

DEUTERIUM OXIDE (D_2O) ENHANCES THE PHOTOSENSITIVITY OF *STENTOR COERULEUS*

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ABSTRACT *Stentor coeruleus* exhibits negative phototaxis and step-up photophobic response (avoiding reaction) to visible light (maximum at 610–620 nm in both responses). In the presence of deuterium oxide (D_2O) the step-up photophobic response was markedly enhanced, whereas the phototactic orientation response was inhibited. The induction time for the step-up photophobic response was longer in D_2O than in H_2O , and the duration of ciliary reversal for the response was also longer in D_2O than in H_2O , indicating that certain steps of the sensory transduction chain are subject to solvent deuterium isotope effects. The enhancement of the step-up photophobic response in D_2O was canceled by $LaCl_3$, while the inhibition of the phototactic orientation response in D_2O was partially removed by $LaCl_3$, even though $LaCl_3$ did not affect the phototactic orientation response. These results suggest that the sensory transduction mechanisms for the two photoresponses are different, although the photoreceptors (stentorin) are the same.

INTRODUCTION

Stentor coeruleus is a ciliate protozoan that shows two types of photoresponses to visible light stimuli. When a forward-swimming organism of *S. coeruleus* encounters a lighted area, it exhibits a sudden avoiding reaction (step-up photophobic response) due to a transient reversal of the beating direction of cilia (1–3). The organism also detects the light direction (phototactic orientation response), orienting itself with respect to the direction of the light stimulus, and it then swims away from the light source (negative phototaxis). Thus, *S. coeruleus* accumulates in the darker region by means of these two types of photoresponses. The photoreceptors for these responses are stentorin, with a hypericinlike chromophore, which gives the organism its characteristic blue-green color (3–6). The photoreceptor proteins are located in pigment granules that reside just beneath the outer pellicle, according to the electron microscopic study (7).

Several observations from the spectroscopic studies of the photoreceptor-entrapped liposomes (8, 9) and the protonophore effect on the photoresponses of live *Stentor* (3, 4), support the hypothesis that protons released from the excited photoreceptors play a primary role as an initial transduction signal after light perception by *S. coeruleus*. Though most previous observations (10–12) revealed that D_2O usually inhibited or slow down physiological and growth responses, it was of interest to examine whether or not D_2O affected the photoresponses of the organism, because proton transfer processes could be facilitated or retarded, depending upon specific acid-base catalytic mechanisms involved (13). For example, deuterium transfer

from the deuterated stentorin chromophore to a basic group on the apoprotein is expected to be slower than proton transfer, if the proton dissociation is rate-limiting. On the other hand, the rate can be enhanced if the conjugate acid (protonated base) plays a major role in the proton transfer and release (to the medium). To elucidate the role of proton transfer in the photosensory transduction, particularly the primary photoprocess in *S. coeruleus*, we carried out the following experiments.

MATERIALS AND METHODS

S. coeruleus was grown in 20-liter vessels containing 2 liters of mineral salt media as previously described (5). The culture was kept at 21°C under room light and dark cycles for 7–10 d. The photoresponses of the organism were monitored via an IR-sensitive camera (model TC 1005; RCA, Lancaster, PA) and recorded on a videotape for analysis (model NV 8950; Panasonic, Secaucus, NJ). Since higher concentrations of D_2O caused a contraction or death of the cell, we used 50% D_2O (redistilled, v/v) as D_2O solution. Monochromatic light was obtained by placing an interference filter (620-3C-S6-28-6-9, half-band width is 10 nm; Corning Glass Works, Corning, NY) in front of the light source. White light (300–700 nm) was obtained by placing an I-R cut-off filter (model HA-30; Hoya Glass Co., Tokyo, Japan) in front of the tungsten lamp to eliminate thermal radiation. During phototactic orientation experiments, collimated white light was used. Since the recording of the phototactic orientation response was performed under dark-field illumination, relatively high intensity of white light was used instead of monochromatic light. All the experiments were carried out with the organisms obtained from 3–4 h light-adapted cultures at 21°C.

RESULTS AND DISCUSSION

Interestingly, the fluence-response curves (Fig. 1 a) show that D_2O enhances the step-up photophobic response in *S. coeruleus*. Thus the organism is about ten times more

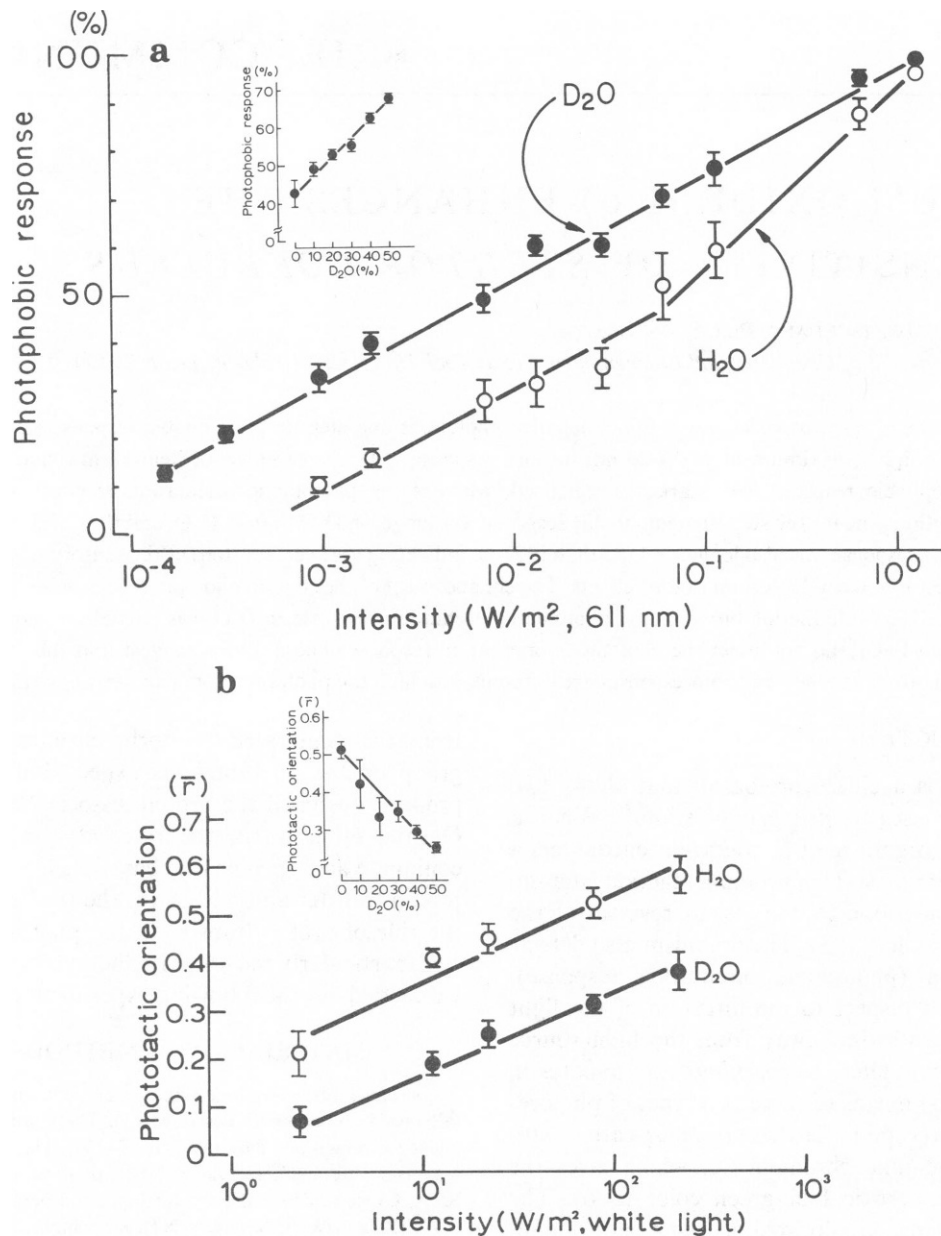


FIGURE 1 The fluence rate response curves for the step-up photophobic response (a) and the phototactic orientation response (b) in *S. coeruleus*. All the organisms were gently washed with a standard saline solution adjusted at pH 7.8 (5). (a) The photophobic response was analyzed by monitoring the cell on a microscope connected with the video equipment (infrared sensitive Newvicon tube of a video camera and video cassette recorder). We measured the phobic response with the light-trap method (3). Monochromatic light was obtained by interference filters with half-bandwidths of ~ 10 nm. *Inset*: The effect of D₂O concentration on the step-up photophobic response. The light intensity of the light-trap was 0.1 W/m² monochromatic light of 611 nm. The vertical line on each plot shows the standard error obtained from four series of measurements. We measured at least 150 specimens at each data point. (b) The phototactic orientation response was observed under low power magnification in a cuvette (75X10X7 mm). The response was recorded on video tape for analysis. The tracks of the moving organisms were traced on TV monitor and the deviation angles from the actinic light ray were determined. The phototactic orientation response was quantified by directional statistics ($0 < \bar{r} < 1$) (16). *Inset*: The effect of D₂O concentration on the phototactic orientation response. Light intensity was 110 W/m² of white light. The vertical line on each plot shows the standard error obtained from three series of measurements. We measured at least 400 specimens at each data point. Note that there is no overlap in the ranges of fluence rates used in the two sets of photoresponses, since the phototactic orientation response was monitored with white light. The same degree of inhibition of the phototactic orientation response in D₂O was confirmed with monochromatic light irradiations (data not shown).

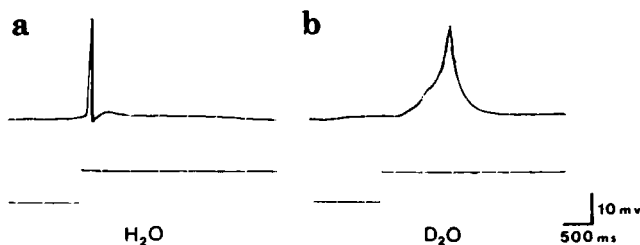


FIGURE 2 The action potential for the step-up photophobic response in both H₂O (a) and D₂O (b). The organism was impaled with microelectrode filled with 1 M KCl, according to Naitoh et al. (17, 18). All the electrophysiological recordings were made via DC amplifier (model P-18D, Grass Instrument Co., Quincy, MA) and a dual channel storage oscilloscope (model 564B; Tektronix Inc., Beaverton, OR). The upper trace shows a cytoplasmic action potential elicited by photostimulation (150 W/m²; white light). The step in the lower trace indicates when the light was switched on. (a) 180 ms after initiation of the photostimulation an action potential was produced, and the duration of the action potential was ~190 ms. (b) 350 ms after initiation of the stimulation an action potential was produced, and the duration of the action potential was ~1,200 ms.

sensitive to light in D₂O than in H₂O. With increasing D₂O concentrations, the organism became gradually more sensitive to light (Fig. 1 inset). However, D₂O inhibited the phototactic orientation response as shown in Fig. 1 b. These results suggest that the photosensory transduction mechanisms may be different for the step-up photophobic and phototactic orientation responses. This is surprising, since the action spectra for the step-up photophobic response and the negative phototaxis are apparently identical (3, 4).

Although the photosensitivity is ten times higher, the induction time for the step-up photophobic response is longer in D₂O (339 ms) than in H₂O (209 ms), and the duration of ciliary reversal for the response is also longer in D₂O (989 ms) than H₂O (278 ms). Swimming velocity was slower in D₂O (2.3 mm/s) than in H₂O (3.3 mm/s). Although the viscosity of the D₂O medium is 15% higher than that of H₂O, the viscosity effect on these behavioral parameters was minimal (induction time, 212 ms; duration of ciliary reversal, 299 ms; and swimming velocity, 3.0 mm/s in an isoviscous polyethylene glycol medium).¹ The longer induction time and the slower rise and decay of the light-induced action potential in D₂O (Fig. 2) are consistent with the photobehavior response kinetics in D₂O. However, in the presence of Ca²⁺-flux enhancers, caffeine (14) or phosphatidic acid (15), both the induction time and

¹It was pointed out by one referee that the inhibition of phototactic orientation response might be related to the apparent enhancements of the photophobic transduction step(s), i.e., lengthening of the induction time. Although this is possible, we have no evidence to ascertain it. We proposed the operation of two different transduction mechanisms because the phototactic orientation response was not affected, even when the photophobic response was inhibited by lanthanum ions (Fig. 3). Nonetheless, the referee's proposal warrants a further study.

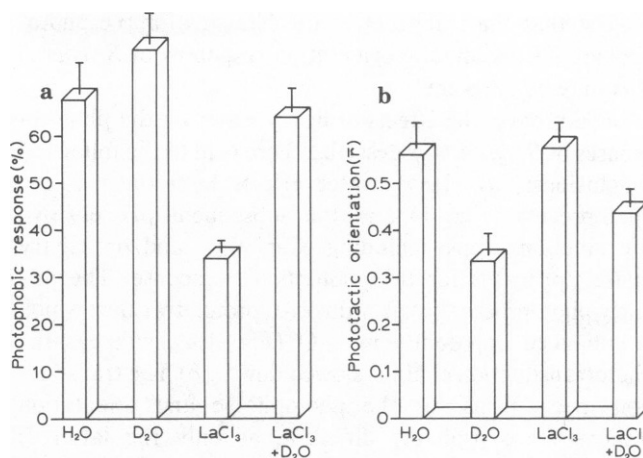


FIGURE 3 The effect of lanthanum (30 μ M) and lanthanum + D₂O on the step-up photophobic response (a) and phototactic orientation response (b). The vertical line of the top of each data point indicates the standard error obtained from three series of measurements. We measured at least 400 specimens at each data point. (a) The light intensity of the light trap was 0.06 W/m² at 611 nm. (b) The light intensity was 110 W/m² of white light.

the duration of ciliary reversal for the phobic response were shortened.

Although lanthanum strongly inhibits the step-up photophobic response (Fig. 3 a) (14), the phototactic orientation response is not affected by lanthanum (Fig. 3 b). Surprisingly, the inhibition of the phototactic orientation response by D₂O was partially reversed by lanthanum ions, whereas the inhibition of the step-up photophobic response by lanthanum ions was removed by D₂O (Fig. 3). Both pH and pD profiles of the photophobic and the phototactic orientation responses were similar (Fig. 4), indicating that the inhibition of the tactic orientation response is not due to nonspecific toxic effects of the solvent. These results again

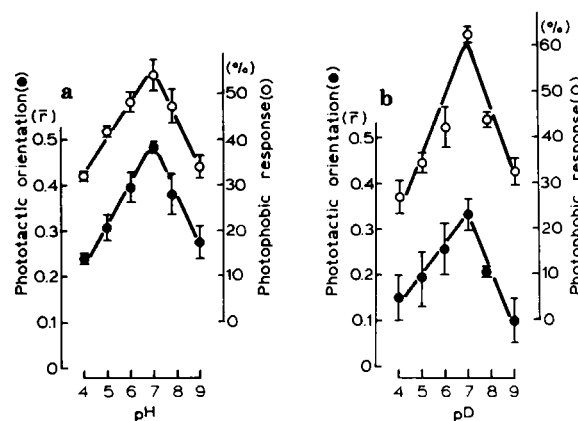


FIGURE 4 The effect of pH (a) and pD (b) on the phototactic orientation response and the step-up photophobic response in *S. coeruleus*. The light intensity of the light trap for the photophobic response was 0.06 W/m² at 611 nm. The light intensity for the phototactic orientation response was 110 W/m² of white light.

suggest that the transduction mechanisms for the photophobic and phototactic orientation responses of *S. coeruleus* may be different.

In summary, the effects of heavy water on the photoreponses of *S. coeruleus* described here lead to the following conclusions: (a) Heavy water affects both the primary photoprocess (Fig. 1) and the subsequent pH-sensitive transduction steps including Ca^{2+} -flux and/or ciliary motor apparatus for the photophobic response. The primary photoprocess possibly involves proton transfer, which is enhanced apparently in D_2O (Fig. 1 a), whereas the phototransduction chain is slowed down. (b) The transduction mechanisms for the photophobic and orientation responses are probably different, as only the latter is inhibited by D_2O that can be restored by lanthanum ions. The enhanced photosensitivity in D_2O is not due to higher fluences perceived by the cell that is moving slower in D_2O than in H_2O , since the size of light trap is sufficiently large to rule out the possibility of attributing the enhanced photosensitivity to higher fluences perceived by the cell moving in D_2O . *Stentor* in both H_2O and D_2O show photophobic response inside the light trap, since the induction time for the response is shorter (209 ms in H_2O , 339 ms in D_2O) than the time necessary to swim through the light trap (303 ms in H_2O , 435 ms in D_2O). Furthermore, the slower moving cell is not likely to sense sharply the spatial change of the light intensity at the light-dark border of the light trap in comparison to fast swimmer.

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