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Poster presentation

Resonance Raman and infrared spectroscopic studies of high-output forms of human soluble guanylyl cyclase

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The allosteric regulator BAY 41-2272 converts the CO-sGC enzyme from a low- to high-output form, with respect to production of cGMP. Resonance Raman (RR) and Fourier Transform Infrared (FTIR) spectroscopic techniques are used to show that the CO adduct of sGC exists as major and minor conformers, both having ν (Fe-CO) and ν (C-O) modes characteristic of six coordinate species. It is further shown that addition of BAY 41-2272 to the CO adduct induces the transition of some fraction of the initial CO-heme adducts into two new CO-heme complexes. This fractional conversion is temperature dependent. One new complex displays vibrational modes characteristic of pentacoordinated CO-adduct and its formation is not affected by temperature. The second complex, although slightly different from the original CO-adducts, is hexacoordinated and its formation is facilitated by temperature. The production of substantial amounts of the five coordinate CO adduct upon addition of BAY 41-2272, reveals the fact that several out-of-plane heme deformation modes are simultaneously activated, an observation similar to that realized upon NO activation. By analogy with earlier studies of other heme proteins, several bands associated with modes attributable to peripheral substituent deformations and methine carbon movements are implicated. The documented formation of *two* new forms upon addition of BAY 41-2272 (a 5-coordinate and a new 6-coordinate form) is discussed with respect to the implications for enzyme activation.

FTIR analysis of the amide I region of sGC enzyme was used to assess conformational changes occurring upon activation with various ligands and regulators. Difference spectra of untreated sGC and the enzyme treated with BAY 41-2272 [sGC/BAY-sGC] or CO [sGC/CO-sGC] alone showed only weak changes in amide I region, interpreted as insignificant changes in protein conformation. However, comparison of conformational signatures occurring in the amide I region upon enzyme's exposure to NO or combined CO/BAY 41-2272 treatment showed a significant change in the conformation. Both [sGC/NO-sGC] and [sGC/CO/BAY-sGC] difference spectra display positive bands at 1653, 1687, 1643 and 1625 cm^{-1}, as well as a negative band at 1650 cm^{-1}. These features are characteristic of amide I modes arising from turns, α-helices and β-sheet secondary structures. This finding suggest that sGC enzyme in a high-output mode achieves a similar confirmation independently on the nature of the stimuli.