deficiency. The same may also apply to many current cases of nonprogressive congenital myopathy and the limp-infant or floppy-baby syndrome, where minor or no histopathologic changes are encountered (*16*).

WILLIAM N. FISHBEIN*
Biochemistry Division,
Armed Forces Institute of Pathology,
Washington, D.C. 20306
VERNON W. ARMBRUSTMACHER
Neuromuscular Pathology Division,
Armed Forces Institute of Pathology
JOE L. GRIFFIN
Experimental Neuropathology Division,
Armed Forces Institute of Pathology

References and Notes

1. W. N. Fishbein, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 1061 (1977); *ibid.* **37**, 331 (1978); _____, V. Armbrustmacher, J. L. Griffin, *Am. J. Pathol.* **86**, 79a (1977).
2. V. Dubowitz, in *Disorders of Voluntary Muscle*, J. N. Walton, Ed. (Churchill Livingstone, London, ed. 3, 1974), pp. 310–359; _____ and M. H. Brooke, *Muscle Biopsy: A Modern Approach* (Saunders, Philadelphia, 1973), pp. 20–33 and 98–102. Fiber typing was based on the adenosine triphosphatase stain after prior treatment with acid.
3. Thirty 16-μm sections were homogenized in microtubes with 0.1 ml of 15 percent sucrose, 10 m*M* tris, *p*H 7, and centrifuged; 2 to 10 μl of the supernatant was assayed by absorption at 555 nm, in a 1-cm cell, at 25°C (*p*H 6.8) in a 1-ml solution containing 0.144 m*M* phenol red, 3.3 m*M* triethanolamine, 300 m*M* KCl, and 1 m*M* AMP. This assay can be used interchangeably for muscle extracts and hemolyzates, and for adenosine deaminase by replacing AMP with adenosine, and decreasing the salt and buffer content threefold. Substrate cross-reactivity was less than 1:2,000 for adenylate deaminase and less than 1:50,000 for adenosine deaminase.
4. Creatine phosphokinase assays were done on undiluted plasma or 200-fold diluted portions of the muscle homogenate supernatants by the method of S. B. Rosalki [*J. Lab. Clin. Med.* **69**, 696 (1967)].
5. R. Wolfenden, Y. Tomazawa, N. Bauman, *Biochemistry* **7**, 3965 (1968); C. L. Zielke and C. H. Suelter, *J. Biol. Chem.* **246**, 2179 (1971); A. Boosman and O. P. Chilson, *ibid.* **251**, 1847 (1976).
6. A. G. Engel, C. S. Potter, J. W. Rosevear, *Nature (London)* **202**, 670 (1964); A. G. Engel, personal communication.
7. N. Ogasawara, H. Goto, T. Watanabe, *Biochim. Biophys. Acta* **403**, 530 (1975); _____, Y. Kawamura, M. Yoshino, *ibid.* **364**, 353 (1974).
8. C.-Y. Lian and D. R. Harkness, *ibid.* **341**, 27 (1974); A. Askari and S. Rao, *ibid.* **151**, 198 (1968); R. Sasaki, K. Ikura, S. Katsura, H. Chiba, *Agr. Biol. Chem.* **40**, 1797 (1976).
9. J. M. Lowenstein, *Physiol. Rev.* **52**, 382 (1972).
10. A. G. Chapman, A. L. Miller, D. E. Atkinson, *Cancer Res.* **36**, 1144 (1976); K. Kaletha, *Acta Biochim. Pol.* **23**, 193 (1976); _____, A. Skladanowski, M. Zydowo, *ibid.*, p. 145; K. Kaletha, A. Stankiewicz, W. Makarewicz, M. Zydowo, *Int. J. Biochem.* **7**, 67 (1976); K. L. Smiley, Jr., A. J. Berry, C. H. Suelter, *J. Biol. Chem.* **242**, 2502 (1967); A. Raggi, C. Bergamini, G. Ronca, *FEBS Lett.* **58**, 19 (1975).
11. D. E. Atkinson, *Cellular Energy Metabolism and Its Regulation* (Academic Press, New York, 1977); *Biochemistry* **7**, 4030 (1968).
12. L. C. Shen, L. Fall, G. M. Walton, D. E. Atkinson, *ibid.*, p. 4041.
13. K. Tornheim and J. M. Lowenstein, *J. Biol. Chem.* **247**, 162 (1972); *ibid.* **248**, 2670 (1973); *ibid.* **249**, 3241 (1974); *ibid.* **250**, 6304 (1975).
14. Blood was drawn with minimal stasis from one arm, then from the other arm after 100 to 150 squeezes in 1 to 2 minutes, and centrifuged at once. Plasma lactate was assayed by the method of P. A. Drewes [in *Clinical Chemistry, Principles and Techniques*, R. J. Henry, D. C. Cannon, J. W. Winkelman, Eds. (Harper & Row, New York, ed. 2, 1974), pp. 1330–1334] and plasma ammonia was assayed by the method of A. Mondzac, G. E. Ehrlich, and J. E. Seegmiller [*J. Lab. Clin. Med.* **66**, 526 (1965)].
15. J. N. Walton, *Lancet* **1956-I**, 6931 (1956); R. D. Adams, D. Denny-Brown, C. M. Pearson, *Diseases of Muscle* (Harper, New York, ed. 2, 1962), pp. 318–320; R. D. Adams, *Diseases of Muscle* (Harper & Row, ed. 3, New York, 1972), pp. 245–258.
16. W. N. Fishbein, *Anal. Biochem.* **46**, 388 (1972).
17. Elements of this work have been reported: W. N. Fishbein, J. L. Griffin, V. W. Armbrustmacher, *J. Cell Biol.* **75**, 321a (1977); _____, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 787 (1978); W. N. Fishbein, V. W. Armbrustmacher, J. L. Griffin, *Clin. Res.* **26**, 20A (1978).
18. Supported in part by PHS research grant AM-10960 (to W.N.F.) under the auspices of Universities Associated for Research and Education in Pathology, Inc.
* Address reprint requests to W.N.F.

7 November 1977; revised 28 December 1977

Cytochrome c Oxidase as the Receptor Molecule for Chemoaccumulation (Chemotaxis) of *Euglena* Toward Oxygen

Abstract. *Chemoaccumulation of* Euglena gracilis *toward oxygen was selectively inhibited, without concomitant effects on cell motility, by cyanide (10^{-6} to 10^{-4} molar) and carbon monoxide (5×10^{-5} to 5×10^{-4} molar). Above these concentrations, motility of the cell was impaired and the chemosensory response was inhibited. Azide did not affect chemoaccumulation even at 5×10^{-3} molar. It is concluded that cytochrome a_3 serves as the chemoreceptor molecule for oxygen-mediated behavioral responses in* Euglena.

Chemoaccumulation of bacteria toward oxygen was discovered almost a hundred years ago (*1*). Although understanding of this response has been advanced (*2, 3*), identification of the binding or receptor protein for oxygen has not been reported. Multiple functions of receptor proteins have been demonstrated in bacteria for the galactose-binding protein (*4*) as well as the ribose-binding protein (*5*). These proteins have also been shown to be the stimulus receptors for the respective chemotactic movements.

We recently discovered that the unicellular alga *Euglena gracilis* exhibits shock responses to spatial gradients of oxygen (*6*). These "chemophobic responses" are expressed as changes of the cell's direction of movement encountering a too high or too low oxygen concentration, and serve to trap the cell in regions of presumably favorable concentrations of oxygen.

Since in eukaryotes, cytochrome c oxidase acts as the terminal oxygen acceptor of the respiratory chain, we reasoned that it might be the receptor molecule for the chemosensory response. This hypothesis was tested by studying the effects of cytochrome inhibitors on chemoresponses.

Euglena gracilis, strain Z, was grown in Bloomington medium (*7*) supplemented with vitamin B_1 (0.5 mg/ml) and vitamin B_{12} (4 μg/ml). Cultures were grown in an incubator at 25°C under constant cool-fluorescent illumination (1.0 W/m^2), and were used for experiments 5 to 7 days after inoculation. At this age, cells were centrifuged, and suspended in supernatant to a density of approximately 10^6 cell/ml. They were observed in a Neubauer chamber (0.1 mm thickness) under an inverted Nikon type M microscope, with constant yellow illumination (interference filter, 577 nm). This wavelength cannot be perceived by the cell's photosensory system, and does not support photosynthesis at the intensity of the experiment. Light intensities were measured with a model 68 Kettering radiant power meter.

The behavioral response of the cells was determined 10 minutes after addition of the chemicals. As an assay for the chemoresponse toward oxygen, we used the spontaneous formation of an expanding ring within 5 minutes after the cells were introduced into the chamber (*6*). This response to oxygen is qualitatively similar to that of motile bacteria (*3*).

Various inhibitors of cytochrome c oxidase were added. There is evidence for the existence of a complex of cytochrome a in *Euglena* (*8, 9*) even though its structure and mechanism of action have not been established. If the two cytochromes a and a_3 could be separated, the cytochrome a_3 moiety would have to be considered the terminal oxidase, since it alone is rapidly autoxidizable (*10*).

Sodium cyanide had no effect at $10^{-7}M$, but at concentrations ranging from $10^{-6}M$ to $10^{-4}M$ it specifically inhibited ring formation in the *Euglena* suspensions. At these concentrations, swimming rates (defined as the time required by the cell to swim its own length) as well as the ability of the cells to respond to photic stimuli (*11*) were not significantly different from those of controls. At $10^{-3}M$ cyanide and above, motility of the cells was impaired and ring formation was inhibited.

Carbon monoxide was ineffective at $5 \times 10^{-6}M$, but at concentrations of $5 \times 10^{-5}M$ and $5 \times 10^{-4}M$ it was found to specifically inhibit ring formation. No ef-

0036-8075/78/0505-0548$00.50/0

fect was found on photic responses or cell motility, the latter becoming impaired at $5 \times 10^{-3}M$ carbon monoxide. Carbon monoxide binds to cytochrome a_3 (*9*), but not to cytochrome a (*12*).

Sodium azide at concentrations as high as $5 \times 10^{-3}M$ had no effect on ring formation even though motility of the cells was significantly impaired at this concentration. While some investigators believe that azide binds to the oxidized form of cytochrome a_3 (*10, 13*), others maintain that azide binds to the reduced form of cytochrome a (*14*) (Table 1).

Sodium cyanide and carbon monoxide inhibit the chemosensory response of *Euglena* toward oxygen at concentrations where neither the movement of the cells, nor their ability to respond to photosensory stimuli are impaired. One might argue that these inhibitors affect respiration rather than chemoaccumulation, and that they act by inhibiting oxygen consumption and thus the formation within the sample chamber of the oxygen concentration gradient which triggers the chemophobic responses in the first place. That this is not the case at the concentrations used by us is evident from the fact that the cells move normally, and thus have available their usual source of metabolic energy. In addition, previous studies in vivo under experimental conditions very similar to our own have shown that cellular respiration of intact *Euglena gracilis* is only marginally inhibited by carbon monoxide (*15*), while cyanide even at $10^{-3}M$ inhibits oxygen consumption of whole cells by no more than 57 percent (*9*). However, the isolated a-type cytochrome is completely inhibited by $10^{-3}M$ cyanide (*9*), indicating that, while cytochrome oxidase does get inactivated by cyanide (which is in agreement with our hypothesis), in intact cells of *Euglena gracilis* there is an alternative pathway for the reoxidation of cytochrome b, and thus for the generation of metabolic energy. This explains the retention of motility in our cells at high inhibitor concentrations.

Since the photosensory responses of *Euglena* were unaffected by carbon monoxide and sodium cyanide at the concentrations studied by us, the effects we are observing do not reflect a generalized inhibition of sensory transduction in *Euglena* at the level of signal processing or effector mechanisms (*11*). We conclude that these results implicate the cytochrome a_3 moiety of cytochrome c oxidase as the receptor molecule for oxygen-mediated chemosensory responses in *Euglena*. In addition, it might be argued that our data support the contention (*14*) that azide, which is ineffective behaviorally even at a concentration at which motility of the cells is inhibited, inhibits cytochrome c oxidase by binding to cytochrome a rather than to cytochrome a_3. The main interest of this inference is that it is based entirely on studies of the behavior of *Euglena* rather than on direct observation of biochemical changes within the cell.

Table 1. Effects of inhibitors (sodium cyanide, carbon monoxide, and sodium azide) of cytochrome c oxidase on chemoaccumulation toward oxygen and motility of *Euglena gracilis*.

Concentration (*M*)	Effect on	
	Ring patterns	Motility (% of control)
	Sodium cyanide	
10^{-7}	None	None
10^{-6}	Inhibited	None
10^{-5}	Inhibited	None
10^{-4}	Inhibited	None
10^{-3}	Inhibited	40
	Carbon monoxide	
5×10^{-6}	None	None
5×10^{-5}	Inhibited	None
5×10^{-4}	Inhibited	None
5×10^{-3}	Inhibited	40
	Sodium azide	
5×10^{-3}	None	50

S. MILLER, B. DIEHN

Department of Chemistry, University of Toledo, Toledo, Ohio 43606

References and Notes

1. T. W. Engelmann, *Bot. Zeitung Leipzig* **39**, 441 (1881).
2. J. C. Sherris, N. W. Preston, J. G. Shoesmith, *J. Gen. Microbiol.* **16**, 86 (1957).
3. J. Adler, *Science* **153**, 708 (1966).
4. G. L. Hazelbauer and J. Adler, *Nature (London) New Biol.* **230**, 101 (1971).
5. R. Aksamit and D. E. Koshland, Jr., *Proc. Natl. Acad. Sci. U.S.A.* **73**, 762 (1971).
6. G. Colombetti and B. Diehn, *J. Protozool.*, in press.
7. R. C. Starr, *Am. J. Bot.* **51**, 1013 (1964).
8. F. Perini, M. D. Kamen, J. A. Schiff, *Biochim. Biophys. Acta* **88**, 74 (1964).
9. J. K. Raison and R. M. Smillie, *ibid.* **180**, 500 (1969).
10. D. S. Bendall and W. D. Bonner, in *Hemes and Hemoproteins*, B. Chance, Ed. (Academic Press, New York, 1966), pp. 485–502.
11. B. Diehn, *Science* **181**, 1009 (1973).
12. Q. H. Gibson and C. Greenwood, *Biochem J.* **86**, 541 (1963).
13. T. Horie and M. Morrison, *J. Biol. Chem.* **238**, 2859 (1964).
14. D. F. Wilson and B. Chance, *Biochem. Biophys. Res. Commun.* **23**, 751 (1966).
15. F. Perini, M. D. Kamen, J. A. Schiff, *Biochim. Biophys. Acta* **88**, 91 (1964).

16. Supported by NSF grant PCM 74-19786. Address correspondence to B.D.

13 October 1977; revised 28 December 1977

Ellipsosomes: Organelles Containing a Cytochrome-Like Pigment in the Retinal Cones of Certain Fishes

Abstract. *Ellipsosomes are dense spherical bodies containing a very large concentration of a heme pigment spectroscopically resembling pure cyctochrome c. They are located at the outer ends of the inner segments of the cones of certain fishes. Although, superficially, they resemble the similarly located oil droplets in the cones of birds and reptiles, their ultrastructure and staining properties resemble those of the neighboring mitochondria. However, like the oil droplets, they may serve as intracellular color filters.*

The outer part of the inner segment of the cone cells of the vertebrate retina is commonly known as the ellipsoid. This region is packed with large mitochondria and, in some avian and reptilian species, contains a large, clear or strongly colored oil droplet immediately in front of the light-absorbing outer segment (*1*). This droplet acts as a filter which prevents the shorter wavelengths of light from reaching the photosensitive pigment. Structures resembling the oil droplets of birds and reptiles have been described in a variety of fishes (*1, 2*). However, as previously pointed out (*3*), these droplets do not stain with oil-soluble dyes and electron micrographs show that they have an organized structure (which the oil droplets do not) reminiscent of an unusually large mitochondrion (Fig. 1). To prevent their being confused with true oil droplets we call them ellipsosomes. We have found that they have a spectral absorbance that is characteristic of a dense heme pigment, different from that of hemoglobin, and similar to that of reduced cytochrome c (*4*). They also stain positively for some other components of the respiratory chain. Furthermore, they contain no trace of carotenoid pigments (*5, 6*).

Eyes were removed from guppies (*Poecilia reticulata*), mollies (*Poecilia latipinna*), killifish (*Fundulus heteroclitus*), and platies (*Xiphophorus maculatus*). The retinas were dissected out in calcium-free physiological solution [to facilitate retinal detachment and dissociation (*7*)] either in bright light or under infrared illumination. After 45 minutes in calcium-free solution, small pieces of retina were teased apart in a drop of the same solution on a large cover slip, ringed with silicone oil (DC 702 diffusion pump oil), and covered with another cover slip. They were then exam-

0036-8075/78/0505-0549$00.50/0