Dye laser photolysis studies on a cooperative dimeric hemoglobin

LAWRENCE J. PARKHURST

Department of Chemistry, University of Nebraska, Lincoln, NE 68588 (U.S.A.)

Ligand kinetics and equilibria were studied for the binding of CO and O_2 to the hemoglobin (Hb) from the sea cucumber *Thyonella gemmata*. The hemoglobin shows cooperative ligand binding for both ligands. Replacement of oxygen by CO as the bound ligand also appears to be cooperative. Circular dichroism spectra of HbO_2 and HbCO indicate that the structures of the two liganded forms are different. Allosteric models for this dimeric hemoglobin predict that a major part of the cooperation for CO binding must derive from large differences in the dissociation rate constants between the R and T states. Direct studies of CO dissociation, however, show little difference between the R and T states. Dye laser studies provide no evidence for a quickly reacting Hb* form. Such measurements reveal complex kinetics but do give the rate of the first-order conformational change between the two unliganded forms generated from HbO_2 and HbCO.

This work was supported by Grants NIH HL 15, 284-09 and NSF PCM 8003655.

In vivo microspectrofluorimetry of photoreceptor pigments

G. COLOMBETTI, F. GHETTI, F. LENCI, E. POLACCO and M. QUAGLIA

Istituto per lo Studio delle Proprieta', Fisiche di Biomolecole e Cellule, Consiglio Nazionale delle Richerche, Via S. Lorenzo 24 - 28, Pisa (Italy)

Many micro-organisms, such as flagellated algae, are capable of responding to variations in the external illumination conditions with changes in their motile properties. The identification of the pigments that form the receptor system is one of the main problems encountered in the study of these phenomena. A possible approach to this problem is the *in vivo* investigation of the spectroscopic properties of the receptive structures when they have been identified and localized in specialized subcellular portions or organelles. Unfortunately the pigment content of these structures is usually quite low and it is therefore difficult to detect their absorption properties. However, if the receptor pigments are fluorescent, it becomes possible to investigate their fluorescence *in vivo*. We describe an improved version of a dye laser microspectrofluorimeter that we are currently employing to measure the fluorescence excitation spectrum of the photoreceptor of the flagellated alga *Euglena gracilis*.