Suppressed or recovered intensities (SRI) analysis in site-directed ^{13}C NMR: Low-frequency fluctuations in bacteriorhodopsin and D85N mutants revisited

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The first proton transfer of bacteriorhodopsin (bR) occurs from the protonated Schiff base to the anionic Asp 85 at the central part of the protein in the L- to M-states. Low-frequency dynamics with frequency of 10^{-4} to 10^{-5} s accompanied by this process can be revealed by the suppressed or recovered intensities (SRI) analysis of site-directed ^{13}C solid-state NMR spectra of 2D crystalline preparations. First of all, we examined a relationship of fluctuation frequencies available from [1-^{13}C]Val- and [3-^{13}C]Ala-labeled preparations, by taking into account of effective correlation time in the latter in the presence of internal methyl rotations. Further, we analyzed the SRI data of [1-^{13}C]Val-labeled wild-type bR and D85N mutants, as a function of temperature and pH, respectively which are directly related to fluctuation frequencies, based on so-far assigned peaks including newly assigned or revised ones. Global conformational change of the protein backbone, caused by neutralization of the anionic D85 by D85N, can be visualized by characteristic displacement of ^{13}C NMR peaks due to the conformation-dependent ^{13}C chemical shifts. Concomitant dynamics changes if any, with fluctuation frequencies in the order of 10^4 Hz, were evaluated by the decreased peak-intensities in the B-C and D-E loops of D85N mutant. The resulting fluctuation frequencies, owing to subsequent, accelerated dynamics changes in the M-like state by deprotonation of the Schiff base at alkaline pH, were successfully evaluated based on the SRI plots as a function of pH, which were varied depending upon the extent of interference of induced fluctuation frequency with frequency of magic angle spinning or escape from such interference. Distinguishing fluctuation frequencies between the higher and lower than 10^4 Hz is now possible, instead of simple description of the data around 10^4 Hz available from one-point data analysis previously reported. Significance of such low frequency fluctuation was also discussed in relation to photo-cycle of bR.