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TIME-RESOLVED ANISOTROPY OF MULTIPLE FLUORESCENT-PROBES BOUND TO HORSERADISH-PEROXIDASE

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Abstract

Previous fluorescence studies of horseradish peroxidase (HRP) conjugated with protoporphyrin IX (PPIX) suggested that the protein behaved hydrodynamically like a prolate ellipsoid of axial ratio 3 to 1. The present study exploits a series of probes, noncovalently bound to the heme binding site of apohorseradish peroxidase, having different orientations of the excitation and emission transition moments with respect to the protein's principle rotational axes. The probes utilized included PPIX and the naphthalene probes ANS, TNS and Bis-ANS. Time-resolved data were obtained using multifrequency phase fluorometry. The Global analysis approach to the determination of molecular shape using multiple probes, proposed by L. Brand and coworkers, was evaluated by utilizing alldata sets while maintaining a constant molecular shape for the HRP. The results indicate that, in such analyses, probes exhibiting a single exponential decay and limited local motion have the major weight in the evaluation of the axial ratio. Probes which show complex decay patterns, such as the naphthalene derivatives, give rise to significant statistical uncertainties in such Global treatments. Supported by NSF-SDC grant INT-8916623 and FONDECYT-Chile grant 91-539.