Soluble Guanylate Cyclase from Bovine Lung: Activation with Nitric Oxide and Carbon Monoxide and Spectral Characterization of the Ferrous and Ferric States†

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Received January 20, 1994®

ABSTRACT: Nitric oxide (•NO) is a recently discovered signaling agent which plays a role in many biological processes such as vasodilation and neuronal synaptic transmission. The only receptor characterized thus far for •NO is the soluble form of guanylate cyclase (sGC). •NO increases the V_{max} of sGC by 100–200-fold, probably by interacting with a heme moiety on the enzyme. Although several procedures exist for purifying sGC, these procedures result in preparations with low heme contents. Using a novel procedure, the enzyme has been purified to homogeneity from bovine lung with a heme content of approximately 1 heme/heterodimer. The UV–visible spectrum of the enzyme contains a Soret peak centered at 431 nm and a single broad α/β peak at 555 nm indicative of a 5-coordinate ferrous heme with histidine as the axial ligand. The heme moiety does not bind oxygen but will readily bind •NO to form a 5-coordinate complex or carbon monoxide (CO) to form a 6-coordinate complex. Oxidation of the heme with ferricyanide shifts the Soret to 393 nm, due most likely to the formation of a 5-coordinate ferric heme. In the ferric state, the heme will apparently not bind water but will bind cyanide with reduced affinity compared to methemoglobin and metmyoglobin. Purified enzyme containing 1 heme/heterodimer is activated 130-fold by •NO and 4.4-fold by CO.

Nitric oxide (•NO)[1] is a free radical that has recently been shown to mediate cell–cell communication in such processes as vasodilation and neuronal signal transduction [for recent reviews, see Calver et al. (1993), Moncada et al. (1991), and Nathan (1992)]. The only receptor for •NO characterized thus far is the soluble form of guanylate cyclase (sGC) (Waldman & Murad, 1987). sGC catalyzes the cyclization of guanosine 5′-triphosphate (GTP) to guanosine 3′,5′-cyclic monophosphate (cGMP). sGC is a heterodimer possessing an α subunit with a mass ranging from 73 to 88 kDa and a β subunit with a mass of 70 kDa. The enzyme has been reported to contain one protoporphyrin-IX type heme/heterodimer (Gerzer et al., 1981). Previous reports have suggested that •NO activates sGC via formation of a ferrous–nitrosyl–heme complex on the enzyme with a 100-fold increase in the V_{max} of the cyclase reaction (Ignarro et al., 1984b).

If •NO truly activates sGC via formation of a ferrous–nitrosyl complex, then two issues must be addressed. The first is that sGC must be able to form such a complex in an aerobic environment. Ferrous hemoproteins which readily form nitrosyl complexes are almost always 5-coordinate high spin such as hemoglobin (Hb) and myoglobin (Mb). However, Hb and Mb do not form a ferrous–nitrosyl complex in an aerobic environment. These proteins will first bind O_2 to the sixth coordination position, followed by a rapid reaction of •NO with this ferrous–oxy species to form nitrate and ferric heme (Doyle & Hoekstra, 1981). Thus for the activation mechanism to hold true, either the heme in sGC must not bind O_2 or the resulting ferrous–oxy species must undergo ligand exchange with •NO rather than reaction. The second issue is deactivation. Organ studies have indicated that, once activated, sGC deactivates within a few minutes (Palmer et al., 1987). However, ferrous–nitrosyl–heme complexes are quite stable, with half-lives for •NO dissociation ranging from 4 min to 3 h at 20 °C (Hille et al., 1979; Sharma & Ranney, 1978). Thus simple dissociation may not be a viable means of deactivation. However, deactivation may involve a protein-catalyzed redox chemical modification of the nitrosyl ligand.

While several schemes have been reported for purifying sGC from bovine lung, all employ the use of techniques which potentially "damage" the enzyme and result in preparations with low heme contents (Waldman & Murad, 1987). Thus a new procedure was developed for the purification of sGC from bovine lung employing only techniques which have a low potential for causing heme loss.

While the Soret regions of electronic spectra for the ferrous protein and its •NO and CO complexes have been reported (Gerzer et al., 1981), the α/β regions of these spectra are less well characterized. Furthermore, the ferric form of the enzyme has not previously been reported. Here we present both the Soret and α/β regions of electronic spectra for ferrous sGC and for its •NO and CO complexes. Also, we report the formation of the ferric enzyme and its cyanide complex.

Recently CO has been hypothesized to be a mediator of cell–cell communication (Maines, 1993; Verma et al., 1993). Much of the weight for this theory rests on reports of CO activating crude preparations of lung sGC (Brüne et al., 1990). However, the ability of CO to activate sGC appeared to diminish markedly upon even partial purification, and purified sGC has not previously been shown to be activated by CO. Here we report that purified sGC containing 1 heme/

† This work was supported by the Hitchings Award of the Burroughs-Wellcome Fund and NIH Grant T32 GM07767. Sequence analyses were supported by General Clinical Research Center Grant M01 RR00042.

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® Abstract published in *Advance ACS Abstracts,* April 1, 1994.

[1] Abbreviations: sGC, soluble guanylate cyclase; •NO, nitric oxide; CO, carbon monoxide; TEA, triethanolamine; EDTA, ethylenediaminetetraacetic acid; IBMX, isobutylmethylxanthine; DTT, dithiothreitol; GTP, guanosine 5′-triphosphate; cGMP, guanosine 3′,5′-cyclic monophosphate; Hb, hemoglobin; Mb, myoglobin; SNP, sodium nitroprusside; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; BSA, bovine serum albumin.

0006-2960/94/0433-5636$04.50/0

Table 1: Purification Summary for sGC

fraction	act. (nmol min^{-1})	protein[a] (mg)	sp act. (nmol min^{-1} mg^{-1})	purifn factor
supernatant	1142	40600	0.0281	1.0
DEAE-Sepharose	3320	5670	0.586	20.8
ATP-agarose	912	33.1	27.6	982
GTP-agarose	464	3.45	134	4770
Ultrogel-AcA-34	403	0.509	792	28200

[a] Determined by Bradford microassay, not corrected by quantitative amino acid analysis.

heterodimer is mildly stimulated by high concentrations of CO. A direct comparison between the ability of •NO to activate sGC and the ability of CO to activate sGC is also presented.

MATERIALS AND METHODS

Materials. Research-grade argon (99.9999%), CO (99.5%), and •NO (99.0%) were obtained from Matheson. All other materials unless otherwise stated were obtained from Sigma.

Purification of sGC from Bovine Lung. sGC was purified from bovine lung by a novel procedure based in part on previously published procedures (Garbers, 1979; Wolin et al., 1982). All steps were performed at 4 °C. During the purification, assays for sGC were carried out in 50 mM TEA, pH 7.4, with 2 mM DTT, 5 mM $MnCl_2$, 100 μM GTP, 1 mM IBMX, 5 mM phosphocreatine, and 152 units/L creatine kinase, with or without 100 μM sodium nitroprusside (SNP) in a total volume of 100 μL at 37 °C. The assays were quenched with 400 μL of 125 mM $Zn(CH_3COO)_2$ and 500 μL of 125 mM Na_2CO_3. The amount of cGMP generated was then quantitated via radioimmunoassay (Amersham). After each column, only active fractions which could be activated at least 10-fold with SNP were carried on to the subsequent step.

Fresh bovine lung (1100 g) was homogenized with a food processor in 1100 mL of homogenizing buffer (25 mM TEA, 5 mM DTT, 1 mM PMSF, 1 mM EDTA, 0.2 mM benzamidine, 1 μM pepstatin A, 1 μM leupeptin, pH 7.8). The homogenate was then centrifuged at 100000*g* for 60 min. The resulting supernatant was added to 450 mL of DEAE-Sepharose CL-6B and stirred slowly with an overhead stirrer for 45 min. The buffer was removed by vacuum filtration, and the resin was washed four times by stirring slowly for 15 min with 1200 mL of homogenizing buffer. The resin was then poured into a 2.5 × 100 cm column and packed by washing the column with homogenizing buffer for 1 h at 80 mL/h. sGC was then eluted with 1 L of a 0.0–0.4 M NaCl gradient at 80 mL/h. Active fractions were pooled (140 mL) and dialyzed for 8 h against 4 L of 25 mM TEA, 5 mM DTT, 0.1 mM PMSF, 0.2 mM benzamidine, 1 μM pepstatin A, 1 μM leupeptin, pH 7.4.

The sample was then brought to 5 mM $MnCl_2$ and applied at 25 mL/h to a 75-mL column of ATP-agarose (11-atom spacer attached through C-8). The column was washed at 25 mL/h with 150 mL of buffer A (25 mM TEA, 5 mM DTT, 0.1 mM PMSF, 0.2 mM benzamidine, 5 mM $MnCl_2$, pH 7.4). The column was then washed at 50 mL/h with 200 mL of buffer A containing 10 mM $NaNO_3$, 10 mM creatine, and 0.5 mM ADP followed by 300 mL of buffer A. sGC was then eluted at 50 mL/h with a 2-L gradient running from 5 mM $MnCl_2$ to 1 mM EDTA. Active fractions were pooled (200 mL), concentrated by ultrafiltration to 30 mL on a YM-30 membrane (Amicon), and brought to 5 mM $MnCl_2$.

The sample was then applied at 5 mL/h to a 3.0-mL column of GTP-agarose (11-atom spacer attached through ribose hydroxyls, ICN). The column was washed at 5 mL/h with 10 mL of buffer A, and then sGC was eluted with a 100-mL gradient running from 0.0 to 0.4 M NaCl in buffer A. Active fractions were pooled, concentrated by ultrafiltration to 8 mL on an Omega Series membrane (30 kDa MWCO, Filtron), and applied to a 2.5 × 95 cm column of Ultrogel AcA-34 (IBF) at 20 mL/h. The column was then washed with 400 mL of 25 mM TEA, 5 mM DTT pH 7.8 at 20 mL/h. Active fractions were pooled (20 mL), provided with additional DTT (5 mM final), brought to 1.0 M NaCl, and concentrated by ultrafiltration to 1.0 mL on an Omega Series membrane. The sample was brought to 50% glycerol and stored as aliquots under nitrogen at −70 °C.

Heme Content. Heme concentrations were determined by the pyridine–hemochromagen assay using myoglobin as the standard as previously described (White & Marletta, 1992). All sGC samples were desalted in 25 mM TEA, pH 7.4, to remove thiol just prior to analysis.

Activation of Purified sGC with •NO and CO. •NO was purified of other nitrogen oxides by bubbling through saturated KOH. Buffer containing 50 mM TEA, pH 7.4, 2 mM DTT, 4.5 mM $MgCl_2$, and 1.5 mM GTP was placed in a gas-tight vial under 1 atm of the appropriate gas and warmed to 37 °C. The reaction was initiated by the injection of 4 μL of sGC with a gas-tight syringe to give a final reaction volume of 1.0 mL with an enzyme concentration of 0.75 μg/mL. At time intervals, 100 μL aliquots were removed with a gas-tight syringe and then quenched and assayed for cGMP as described above.

Protein Determination. Protein concentrations were determined with the Bradford microassay (Bio-Rad) using BSA as the standard. The assay was calibrated using quantitative amino acid analysis as performed by the University of Michigan Protein and Carbohydrate Structure Facility. The correction factor obtained was applied to all specific activity and heme content determinations.

Electronic Spectroscopy. All electronic spectra were obtained on a Cary 3E UV–visible spectrophotometer at 10 °C. Buffer conditions were as described in the figure captions.

Electrophoresis. Reducing SDS polyacrylamide gel electrophoresis (SDS–PAGE) of purified sGC was performed using the Bio-Rad mini gel system following the procedure provided by the manufacturer. The running gel contained 12% acrylamide, and the protein was visualized via silver stain (Bio-Rad).

RESULTS

Purification. The novel purification scheme described here results in the isolation of approximately 0.5 mg of sGC from 1100 g of bovine lung (Table 1). Reducing SDS–PAGE of the purified protein reveals two bands of approximately equal intensity with apparent masses of 69 and 78 kDa (Figure 1). As seen previously, the total activity increases upon placing supernatant over the ion-exchange column (Wolin et al., 1982).

Heme Content. Quantitative amino acid analysis reveals that the Bradford microassay with BSA as the standard overestimates the amount of sGC by a factor of 1.66 ± 0.05

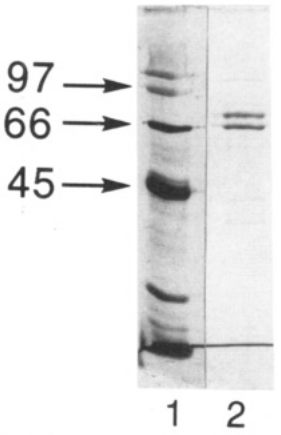

FIGURE 1: 12% reducing SDS–PAGE of sGC: lane 1, molecular weight markers (kDa); lane 2, sGC.

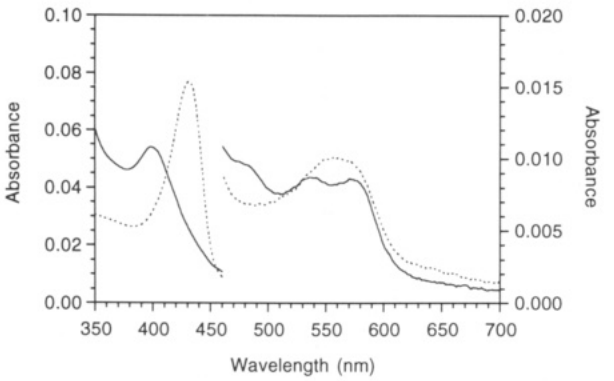

FIGURE 2: Electronic absorption spectra of sGC in 12.5 mM TEA, pH 7.8, 5 mM DTT, 0.5 M NaCl, 50% glycerol under air (···) and •NO (—). The scale on the left refers to the Soret region, and the scale on the right refers to the α/β region.

(n = 4). Using this correction factor and the pyridine–hemochromagen assay, the purified protein was found to contain 1.08 ± 0.17 hemes/heterodimer (n = 3 preparations). It was observed that the peak activity after each of the first three columns (ion exchange, ATP-agarose, and GTP-agarose) contained multiple populations of guanylate cyclase activity with some active fractions that were highly activated (>10-fold) with 100 μM SNP and some that were activated poorly (<10-fold) using Mn^{+2} as the required divalent metal. In order to obtain enzyme with a high heme content, only those fractions activated >10-fold with SNP were carried on to the next step.

Spectral Studies. The UV–vis spectrum of the enzyme in 5 mM DTT under air (Figure 2) depicts a sharp Soret at 431 nm and a single broad peak in the α/β region at 555 nm. The spectrum is unchanged by placing the enzyme under argon or ~100% O_2 or by removing the DTT by desalting. Upon placing anaerobic enzyme under 1 atm of •NO, the Soret shifts to 398 nm with the appearance of a shoulder at 485 nm and peaks at 537 and 572 nm (Figure 2). Upon placing aerobic enzyme under 1 atm of CO (Figure 3), the Soret shifts to 423 nm and the single broad α/β peak is replaced by peaks at 567 and 541 nm.

Addition of 5 μM ferricyanide to thiol-free sGC shifts the Soret from 431 nm to 393 nm (Figure 4). This spectral transition is reversed with dithionite. Addition of 1 mM KCN to the oxidized species has a minimal effect on the spectrum, but addition of 10 mM KCN shifts the Soret to 418 nm (Figure 4). Table 2 summarizes the peak positions and extinctions for all species analyzed.

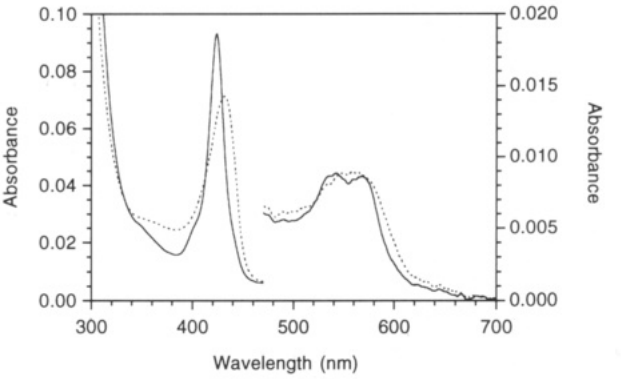

FIGURE 3: Electronic absorption spectra of sGC in 12.5 mM TEA, pH 7.8, 5 mM DTT, 0.5 M NaCl, 50% glycerol under air (···) and CO (—). The scale on the left refers to the Soret region, and the scale on the right refers to the α/β region.

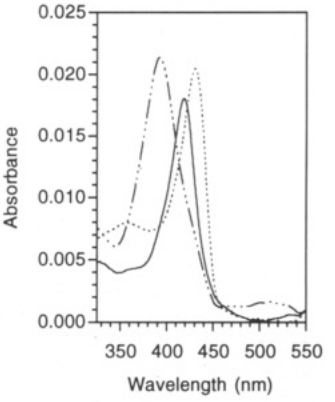

FIGURE 4: Electronic absorption spectra of sGC in 25 mM TEA, pH 7.4: ferrous (···), ferric (–···–), and ferric–cyano (—).

Table 2: Peak Positions and Extinctions for sGC[a]

sample	Soret	β	α
ferrous	431 (111)	555 (14)	
ferrous–NO	398 (79)	537 (12)	572 (12)
ferrous–CO	423 (145)	541 (14)	567 (14)
ferric	393 (118)		
ferric–CN	418 (99)		

[a] All peak positions are in nm and all extinctions are in mM^{-1} with a standard deviation of ±10%. All values were determined in 25 mM TEA, pH 7.4.

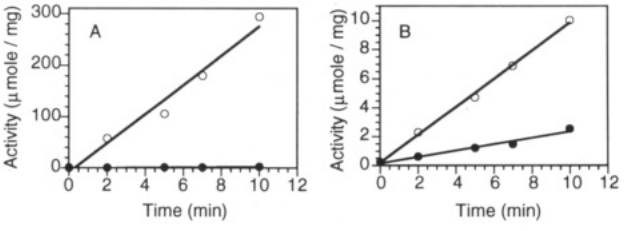

FIGURE 5: Activation of sGC with •NO and CO. sGC was assayed as described in Materials and Methods. Panel A: argon (●), 0.5% •NO in argon (○). Panel B: argon (●), CO (○). Note the difference in the ordinate scale between the two panels.

Activity Studies. As observed previously, the nonactivated (basal) specific activity is significantly lower when Mg^{2+} is used as the required divalent metal rather than Mn^{2+} (Waldman & Murad, 1987). Note that for each gas tested (~100% argon, ~100% CO, and 0.5% •NO in argon), enzyme activity was linear for the 10 minutes assayed (Figure 5). There does not appear to be any lag for activation with either •NO or CO nor do the activated rates diminish during the 10 minutes observed. Purified enzyme containing approximately

Table 3: Activation of sGC with •NO and CO

gas in headspace	sp act. (nmol min^{-1} mg^{-1})	fold activation
argon	221 ± 23	
CO	966 ± 39	4.4 ± 0.4
0.5% •NO in argon	28200 ± 2800	128 ± 17

1 heme/heterodimer is activated 130-fold by 0.5% •NO and 4–5-fold by ~100% CO (Table 3).

DISCUSSION

The new purification scheme detailed here provides for the purification of enzyme containing significant levels of heme, approximately 1 heme/heterodimer. This is a result of eliminating procedures which are believed to cause heme loss, namely, isoelectric focusing, isoelectric precipitation, preparative electrophoresis, ammonium sulfate precipitation, and dye-ligand chromatography (Waldman & Murad, 1987). However, it is still not clear what the final heme stoichiometry will be since the two subunits share significant homology to each other (Koesling et al., 1990; Nakane et al., 1990). For example, each subunit contains a C-terminal "cyclase" domain which is homologous to the catalytic domains of particulate guanylate cyclases and adenylate cyclases. Also each contains a putative N-terminal regulatory domain which shows 31% identity and 54% overall homology between the two subunits but no homology to any other known protein sequence. Currently heme is presumed to bind in this regulatory portion of the subunits. Since heme binding cannot be localized to any particular sequence in the region and with the high degree of homology, it is quite possible that the enzyme will contain 2 hemes/heterodimer. Reconstitution experiments and further manipulations of the purification scheme are currently under way to answer this question.

The spectral experiments carried out clearly show that the heme in sGC is highly unusual. The UV–vis spectrum of the isolated protein reveals a Soret centered at 431 nm and a single broad α/β peak at 555 nm. This spectrum is characteristic of a 5-coordinate high-spin ferrous heme with imidazole as the axial ligand such as in deoxyMb (Antonini & Brunori, 1971). Nearly all hemoproteins which form such a complex do so only in an anaerobic environment since oxygen will readily bind to the sixth coordination position. Interestingly, the ferrous heme of sGC appears to be 5-coordinate even under air. In fact the spectrum under argon is identical to that under 100% O_2. Thus the heme of sGC appears to have an unusually low affinity for O_2.

The heme in sGC will form a complex with •NO when placed under 1 atm of the gas. The Soret shifts to 398 nm, and a prominent shoulder at 485 nm appears. These spectral characteristics are indicative of a 5-coordinate ferrous–nitrosyl–heme complex (Suzuki et al., 1987; Yoshimura & Ozaki, 1984). Thus the bond of the axial imidazole ligand to the iron appears to be broken as •NO binds to the heme. 6-Coordinate nitrosyl complexes tend to have a Soret maximum centered around 420 nm and lack a shoulder at 485 nm (Yoshimura, 1986). Although the nitrosyl complex of sGC was formed under 1 atm of •NO, the complex can be formed with lower concentrations of •NO (data not shown). Currently the dissociation constant (K_d) of •NO for the heme of sGC is unknown. The heme in sGC also forms a complex with CO as shown previously (Gerzer et al., 1981). Under 1 atm of CO, the Soret shifts to 423 nm, and the α/β peak splits. This spectrum is indicative of a 6-coordinate complex where CO is one axial ligand and imidazole the other (Antonini & Brunori, 1971). Therefore, the coordination complexes generated with •NO and CO are significantly different.

As isolated in the presence of 5 mM DTT, the heme is in the ferrous state. Upon removal of DTT by desalting, the heme remains in the ferrous state for at least 2 h at 10 °C. However, the heme can be oxidized with 5 μM ferricyanide. The position of the ferric Soret at 393 nm is unusual for a ferric–imidazole heme. For metHb and metMb, the iron is always 6-coordinate because, if no exogenous ligand is provided, water will coordinate at the sixth position. Correspondingly, the Sorets range from 410 to 425 nm for the 6-coordinate low-spin complexes and from 405 nm to 410 for the 6-coordinate high-spin complexes (Antonini & Brunori, 1971). However, the prokaryotic cytochrome c' contains a 5-coordinate ferric heme with imidazole as the sole axial ligand (Finzel et al., 1985). Water does not bind to the ferric heme of cytochrome c', and the Soret positions are 390–400 nm (Yamanaka, 1992). Thus, at present, it is most likely that, even in the ferric state, the heme of sGC is 5-coordinate high spin with the unusual property of not binding water. The spin state and coordination number of ferri-sGC will be studied further with other spectroscopic techniques.

In the presence of 10 mM KCN, the Soret of ferric sGC shifts from 393 nm to 418 nm. This latter position is in good agreement with those of 6-coordinate cyanide complexes of metHb and metMb (Antonini & Brunori, 1971). Thus the ferric heme of sGC is able to accommodate ligand at the sixth coordination site. Cyanide is the first ionic ligand shown to bind to the heme of sGC. However, the K_d appears to be between 1 and 10 mM, which is 6 orders of magnitude higher than for metHb and metMb (Antonni & Brunori, 1971). Therefore the ferric heme of sGC has a much reduced affinity for cyanide compared to most other ferric imidazole hemes. Interestingly, for cytochrome c' the K_d for cyanide appears to be around 50 μM, which is 4 orders of magnitude higher than for metHb and metMb (Kassner et al., 1985). It is possible that the structural aspects of cytochrome c' and sGC which result in a 5-coordinate ferric heme also result in reduced cyanide affinity.

Cytochrome c' is a prokaryotic hemoprotein of unknown function (Meyer and Kamen, 1982; Yamanaka, 1992). In both the ferrous and ferric states, it is 5-coordinate with histidine as the only axial ligand. The ferrous state readily binds •NO to form a 5-coordinate ferrous–nitrosyl complex (Suzuki et al., 1987). On the basis of this latter observation, this protein has been hypothesized to be a physiologic receptor for •NO (Ferguson, 1991). Interestingly the UV–vis spectral properties and ligand-binding properties of the hemes of sGC and cytochrome c' are very similar. Ferrous cytochrome c' has been reported to bind •NO and CO but not O_2 (Meyer & Kamen, 1982), just as is the case for sGC. Also both proteins form a 5-coordinate ferrous–nitrosyl complex. In the ferric state, both exhibit Sorets in the 390–400-nm region, and both bind cyanide with much reduced affinity. However, unlike sGC, cytochrome c' displays a d-type hyperporphyrin spectrum in both the ferric and ferrous states (Yoshimura et al., 1985). Also, the ferric state of cytochrome c' is 5-coordinate but not actually high spin. The axial ligand field is so weak that an admixed intermediate spin state ($S = {}^3/_2, {}^5/_2$) exists (Scheidt & Reed, 1981). The spin state of ferri-sGC has yet to be determined. There appears to be no homology between the protein sequences of cytochrome c' and sGC, and thus the similarities in spectral and ligand-binding properties are probably a result of convergent evolution. In the case of cytochrome c', three explanations

for its unusual heme properties have been put forward (Kassner, 1991): (1) the sixth coordination position is sterically hindered, (2) the imidazole–iron bond is relatively weak, or (3) there is no H-bonding group near the sixth coordination position. Which, if any, of these factors holds true for sGC remains to be determined.

It has been hypothesized that •NO activates sGC by forming a nitrosyl–heme complex on the enzyme (Ignarro et al., 1984b). This hypothesis is based on the observation that activation with •NO is heme dependent (Craven & DeRubertis, 1978; Ignarro et al., 1982). Enzyme deficient in heme is not activated nearly as well as enzyme containing heme. Furthermore, heme-deficient enzyme is extensively activated by preformed ferrous–nitrosyl–heme complexes (Craven et al., 1979; Ignarro et al., 1982). One problem with this activation mechanism is that it requires the enzyme to form a ferrous–nitrosyl complex in an aerobic environment. Ferrous hemoproteins which readily form nitrosyl complexes are almost always 5-coordinate high spin such as Hb and Mb. However, Hb and Mb cannot form a ferrous–nitrosyl complex in an aerobic environment because they first bind O_2 to the sixth coordination position. •NO then rapidly reacts with this ferrous–oxy species to form nitrate and ferric heme (Doyle & Hoekstra, 1981). Thus for the activation mechanism to hold true, either the heme in sGC must not bind O_2 or the ferrous–oxy species must undergo ligand exchange with •NO rather than reaction. Here we have shown that the heme in sGC has an unusually low affinity for O_2, making it possible for sGC to form a ferrous–nitrosyl complex in an aerobic environment. Although the observations reported here support the activation mechanism as proposed, further experiments are required to determine if the mechanism is valid.

Recently CO has been hypothesized to be a mediator of cell–cell communication in the brain with sGC being the receptor (Maines, 1993; Verma et al., 1993). Much of the weight for this argument rests on the observation that crude preparations of sGC from bovine lung are stimulated by CO (Brüne et al., 1990). However, the ability to activate sGC with CO appeared to diminish markedly upon even partial purification of the enzyme, and pure sGC has not previously been shown to be activated by CO. Here we report that sGC containing 1 heme/heterodimer is activated 4.4-fold by ~100% CO. However, the activation is very small compared to that achieved with 0.5% •NO (130-fold). Furthermore, it is unclear whether CO at physiological concentrations will have any effect on sGC. In addition to the *in vitro* activation of sGC by CO, the CO signaling hypothesis has gained support from a report of an inhibitor of heme oxygenase lowering cGMP levels in cell culture (Verma et al., 1993). However, the heme oxygenase inhibitor employed, Zn-protoporphyrin-IX, has already been shown to directly inhibit sGC (Ignarro et al., 1984a). Thus, at present, the notion that CO may be a mediator of cell–cell communication by stimulating sGC must be viewed with some skepticism. However, it cannot be ruled out that there exists in the brain an isoform of sGC that is markedly stimulated by low concentrations of CO.

In summary, sGC has been purified from bovine lung by a novel procedure that results in a sample containing approximately 1 heme/heterodimer. The heme appears to be 5-coordinate high spin in both the ferric and ferrous states. The ferrous heme binds •NO to form a 5-coordinate complex and CO to form a 6-coordinate complex. The ferric heme binds cyanide with reduced affinity to form a 6-coordinate complex. Purified enzyme is activated 130-fold by •NO and 4.4-fold by CO.

ACKNOWLEDGMENT

We thank the University of Michigan Protein and Carbohydrate Structure Facility for performing the quantitative amino acid analysis.

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