax* [16] and *Saccharomyces cerevisiae* [17], this temperature span was 15 and 8 to 16 degrees, respectively, below the growth temperature.

At a first glance, discontinuities of the Arrhenius plot may be open to speculations. It may be argued that they reflect temperature-dependent changes of the rate-determining enzymes. Kawada *et al.* (1981) have shown that the purified NADH oxidase of *Bacillus caldotenax* displays a straight line in the Arrhenius plot, whereas the membrane-bound enzyme has two break points [16]. Furthermore, the shift of the discontinuities depending on growth temperatures renders unlikely that enzymes contribute to the data measured here by, *e.g.*, changing their configuration.

Another explanation for the discontinuities may be the direct interaction between lipids and membrane proteins, independent of a phase separation. The change of apparent activation energy may be due to their lipid-protein interaction, not to the phase transition of the membrane. To discuss this possibility, it should be mentioned that a phase separation had been determined by chlorophyll fluorescence [12] or spin-label experiments [4], which are independent of enzyme activities. The molecular mobility of spin-labeled substances in thylakoids of tomato chloroplasts showed a dramatic change of the activation energy below 12 °C. The Arrhenius plots of the EPR measurements yielded a biphasic curve with a break point at 12 °C, which was also obtained with the Arrhenius plot of photosynthetic $NADP^+$ reduction [4]. Similar results had been reported for the photosynthetic electron transport chain of *Anacystis nidulans* [13, 20] as well as for the respiratory activity of sheep-liver mitochondria [4]. It has been demonstrated further that a change of apparent activation energy corresponds with the (upper) phase-separation temperature of the membrane. Membranes of *Anabaena*

variabilis, however, have not been examined with spin-label experiments.

Obviously, the break points reported in this study can be convincingly interpreted as being due to phase separation of the membrane. The two discontinuities can be explained as indicating the temperature range of the phase separation. A decrease of growth temperature is followed by a change of lipid composition of the membranes. In *Anabaena variabilis* [19] as well as in *Synechococcus lividus* [12], an increase of short and unsaturated lipids was reported, causing higher membrane fluidity [20] when the growth temperature was decreased.

The photosynthetic break points observed with *Anabaena variabilis* are about 10 degrees apart. In most cases, however, the phase separation of biological membranes covers a wider temperature range [5, 11, 18]. This cannot be explained at the moment. Respiration of *Anabaena* exhibits only one break point. Presumably there is a second one, but the lower phase-separation temperature apparently is beyond our possibilities to measure it. As already mentioned, two break points have been reported with (membrane-bound) NADH oxidase of the thermophilic *Bacillus caldotenax* [16]. – Therefore, the data imply that the temperature ranges of phase separation of photosynthetic and respiratory redox activity are different, most probably depending on different lipids associated with these two redox systems.

Recently, Almon and Böhme [21] found in our laboratory that photophosphorylation of *Nostoc muscorum* spheroplasts was strongly inhibited by NH_4^+, while aerobic phosphorylation in the dark was not affected. This was explained as evidence that respiration is located in the cytoplasmic membrane [21].

Conclusively, respiration and photosynthesis are most likely located on different membranes (cytoplasmatic and thylakoid membrane) or in different areas of the thylakoids. Our results exclude the alternative, namely that photosynthesis and respiration share a *common* electron-transport chain. The findings, however, do not exclude an interaction between both electron-transport system via mobile components.

Acknowledgements

This study was supported by the Deutsche Forschungsgemeinschaft (SFB 138). We thank Profs. Dr. G. Adam and Dr. H. Böhme, both of the Faculty of Biology of this University, for many helpful discussions and for carefully reading the manuscript.

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