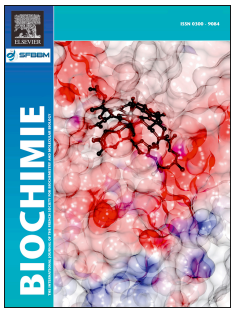

Phosphorylation-Induced Conformational Changes of Photoactivated Rhodopsin Probed by Fluorescent Labeling at Cys^{140} and Cys^{316}

Sheerly Rodríguez, May-Li Silva, Gustavo Benaím, José Bubis

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Abstract

In order to monitor conformational changes following photoactivation and phosphorylation of bovine rhodopsin, the two reactive sulfhydryl groups at Cys^{140} and Cys^{316} were specifically labeled with the monobromobimane (mBBr) fluorophore. Although alterations in conformation after light exposure of rhodopsin were not detected by fluorescence excitation scans (300-450 nm) of the mBBr-labeled protein, the fluorescence signal was reduced ~ 90% in samples containing photoactivated phosphorhodopsin. Predominant labeling at either Cys^{140} or Cys^{316} in light-activated and phosphorylated rhodopsin merely generated a decrease of ~ 38% and 28%, respectively, in the fluorescence excitation intensity. Thus, neither mBBr-modified Cys^{140} nor mBBr-modified Cys^{316} were involved single-handedly in the remarkable fall seen on the signal following phosphorylation of the protein; rather, the incorporation of phosphate groups on the mBBr-labeled light-activated rhodopsin appeared to affect its fluorescence signal in a cooperative or synergistic manner. These findings demonstrated that the phosphorylation of specific hydroxyl groups at the carboxyl terminal tail of rhodopsin causes definite conformational changes in the three-dimensional fold of the protein. Apparently, amino acid residues that are buried in the interior of the inactive protein become accessible following bleaching and phosphorylation of rhodopsin, quenching in turn the fluorescence excitation signal of mBBr-modified rhodopsin.