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Dynamic ligand exchange as mechanism for sGC selectivity, NO activation and its reversal

Emil Martin*, Vladimir Berka, Ah-Lih Tsai and Ferid Murad

Address: Institute of Molecular Medicine, Houston, Texas, USA

Email: Emil Martin* - emil.martin@uth.tmc.edu

* Corresponding author

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Nitric oxide is the main physiologic ligand of soluble guanylyl cyclase (sGC), a hemoprotein with high affinity for NO. Purified sGC binds NO with an estimated picomolar Kd for NO, which corresponds to only a few molecules of NO per cell. The His105 residue of the β subunit is the proximal ligand of the heme (His105-heme complex with a 430 nm Soret band). Binding of NO to the sGC heme forms a six coordinate (6C) complex (His105-heme-NO), which is then converted into a five coordinate (5C) complex (His105 and heme-NO) [1]. This transformation coincides with several hundred fold activation of the cyclase activity. The enzyme displays NO-concentration dependence not only in the NO binding, but also in the 6C sGC-NO→5C sGC-NO complex conversion [1]. Previous studies showed that the sGC-NO complex obtained under stoichiometric NO amounts is not fully activated, while addition of GTP substrate or additional NO results in a fully activated sGC [2].

In this current report we investigated the molecular basis of this NO- and GTP-dependence of enzyme activation. Stop-flow techniques were used to monitor the kinetics of NO-sGC complex formation and its fate under stoichiometric and excess NO conditions or in the presence of GTP. Under both stoichiometric and excess NO conditions 6C His105-heme-NO complex (~420 nm Soret band) is formed at a diffusion limited rate independently of the presence or absence of GTP. Although in all tested conditions the His105-heme-NO complex is converted into five coordinate complex (free His105 and heme-NO complex; 399 nm Soret band), the fate of this complex depends on the amount of NO applied or the presence of GTP substrate.

Under stoichiometric conditions, the heme-NO reverts back to a 430 nm species with sub-second kinetics, presumably due to the loss of NO. The newly formed His105-heme complex (sGC*) may rebind NO and forms a 5C sGC-NO complex (399 nm species), but with much slower kinetics (minutes vs. sub-millisecond kinetics for intial sGC). This secondary sGC-NO* complex is, most probably, the weakly activated sGC-NO complex described previously [2]. However, even a two-fold molar excess NO or the presence of GTP generates a 5C NO-heme complex with no sign of NO loss.

Spectral properties of the sGC* state generated after the loss of NO (430 nm Soret peak) indicate that the heme moiety remains coordinated by a histidine residue. Different NO-binding properties, however, suggest that significant changes in the properties of the enzyme occurred. We investigated whether a change of proximal ligands may explain such transformation during the conversion of 5C sGC-NO to sGC*. We substituted the βHis107 position, which is conserved in all mammalian β1 subunits, to leucine. Similar to wild type sGC mutant αβHis107→Leu enzyme also contains heme with a Soret peak at 430 nm. The mutant has different NO-binding kinetics, since it forms a stable sGC-NO complex, which does not loose NO even under sub-stoichiometric NO conditions.

These data support the notion that an exchange of the proximal hisitidyl ligand may occur at substoichometric NO concentrations. Such proximal ligand exchange may be essential for the discrimination between a random NO molecule (inhaled NO, dietary nitrite) and the activating NO signal (NOS-generated) and may be a process critical for the deactivation of sGC.

References

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