Effects of YC-1 and GTP on NO-bound Heme Structure of Soluble Gyanylyl Cyclase

Teizo Kitagawa,[a)] Biswajit Pal,[a)] Shigeo Takenaka,[b)] and Shingo Tsuyama,[b)]

[a)]Okazaki Institute for Integrative Bioscience, National Institutes of Natural Sciences, Myodaiji, Okazaki, 444-8787 Japan

[b)]Department of Veterinary science, Osaka Prefecture University, Sakai, 599-8531 Japan.

Soluble guanylate cyclase (sGC) is a heterodimeric, cytosolic enzyme that catalyses the conversion of GTP into cyclic GMP (cGMP), which acts as a messenger in cellular systems. This enzyme has an iron-protoheme bound to the conserved histidine (His-β105), and it serves as a physiological receptor for nitric oxide (NO) produced by NO synthase. Resting sGC has a five-coordinate (5c) high-spin Fe^{II}-heme. NO binds to its distal side, causing cleavage of the original Fe-His bond due to its strong negative *trans* effect and raises the enzymatic activity a few hundreds fold. Contradicting models have been proposed to explain this. Also, a xenobiotic, YC-1, was recently found to further stimulate the activity of sGC in the presence of NO or CO.

We have applied resonance Raman (RR) spectroscopy to explore details of structural changes of the NO-heme as well as the CO-heme by YC-1 and GTP (or cGMP). The N-O stretching was identified at 1681 cm^{-1} for purified sGC, at 1688 cm^{-1} in the presence of YC-1, and split into 1681 and 1699 cm^{-1} by GTP (or cGMP), while the Fe-NO stretching frequency remains unchanged. Previously, we have shown that binding of YC-1 to CO-bound sGC takes place within the heme pocket in proximity of the heme vinyl groups.[1)] In the absence of any effectors, the Fe-CO stretching and the heme vinyl bending modes appeared at 473 and 424 cm^{-1}, respectively. There was no band around 372 cm^{-1}. However, in the presence of YC-1 and GTP, the Fe-CO stretching mode was shifted to 489 cm^{-1} and the vinyl mode appeared at 400 cm^{-1}. The propionate mode appeared at 372 cm^{-1} as a sharp band. The vinyl modes at 424 and 400 cm^{-1} are assigned to the vinyl-2 and vinyl-4 C_β-C_a=C_b bending vibrations that are strongly coupled with ring modes of pyrroles-I and II, respectively,[2)] while the propionate mode is coupled with ring modes of pyrroles-III and IV. These observations were similar to those for the NO-heme, suggesting that rotation of NO around the Fe-N bond upon binding of YC-1 and further disposition to the proximal side upon binding of GTP (or cGMP).

1) Li, Z., Pal, B., Takenaka, S., Tsuyama, S. and Kitagawa, T. (2005) Biochemistry 44, 939-946.
2) Mak, P. J., Podstawka, E., Kincaid, J. R., Proniewics, L. M. (2004) Biopolymer, 75, 217-218.