Distal Pocket Polarity in the Unusual Ligand Binding Site of Soluble Guanylate Cyclase: Implications for the Control of •NO Binding

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The only known receptor for the intercellular signaling agent nitric oxide (•NO) is the soluble isoform of guanylate cyclase (sGC). The rate of GTP to cGMP conversion by sGC increases about 400-fold over the basal level activity upon •NO binding.[1] sGC is a heterodimer, and each subunit contains one protoporphyrin IX type heme[1] which is the target for NO binding.[2] The heme Fe^{II} is 5-coordinate high spin with histidine as the axial ligand.[3] However, unlike other 5-coordinate high-spin heme proteins, sGC shows unusual behavior toward small ligands such as O_2,[3] CO,[4] nitrosomethane,[4] and •NO.[2] The affinity for O_2 is so low that in air-saturated buffer the heme Fe remains 5-coordinate.[3] This is physiologically important because most hemes will rapidly bind oxygen and then react with •NO.[5] CO binds to the heme of sGC, but the affinity is much lower than for typical 5-coordinate ferrous imidazole-ligated heme proteins such as Hb and Mb. This reduced affinity for sGC is primarily due to an increased off-rate (3.5 s^{-1}).[4] •NO binds to the heme Fe of sGC to form a transient 6-coordinate heme.[2] In this complex, however, the Fe−His bond is unstable and the breakage of this bond is believed to be linked to activation of the enzyme.[2] While the binding of •NO to heme is virtually irreversible in both model systems and heme proteins, dissociation of •NO from heme of sGC appears to be fast as hypothesized from whole organ studies.[6] Recent kinetic studies using purified sGC provide molecular evidence to support this speculation.[2] A similar ligand, nitrosomethane (CH_3NO), which typically binds irreversibly to high-spin ferrous heme proteins to form a 6-coordinate complex, also binds to the heme of sGC but in a reversible manner again due to a fast off-rate.[4] The observations for all these ligands deviate from what is commonly observed for heme proteins and indicate that the heme environment of sGC is distinct from that of other heme-containing proteins.

We recently reported resonance Raman measurements on unligated, CO-bound, and NO-bound sGC.[7] In that study we assigned a mode at 204 cm^{-1} for ferrous unligated sGC to ν(Fe−His) and suggested that the proximal histidine is neutral. In the same study we showed that CO binding to the heme results in a 6-coordinate heme Fe, leaving the Fe−His bond intact. The stretching frequency we observed for the Fe−CO bond at 472 cm^{-1} is the lowest frequency thus far reported for a CO-bound heme. Vibrational measurements on CO-bound heme proteins are particularly useful to infer the status of the proximal ligand and the environment of the distal pocket because the correlation of ν(Fe−C) to ν(C−O) is well established in both model porphyrins and heme proteins. This correlation is understood on the basis of competition for metal σ bonding between the bound CO and the trans ligand, superimposed on variations in Fe $d_\pi \rightarrow$ CO π^* back-donation.[8] When the axial ligand is held constant (therefore the trans competition for metal σ bonding is fixed), there is an inverse linear relationship between ν(Fe−C) and ν(C−O) due to Fe $d_\pi \rightarrow$ CO π^* back-donation: as the extent of back-donation increases, an increase occurs in the Fe−C bond order in concert with a decrease in the C−O bond order. The extent of back-bonding is influenced mainly by the polarity of the environment around the bound CO.[8−10] In addition, hydrogen bonding to the carbonyl oxygen enhances this effect greatly.[11−14]

On the basis of the weak proximal Fe−His bond in sGC (less competition for metal σ bonding), a relatively high frequency for the Fe−CO stretch was expected.[7] Surprisingly the observed frequency for the Fe−CO stretch was unusually low, leading us to the conclusion that proximal effects alone cannot explain our observation and that the influence of the distal pocket environment is largely responsible for the low ν(Fe−CO).[7] Assuming that the proximal histidine is neutral, ν(FeC−O) of CO−sGC can be calculated from the back-bonding correlation curve made by Li and Spiro,[15] and, on the basis of that correlation curve, a C−O stretching frequency close to 2000 cm^{-1} is predicted.[7] However, sGC shows strikingly different ligand binding behavior relative to other heme proteins, which raises the possibility that additional protein-induced constraints may alter the vibrational properties of the CO ligand. A precedent for such effects comes from the CO−cytochrome *c* oxidase complex, which deviates markedly from the ν(Fe−CO)/ν(C−O) correlation curve for histidine-ligated carbonmonoxy hemes and heme proteins.[8] The deviation in the heme/copper terminal oxidases has been attributed to unusual distal effects brought about by the nearby Cu_B species.[16] To investigate the extent to which protein effects beyond those imposed by distal pocket polarity influence ligand binding properties, it is essential to measure the C−O stretching vibration in sGC directly. Here we report FTIR measurements on CO−sGC that provide additional insight into the extent of metal to ligand π^* back-bonding. The frequency of the Fe−CO stretching vibration is very high and agrees well with the predictions we made previously. Distal pocket polarity effects, therefore, provide a primary explanation for CO binding to the heme Fe in sGC.

Figure 1 shows FTIR[17] spectra of CO-bound sGC in the 1800−2050 cm^{-1} region. With natural isotopic abundance CO, a single peak is observed at 1987 cm^{-1}. This mode shifts to 1943 cm^{-1} with $^{13}C^{16}O$ and to 1895 with $^{13}C^{18}O$. The observed isotope shifts are as expected for a C≡O harmonic oscillator,

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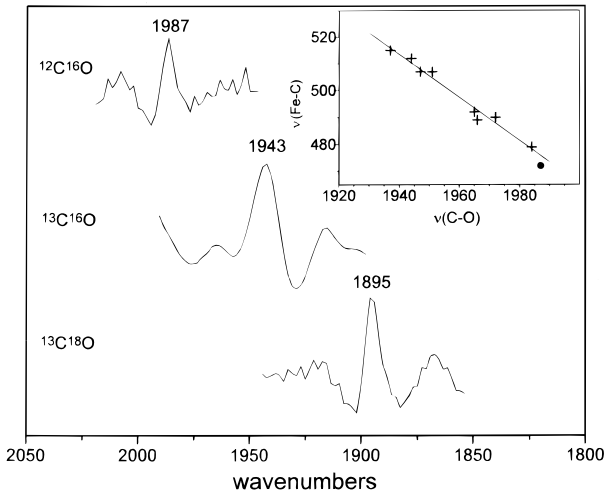

Figure 1. FTIR spectra of CO-bound sGC: (top left) $^{12}C^{16}O$; (center) $^{13}C^{16}O$; (bottom right): $^{13}C^{18}O$. Smooth polynomials were subtracted from the original data to correct for the base line. The inset shows the Fe−C stretching frequency versus the C−O stretching frequency for a number of histidine ligated heme proteins (open circles)[8,15] and sGC (closed circle). The solid line is a best-fit line to all the data points except sGC.

and therefore, the mode can be unambiguously assigned to the C≡O stretching frequency. In the inset of Figure 1 we have plotted the Fe−CO stretching frequency versus the C−O stretching frequencies for sGC and other histidine-ligated heme proteins. The measured frequencies for sGC fall on the same curve as those of other proteins with histidine as the proximal ligand. This provides additional strong evidence that the heme Fe in sGC is indeed ligated by histidine. The presence of a proximal histidine ligand was previously concluded on the basis of the absorption spectrum[3] and a Raman active mode observed at 204 cm^{-1} which was ascribed to Fe−His stretch.[7] This is also consistent with our previous interpretations that the histidine ligand is neither anionic nor hydrogen bonded because in those cases the ν_{FeC}/ν_{CO} points fall below the imidazole correlation curve due to the weakened Fe−Cσ bond as shown in a model compound and in horseradish peroxidase at high pH.[13] The position along the correlation curve is mainly determined by the polarity of the distal pocket.[8] sGC falls on this curve at the lowest point so far observed. The protein nearest to sGC on the curve is the porcine myoglobin mutant [H64V-V68T], in which back-bonding is inhibited due to the negative polarity of the distal pocket in the absence of a hydrogen-bonding donor (His 64).[10] Since sGC falls on this same back-bonding curve, we infer that there is negative polarity in the distal pocket of sGC. Introducing negative polarity in the distal pocket increases not ony the stretching frequency of CO bound to the heme but also the off-rate of both O_2 and CO binding, as has been shown in both V68T (3-fold for O_2 and 4-fold for CO) and H64V-V68T (300-fold for O_2 and 3-fold for CO) mutants.[10] The general trend in sGC is the same, showing an increased ν(C−O) and a very high off-rate for CO (3.5 s^{-1}),[4] which further supports the idea that there is negative polarity in the distal pocket of the heme in sGC and that it is a major determinant for the ligand binding properties of sGC.

In general, ligand binding affinity to hemes increases when going from O_2 to CO and to •NO. For sGC, a strong decrease for O_2 binding, an increased dissociation rate for CO, and an apparent relative ease of •NO dissociation are observed, indicative of an overall decreased affinity for these small ligands. It is very likely that sGC uses the negative polarity to achieve the reduced O_2 binding and the relative ease of •NO dissociation. Both these are necessary for proper functioning of the enzyme.

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(17) FTIR spectra were acquired on a Nicolet 740 FTIR spectrometer. sGC was purified as described previously[1] except that the last desalting step was done with 25 mM TEA, 5 mM DTT, 50 mM NaCl, pH 7.4 and that no glycerol was added to the sample. The desalted sample was further concentrated on the Ultrafree-MC filter unit (30 kDa MWCO, Millipore). The concentrated sample (∼50 μL, ∼80 μM heme) was then placed in a CaF_2 cell (50 μm path length) and thermostated at 3 °C in a cryostat with AgCl and Ge windows. 20 000, 11 000, and 20 000 scans were averaged for $^{12}C^{16}O$, $^{13}C^{16}O$, and $^{13}C^{18}O$, respectively. The binding of CO was confirmed prior to FTIR by a Soret shift from 431 to 423 nm in the absorption spectrum.[3]