POSSIBLE ROLE OF SINGLET MOLECULAR OXYGEN IN THE CONTROL OF THE PHOTOTACTIC REACTION SIGN OF *ANABAENA VARIABILIS*†

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(Received January 5, 1984)

Summary

In *Anabaena variabilis* the phototactic reaction sign at high photon fluence rates is reversed from negative to positive by the addition of 10^{-3} M sodium azide. This effect has been interpreted to indicate that a hypothetical phototactic reaction sign reversal generator in the sensory transduction chain is controlled by the level of singlet oxygen which is quenched by azide. To confirm this hypothesis, experiments with other quenching agents, at various pH values, as well as in nitrogen and oxygen atmospheres, have been carried out. Experiments with a phosphate buffer at various pH values show that the transition point from positive to negative phototaxis is shifted from 5.7 W m^{-2} at pH 7 to about 15 W m^{-2} at pH 6. At pH 5.5 only positive responses occur even at 120 W m^{-2}. At lower pH values movement ceases. However, at alkaline pH (8.0) the transition point is shifted to lower fluence rates (4.5 W m^{-2}). When imidazole buffer (pH 6) is used, the reaction sign reversal is even more pronounced. L-histidine, a $^1O_2$ quencher, decreases negative phototaxis at 13.5 W m^{-2} with increasing concentration, but does not reverse the reaction sign. 1,4-diazabicyclo[2.2.2]octane reverses the reaction sign at 13.5 W m^{-2} from negative to slightly positive, but is cytotoxic at higher concentration. When the Petri dishes are gassed with nitrogen and oxygen, only positive reactions are observed up to 54 W m^{-2}. These results support the hypothesis that in *Anabaena* the phototactic reaction sign is controlled by the $^1O_2$ concentration via a reaction sign reversal generator. This mechanism enables the *Anabaena* filaments to escape dangerous light conditions. 2×10^{-2} M KI inhibits both positive and negative phototaxis.

1. Introduction

In earlier papers [1, 2] it has been reported that in the cyanobacterium *Anabaena variabilis* negative phototaxis, *i.e.* movement away from the light

†Paper presented at the COSMO 84 Conference on Singlet Molecular Oxygen, Clearwater Beach, FL, U.S.A., January 4 - 7, 1984.

0047-2670/84/$3.00

source caused by high photon fluence rates, is reversed to positive phototaxis, *i.e.* movement towards the light source, by the addition of 10^{-3} M sodium azide. Since the azide ion is an effective quencher of $^1O_2$ [3], it has been proposed that the phototactic reaction sign is controlled by the level of $^1O_2$ produced in photosynthesis. If at high fluence rates the quenching capacity of the naturally occurring $^1O_2$ quenchers, such as carotenoids, is not sufficient, the phototactic reaction sign becomes negative. Addition of azide, an artificial $^1O_2$ quencher, lowers the level of $^1O_2$, thus allowing positive reactions even at high photon fluence rates. If this hypothesis were correct, any change in the environment of the cyanobacterium which prevents or decreases the production of $^1O_2$ should reverse the reaction sign from negative to positive or at least shift the transition point, *i.e.* the fluence rate at which the sign of the phototactic response is changed, to higher fluence rates. Conversely, any treatment of the cyanobacterium with $^1O_2$ enhancing methods should shift the transition point to lower fluence rates. Therefore, to verify this hypothesis, the *Anabaena* trichomes were exposed to various environmental conditions of both types. The results of these experiments are reported here.

2. Material and methods

All experiments were carried out with the cyanobacterium (blue–green alga) *Anabaena variabilis* Kütz strain B 377 which was obtained from the culture collection of algae at Indiana University. It was grown at 21 °C under continuous fluorescent light (1 W m^{-2}) in Petri dishes on membrane filters (type 307; Sartorius, Göttingen, F.R.G.) in the medium D designed by Sheridan [4].

For the phototactic experiments Petri dishes were filled with 6 mM Soerensen phosphate buffer (pH 7.0) agar with and without $^1O_2$ quenching agents or other chemicals. In one series of experiments the pH was varied between 5.0 and 8.0; in another series imidazole buffer (pH 6.0) was used. The agar plates were inoculated in the centre with about 20 mg cyanobacterial mass (fresh weight) of a culture 5 days old and then exposed to unilateral irradiation. Possible effects of the tested chemicals and the experimental conditions on motility and photokinesis were controlled as described by Nultsch *et al.* [1]. In general, the experiments were carried out in air. To study the phototactic behaviour in pure nitrogen or oxygen, special Petri dishes with inlet and outlet tubes were used which were gassed with nitrogen and oxygen respectively.

For the evaluation of phototaxis the movement tracks of the trichomes towards the light source or away from it were measured. The distances traversed per time unit in either direction were calculated and subtracted from each other (for further details see Nultsch *et al.* [5]).

In some experiments it was found that a few trichomes moved in one direction, whereas the bulk of the organisms moved in the opposite

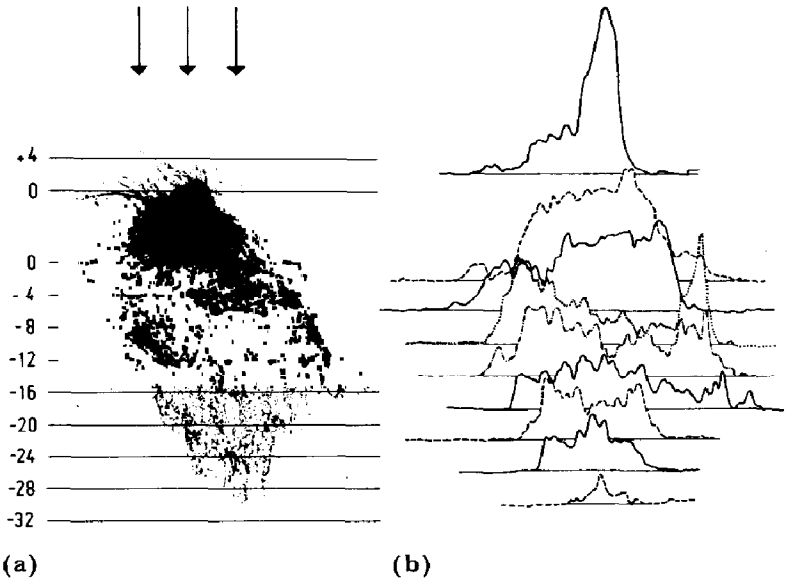

Fig. 1. (a) Photogram of *Anabaena* trichomes spreading on the agar surface away from the light source; (b) densitometric recordings of scanning areas 4 mm wide.

direction. In these cases the distribution of the organism was measured by scanning the whole spreading area with a densitometer (B. Lange, Berlin, F.R.G.), as shown in Fig. 1. The records were evaluated by planimetry, and the distances weighted by multiplication with a distance factor. The sum of the planimetric values of the negative spreading area was subtracted from that of the values of the positive spreading area, and the difference was used as a relative measure for positive and negative movement.

In general, white light was produced by Prado projectors (Leitz, Wetzlar, F.R.G.) with quartz–iodine lamps (Osram; 24 V, 250 W). Different fluence rates were obtained by inserting neutral density filters. In the red light experiments RG 2 cut-off filters (2 mm; Schott and Gen., Mainz, F.R.G.) were used. The energy was measured with a thermopile (Kipp and Zonen, Delft, Holland) coupled with a microvoltmeter (Keithley, Cleveland, OH, U.S.A.). The temperature during the experiments was 20 °C.

The following chemicals were used: KI (pro analysi), L-histidine and imidazole (Merck, Darmstadt, F.R.G.); 1,4-diazabicyclo[2.2.2]octane (DABCO) (EGA-Chemie, Steinheim, F.R.G.).

3. Results

3.1. pH *experiments*

As has been shown by Bonneau *et al.* [6] and Pottier *et al.* [7], the quantum yield of the $^1O_2$ production in an aqueous medium depends on the pH. This has been confirmed by Ito [8], who found that the photodynamic

inactivation rates of yeast cells treated with toluidine blue increased with an increase in pH from 5.8 to 7.8. Therefore, we tested the effects of various external pH values between 5.0 and 8.0 (phosphate buffer) on the phototactic reaction sign. The effect of the external pH on the intracellular pH is controversial. Whereas in several plants the cytoplasmic pH is relatively insensitive to external pH changes [9], Kaplan [10] attributed the observed inhibition of photosynthesis by strongly alkaline external pH in *Anabaena variabilis* to a change in the internal pH. In our experiments the pH dependence of the phototactic reaction sign is clearly demonstrated. As shown in Fig. 2, the transition point from positive to negative phototaxis is shifted from 5.7 W m^{-2} at pH 7.0 to 15 W m^{-2} at pH 6.0. At pH 5.5 no negative

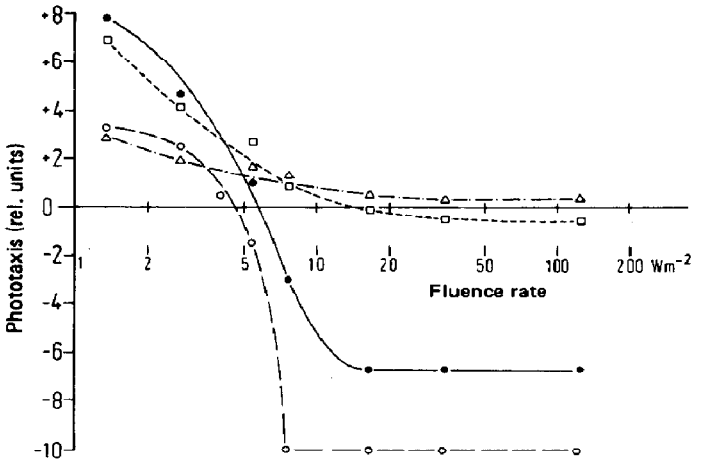

Fig. 2. Phototactic fluence rate–response curves measured at various values: △, pH 5.5; □, pH 6; ●, pH 7; ○, pH 8. (The phototaxis was calculated from densitometric measurements.)

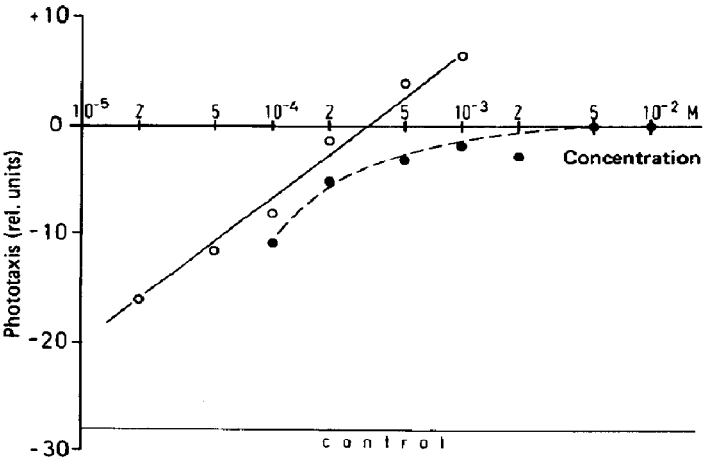

Fig. 3. Effects of imidazole buffer (pH 6) and L-histidine on phototaxis at 13.5 W m^{-2}: ○, imidazole; ●, L-histidine.

reactions occur up to 120 W m^{-2}. No experiments at pH < 5.5 are possible, since motility ceases. At pH 8.0 the transition point is shifted to lower fluence rates (4.5 W m^{-2}). The pH effect is more pronounced when imidazole buffer is used instead of the phosphate buffer, because imidazole itself has a quenching effect. As shown in Fig. 3, the negative response at 13.5 W m^{-2} is decreased in imidazole buffer (pH 6) with increasing imidazole concentration. Above 3×10^{-4} M imidazole the reaction becomes positive. These experiments strongly support the $^1O_2$ hypothesis.

3.2. Effects of $^1O_2$ quenchers

Among the various $^1O_2$ quenchers, L-histidine [11, 12] is perhaps the most compatible agent physiologically. It does not influence positive phototaxis at 1.35 W m^{-2}, but decreases negative phototaxis at 13.5 W m^{-2} with increasing concentration (Fig. 3). Above 2×10^{-3} M no negative responses occur so that the trichomes spread randomly from the inoculation spots forming circular patches. Thus, a reversion of the reaction sign to positive phototaxis was not observed.

DABCO is also an effective quencher of $^1O_2$ [13, 14]. In our experiments up to 2 mM DABCO did not influence negative phototaxis of *Anabaena* at 13.5 W m^{-2} (Fig. 4). At 5 mM, however, slightly positive phototactic reactions were observed. At 10 mM movement ceased. These results also support the $^1O_2$ hypothesis, but confirm the observation of Ito [3] that DABCO is not a suitable substance to protect living cells from photodamage because of its toxicity.

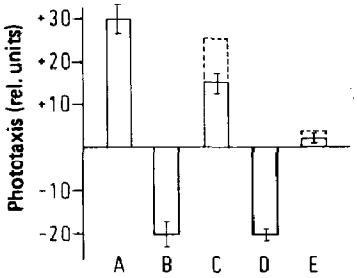

Fig. 4. Effect of DABCO on phototaxis: A, control at 1.35 W m^{-2}; B, control at 13.5 W m^{-2}; C, 10^{-3} M azide at 13.5 W m^{-2}; D, 2 mM DABCO at 13.5 W m^{-2}; E, 5 mM DABCO at 13.5 W m^{-2}; — — —, photokinetically corrected values; I, standard deviations.

3.3. Effects of nitrogen and oxygen gassing

As already shown by Mathews and Sistrom [15], the photodynamic effect of toluidine blue is prevented by nitrogen gassing. Similar results were obtained by Ito and Kobayashi (*cf.* ref. 3) with acridine orange as photosensitizer. Therefore, we expected that the gassing of the Petri dishes with nitrogen would also decrease the $^1O_2$ concentration and, hence, reverse the reaction sign. As expected, in the nitrogen-gassed Petri dishes the phototactic reaction was positive up to a fluence rate of 54 W m^{-2}, but negative above 5 W m^{-2} in the control (Fig. 5). In another series of experiments the Petri

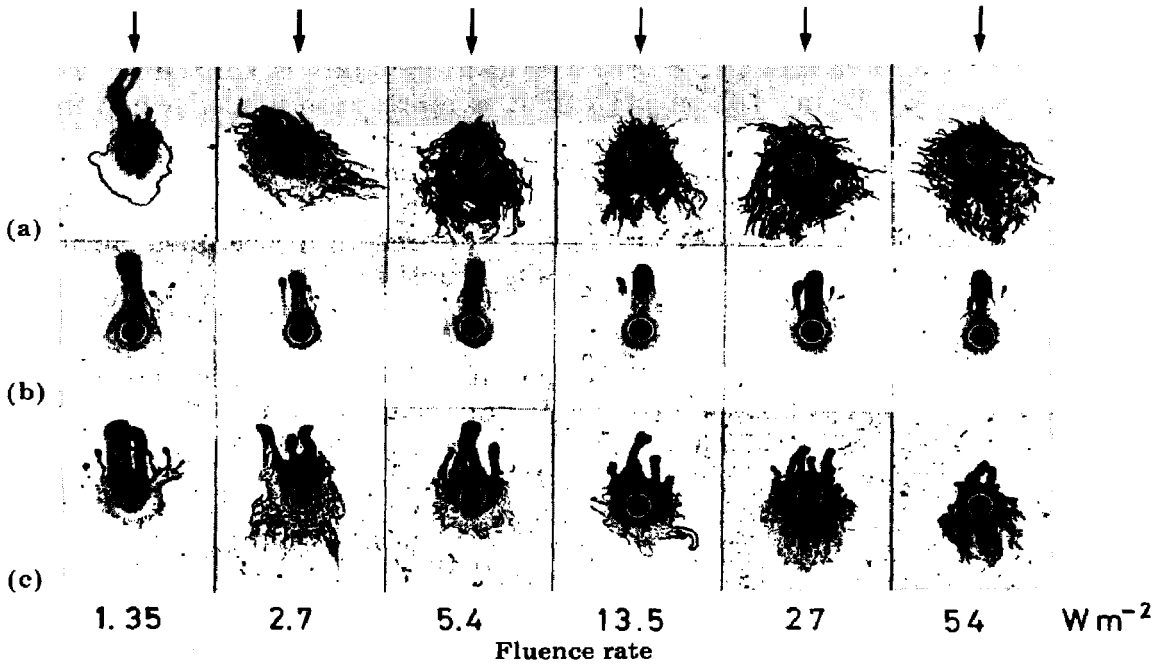

Fig. 5. Effect of nitrogen and oxygen gassing on phototaxis: (a) air control (no gassing); (b) nitrogen gassing; (c) oxygen gassing. The inoculation spots at the beginning of the experiments are marked by white circles. The direction of light is indicated by arrows (top).

dishes were gassed with $^3O_2$, since we expected that the higher $^3O_2$ availability would increase the $^1O_2$ production. However, as seen in Fig. 5, this is not true. On the contrary, even in the $^3O_2$-gassed Petri dishes a significant reaction sign reversal from negative to positive was observed at higher fluence rates. Apparently the decreased availability of oxygen is not the cause of the reaction sign reversal in nitrogen-gassed samples, as is the case in the above-mentioned experiments [3, 15]. Since we assume that $^1O_2$ is produced in side reactions of photosynthesis, the sign reversal is rather the result of $^1O_2$ removal by the gas stream. As this can be achieved by any gas, even gassing with $^3O_2$ causes a reaction sign reversal at higher fluence rates.

3.4. KI

The iodide ion is also a quencher of $^1O_2$ [16]. In addition, however, KI quenches singlet and triplet states of some sensitizers responsible for $^1O_2$ production. Therefore, the effect of KI on phototaxis was also investigated. 2×10^{-2} M KI, which decreases motility by only 25%, inhibits phototaxis completely (Fig. 6). However, this inhibitory effect is not specific, since positive as well as negative phototaxis is inhibited to the same extent (Fig. 7). This suggests that the excited states of the photoreceptor molecules involved are quenched by KI, rendering the organisms incapable of reacting phototactically. Although the KI experiments do not give direct evidence for the $^1O_2$ hypothesis, they fit well in the hypothetical scheme of the reaction chain [1].

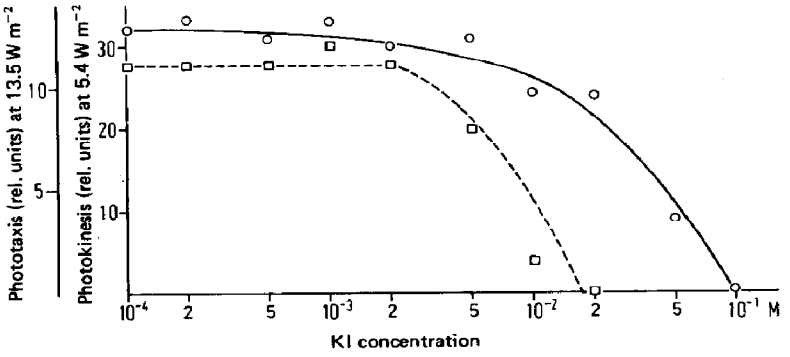

Fig. 6. Effect of KI on photokinesis (○, ——) and phototaxis (□, — — —).

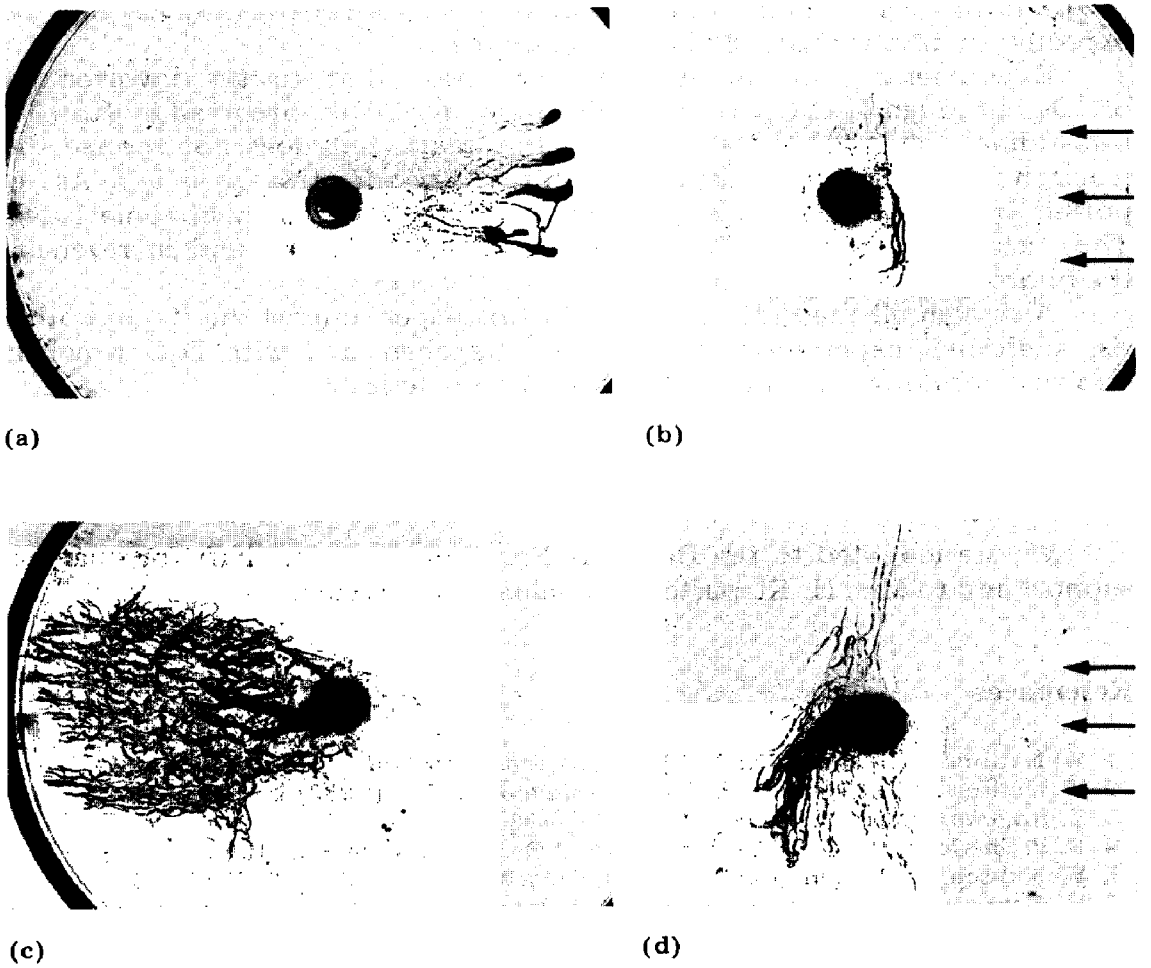

Fig. 7. Photograms of spreading areas of *Anabaena* trichomes formed under unilateral irradiation (←) with low and high fluence rates: (a) 1.35 W m^{-2} in the absence of KI; (b) 1.35 W m^{-2} in the presence of 2×10^{-2} M KI; (c) 13.5 W m^{-2} in the absence of KI; (d) 13.5 W m^{-2} in the presence of 2×10^{-2} M KI.

4. Discussion

The phototactic reaction sign reversal at higher fluence rates by 10^{-3} M azide has been interpreted as being caused by quenching of $^1O_2$ [1]. However, azide is not an absolutely specific quencher of $^1O_2$, since it also quenches triplets of sensitizers [17, 18]. Therefore, additional experiments with other quenchers as well as under conditions altering the intracellular $^1O_2$ concentration were necessary.

As shown in this paper, pH conditions which diminish the quantum yield of $^1O_2$ production shift the transition point of phototaxis to higher fluence rates and vice versa. A steady stream of nitrogen or another gas removes the $^1O_2$ produced in photosynthesis, also causing a shift of the transition point to higher fluence rates. This seems to be possible since it has been shown that $^1O_2$ can migrate through stearate layers 50 nm thick [19] and through sodium dodecylsulphate micelles [20]. Thus, $^1O_2$ may be expected to diffuse through plasma membranes.

Experiments with other quenchers also point to the involvement of $^1O_2$, although DABCO is relatively toxic, as already observed by Ito [3]. L-histidine only suppresses negative phototaxis, but does not reverse the reaction sign. Obviously histidine is not a very effective quencher. As reported by Ito [3], it can even enhance photosensitization in some cases. Thus, azide is the most effective agent in quenching $^1O_2$ and in reversing the phototactic reaction sign.

Although all the data presented in this paper support the $^1O_2$ hypothesis, additional experiments with other quenchers and with D_2O which is known to prolong the lifetime of $^1O_2$ would be desirable.

Acknowledgments

We are indebted to the Deutsche Forschungsgemeinschaft for financial support and to Mrs. H. Klappstein for valuable assistance.

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