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## Resonance raman investigation of effects of YC-1 and GTP on structure of CO-bound heme of soluble guanylate cyclase

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Resonance Raman (RR) spectra of soluble guanylate cyclase (sGC) reported by five independent research groups have been categorized into two types; sGC<sub>1</sub> and sGC<sub>2</sub>. Here we demonstrate that the RR spectra of sGC isolated from bovine lung contains only sGC<sub>2</sub> while both species are observed in the spectra of CO-bound form (CO-sGC). The relative populations of the two forms altered from an initial composition in which the CO-sGC<sub>2</sub> form predominated, with the Fe-CO ( $\nu_{\text{Fe-CO}}$ ) and C-O stretching modes ( $\nu_{\text{CO}}$ ) at 472 and 1985 cm<sup>-1</sup>, respectively, to a composition dominated by the CO-sGC<sub>1</sub> form with  $\nu_{\text{Fe-CO}}$  and  $\nu_{\text{CO}}$  at 488 and 1969 cm<sup>-1</sup>, following the addition of xenobiotic, YC-1. Further addition of a substrate, GTP, completed the change. GDP and cGMP had a similar but significantly smaller effect, while a substrate analogue, GTP- $\gamma$ -S had an effect similar to that of GTP. In contrast, ATP had a reverse effect, and suppressed the effects of YC-1 and GTP. In the presence of both YC-1 and GTP, vinyl vibrations of heme were significantly influenced.

In the absence of any effectors, the Fe-CO stretching and the heme vinyl bending modes appeared at 473 and 424 cm<sup>-1</sup>, respectively. There was no band around 372 cm<sup>-1</sup>. However, in the presence of YC-1 and GTP, the Fe-CO stretching mode was shifted to 489 cm<sup>-1</sup> and the vinyl mode appeared at 400 cm<sup>-1</sup>. The propionate mode appeared at 372 cm<sup>-1</sup> as a sharp band. The vinyl modes at 424 and 400 cm<sup>-1</sup> are assigned to the vinyl-2 and vinyl-4 C <sub>$\beta$</sub> -C <sub>$\alpha$</sub> =C <sub>$\beta$</sub>  bending vibrations that are strongly coupled with ring modes of pyrroles-I and II, respectively, while

the propionate mode is coupled with ring modes of pyrroles-III and IV. In addition, new CO-isotope sensitive bands were observed at 521, 363, and 227 cm<sup>-1</sup>. The 521 cm<sup>-1</sup> band was assigned to the five-coordinate (5c) species from the model compound studies using ferrous iron-protoporphyrin IX in CTAB micelles. Distinct from the 472 cm<sup>-1</sup> species, both the 488-cm<sup>-1</sup> and 521-cm<sup>-1</sup> species were apparently unphotodissociated when an ordinary Raman spinning cell was used with a CW laser source for Raman excitation, indicating rapid recombination of photodissociated CO in the presence of YC-1 and GTP. On the basis of these findings, binding of YC-1 and GTP to the heme pocket is proposed.