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Activation of purified soluble guanylate cyclase by protoporphyrin IX

(enzyme regulation/cyclic GMP and porphyrins/heme/metalloporphyrins)

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ABSTRACT **Soluble guanylate cyclase [GTP pyrophosphate-lyase (cyclizing), EC 4.6.1.2] purified from bovine lung is markedly activated (30- to 40-fold) by protoporphyrin IX (K_a, 15–25 nM) and is inhibited by hematin (K_i, 3.7 μM) when MgGTP is used as substrate. Guanylate cyclase possesses specific activities (μmol of cGMP per min/mg of protein) of 0.1–0.2 (MgGTP) and 0.3–0.5 (MnGTP) and can attain values of 2–8 (MgGTP) or 1–1.4 (MnGTP) in the presence of protoporphyrin IX. Guanylate cyclase purified in this study contains heme and is activated by nitric oxide and nitrosyl-heme to the same magnitude as that by protoporphyrin IX. With the exception of hematoporphyrin IX, close structural analogs of protoporphyrin IX, including precursors and metabolites, do not activate guanylate cyclase. The insertion of iron into protoporphyrin IX to form heme or hematin renders the metalloporphyrin an inhibitor of unactivated or activated guanylate cyclase. The data suggest that protoporphyrin IX and heme could function to modulate guanylate cyclase activity.**

Unpurified soluble guanylate cyclase [GTP pyrophosphate-lyase (cyclizing), EC 4.6.1.2] is activated by nitric oxide and certain nitroso or nitro compounds capable of releasing or forming nitric oxide (1–4). The same compounds have been demonstrated to elevate cyclic GMP levels in a variety of tissues (5–13). These observations may be important pharmacologically because chemically related nitrogen oxide-containing drugs, such as nitroglycerin, amyl nitrite, and nitroprusside, are widely used clinically as vasodilators. In addition, many of these drugs are potent inhibitors of platelet aggregation (11, 14–16). Their actions as vascular smooth muscle relaxants and inhibitors of platelet aggregation are closely associated with their capacities to activate guanylate cyclase and elevate tissue levels of cyclic GMP (6, 9–13). Previous reports suggested that guanylate cyclase activation by nitric oxide, nitroso, and nitro compounds may be dependent on the presence of heme (17). Along these lines, nitric oxide is well known to bind heme iron, resulting in the formation of nitrosyl-heme, which has been shown to bind (18) and activate (17, 18) guanylate cyclase. Moreover, a recent report by Gerzer *et al.* (19) indicated that guanylate cyclase could be purified in a form containing heme and is activated by nitroprusside in the absence of added heme.

The above studies suggest that heme is indeed required for guanylate cyclase activation by nitric oxide and related agents. Nevertheless, the physiological significance of these observations is not apparent. The findings in the present study may shed some light on the potential physiological mechanisms by which soluble guanylate cyclase is activated. We report here that guanylate cyclase, purified to apparent homogeneity from bovine lung, is markedly activated by protoporphyrin IX, a naturally occurring intracellular metabolite in mammalian cells. In contrast, ferroprotoporphyrin IX (heme) and ferriprotoporphyrin IX (hematin) are potent inhibitors of guanylate cyclase.

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MATERIALS AND METHODS

Purification of Guanylate Cyclase. Details of the purification procedures will be described elsewhere. Briefly, fresh bovine lung (0.5 kg) was homogenized in 3 vol of 25 mM triethanolamine·HCl (Et_3N·HCl), pH 7.8/5 mM dithiothreitol. Homogenates were centrifuged at 105,000 × *g* for 60 min, and the supernatant was stirred with 400 g of DEAE-Sepharose CL-6B (Pharmacia), preequilibrated with Et_3N·HCl/dithiothreitol. The resin was extensively washed with Et_3N·HCl/dithiothreitol and packed into a column (2.5 × 80 cm). Guanylate cyclase was eluted with 1.3 liters of a linear NaCl gradient (0–0.4 M) in Et_3N·HCl/dithiothreitol. Fractions with peak activity (0.20–0.23 M NaCl) were pooled, concentrated, and applied to a column (2.5 × 80 cm) of Ultrogel AcA-34 (LKB) equilibrated with Et_3N·HCl/dithiothreitol. Guanylate cyclase was eluted with 300 ml of Et_3N·HCl/dithiothreitol and active fractions were pooled, concentrated, and applied to a column (1.5 × 20 cm) of Matrex gel blue A (Amicon). After incorporating the sample into the resin, flow was stopped for 45 min, and the column was then washed extensively with Et_3N·HCl/dithiothreitol followed with 0.5 M NaCl in Et_3N·HCl/dithiothreitol. Guanylate cyclase was eluted with 400 ml of a linear NaCl gradient (0.5–2 M) in Et_3N·HCl/dithiothreitol. Fractions with peak enzymatic activity (1.2–1.3 M NaCl) were pooled and concentrated. Glycerol was added to 30% (vol/vol), and 0.2- to 0.5-ml aliquots were equilibrated with oxygen-free N_2 and stored at −60°C. Basal specific activities (μmol of cGMP per min/mg of protein) were 0.1–0.2 in the presence of 1 mM GTP and 3 mM Mg^{2+}, and 0.3–0.5 with 1 mM GTP and 3 mM Mn^{2+}.

Guanylate cyclase was purified 6,000- to 10,000-fold from the starting crude supernatant (0.05 nmol of cGMP per min/mg of protein; MnGTP substrate). The purified enzyme migrated as a single band under both nondenaturing and denaturing conditions of polyacrylamide gel electrophoresis. Nondenaturing electrophoresis in 50 mM Tris glycine (pH 8.2) was performed as described by Garbers (20). Denaturing polyacrylamide gel electrophoresis was conducted with 0.3 × 14 × 16 cm vertical slab gels (total acrylamide, 7.5%) in 25 mM Tris glycine, pH 8.3/0.1% sodium dodecyl sulfate. Electrophoresis was conducted at constant temperature (18°C) and current (50 mA). Enzyme samples in 0.1 M Tris·HCl, pH 6.8/2% (wt/vol) sodium dodecyl sulfate/100 mM dithiothreitol/10% (vol/vol) glycerol/0.0013% bromphenol blue were heated at 100°C for 12 min just prior to electrophoresis. The amounts of enzyme protein analyzed ranged from 4 to 20 μg. The M_r of the purified protein was ≈72,000 after polyacrylamide gel electrophoresis under denaturing conditions. These observations are similar to those of

Abbreviation: Et_3N·HCl, triethanolamine·HCl.

Gerzer *et al.* (19), which were made with bovine lung soluble guanylate cyclase. Product formation was linear with time (0–90 min at 37°C; 0.24–0.32 μg of enzyme) and with enzyme concentration (0.1–1 μg of enzyme; 10 min at 37°C) in 1 mM GTP/3 mM Mg^{2+}/2 mM dithiothreitol.

Purified guanylate cyclase contained heme (ferroprotoporphyrin IX), as verified by visible absorption spectroscopy (21). Concentrated enzyme (63 μg in 0.2 ml) in 25 mM Et_3N·HCl, pH 7.8/30% (vol/vol) glycerol/5 mM dithiothreitol/0.1 M NaCl was scanned (320–700 nm) at 10°C under N_2 and revealed a sharp absorbance maximum at 425 nm and a much smaller peak at 560 nm. Addition of excess purified NO under N_2 produced a distinct shift in the major absorbance peak to 401 nm. During the preparation of this manuscript, Gerzer *et al.* (22) reported similar observations.

Guanylate Cyclase and Other Assays. Guanylate cyclase activity was determined as described (21), except that 40 mM Et_3N·HCl, pH 7.4/2 mM dithiothreitol was used as the buffer and enzyme reactions were assayed in 0.2- or 1-ml volumes as indicated. Protein concentrations were determined by the Bio-Rad, Coomassie brilliant blue G-250 method, which is unaffected by large concentrations of Et_3N·HCl, dithiothreitol, or glycerol. The preparation and handling of nitric oxide, nitrosyl-heme, and *S*-nitroso-*N*-acetylpenicillamine have been described (21, 23).

Materials. Reagents for the guanylate cyclase assay have been described (21, 23). Protoporphyrin IX and its dimethyl ester, coproporphyrin I, uroporphyrin I, porphobilinogen, hematin, biliverdin, and bilirubin were obtained from Sigma. Hematoporphyrin IX was purchased from ICN Pharmaceuticals. All other chemicals were of the highest purity available. Heme was prepared from hematin by reduction of the latter with dithionite in 50 mM Tris·HCl, pH 7/10 mM dithiothreitol under an atmosphere of N_2 (18, 21).

RESULTS

Effects of Porphyrins and Related Substances on Guanylate Cyclase Activity. Guanylate cyclase was activated about 30-fold by 0.1 μM protoporphyrin IX when MgGTP was used as substrate (Table 1). Several precursors of protoporphyrin IX such as porphobilinogen, uroporphyrin I, and coproporphyrin I had no effect on enzymatic activity. In contrast to the marked enzyme activation by protoporphyrin IX, heme and hematin inhibited guanylate cyclase activity, whereas biliverdin and bilirubin, two metabolites of heme, elicited no effect. Similarly, the dimethyl ester of protoporphyrin IX was inactive. However, hematoporphyrin IX, which differs from protoporphyrin IX in possessing two hydroxyethyl groups instead of vinyl groups, activated guanylate cyclase. Nitric oxide, nitrosyl-heme, and *S*-nitroso-*N*-acetylpenicillamine also activated guanylate cyclase (Table 1). Larger concentrations of these agents did not cause further activation. The magnitude of activation of purified enzyme by nitric oxide was much greater than that reported previously (24) and is attributed to the presence of heme in guanylate cyclase purified in the present study. Indeed, nitric oxide activated guanylate cyclase to the same magnitude as did preformed nitrosyl-heme, which, unlike nitric oxide, is believed to activate guanylate cyclase in the absence of heme (17). Further, the addition of 0.1–1 μM hematin to reaction mixtures did not enhance, and larger hematin concentrations inhibited, the activation of guanylate cyclase by NO or *S*-nitroso-*N*-acetylpenicillamine (not shown). This is the first report of activation of purified guanylate cyclase by nitrosyl-heme. Guanylate cyclase activity was not observed in the absence of metal whether or not activators were present.

Effects of Protoporphyrin IX on the K_m for GTP and V_{max} of Guanylate Cyclase. In the presence of excess Mn^{2+}, the apparent K_m for GTP was 10 μM, and the extrapolated V_{max} was 0.17 μmol/min per mg of protein (Fig. 1). Absolute values for specific activity corresponding to the points for 1 mM GTP ranged from 0.3 to 0.5. A saturating concentration (5 μM) of protoporphyrin IX increased the V_{max} from 0.17 to 0.5 μmol/min per mg of protein without altering the apparent K_m. The values for both K_m and V_{max} were calculated from the linear portions of the double reciprocal plots in Fig. 1. GTP up to 0.1 mM gave linear plots, but as the GTP concentration was increased to 1 mM, the lines curved downward. In contrast to Mn^{2+}, linear plots were obtained with Mg^{2+} over a range of 0.01–1 mM GTP (Fig. 2). In the presence of excess Mg^{2+}, the K_m for GTP was 100 μM and the V_{max} was 0.1 μmol/min per mg of protein. At saturating concentrations of 0.1 and 1 μM protoporphyrin IX, a marked increase in the V_{max} to 2 μmol/min per mg of protein and a small decrease in the K_m to 56 μM were observed. Specific activities (determined in the presence of 1 mM GTP and 3 mM Mg^{2+}) ranged from 0.1 to 0.2 μmol/

Table 1. Effects of protoporphyrin IX, heme, nitric oxide, and related agents on guanylate cyclase

Test agent*	Concentration, μM	Guanylate cyclase activity, μmol cGMP/min/mg protein
None (basal activity)	—	0.14
Protoporphyrin IX	0.1	3.9
	1	4.3
Porphobilinogen	10	0.15
Uroporphyrin I	1	0.15
Coproporphyrin I	1	0.16
Protoporphyrin IX dimethyl ester	1	0.17
Hematoporphyrin IX	0.1	2.5
	1	3.7
Hematin	10	0.05
Heme	10	0.06
Biliverdin	10	0.14
Bilirubin	10	0.15
Nitric oxide, 0.5 μl	—	5.2
Nitrosyl-heme	1	5.5
S-Nitroso-*N*-acetyl-penicillamine	100	5.4

* Test agents were incubated for 10 min at 37°C in reaction mixtures (0.2 ml) containing 40 mM Et_3N·HCl (pH 7.4), 2 mM dithiothreitol, 1 mM GTP, 3 mM Mg^{2+}, and 0.24 μg of guanylate cyclase.

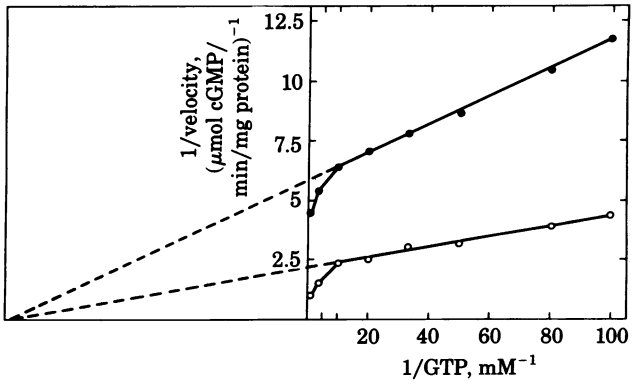

FIG. 1. Effect of protoporphyrin IX on K_m for GTP and V_{max} of guanylate cyclase in the presence of Mn^{2+}. Enzymatic reactions were conducted for 10 min at 37°C in the absence (●) or presence (○) of 5 μM protoporphyrin IX in 40 mM Et_3N·HCl (pH 7.4) containing 2 mM dithiothreitol, 3 mM Mn^{2+}, 0.32 μg of guanylate cyclase, and 0.01–1 mM GTP. Reaction volumes were 0.2 ml or 1 ml in the absence or presence, respectively, of protoporphyrin IX.

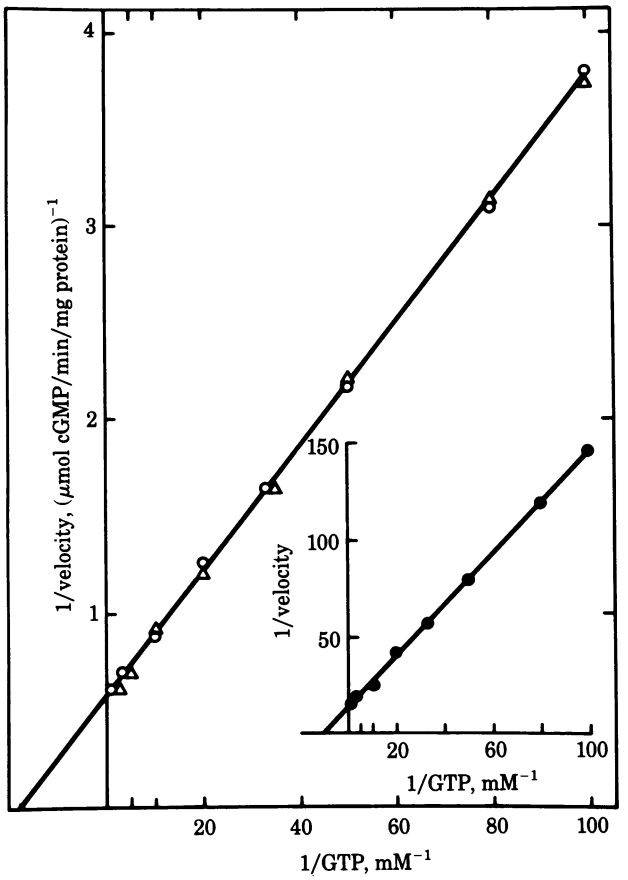

FIG. 2. Effect of protoporphyrin IX on K_m for GTP and V_{max} of guanylate cyclase in the presence of Mg^{2+}. Enzymatic reactions were conducted for 10 min at 37°C in the absence (●; *Inset*) or presence of 0.1 μM (○) and 1 μM (△) protoporphyrin IX in 40 mM Et_3N·HCl (pH 7.4) containing 2 mM dithiothreitol, 3 mM Mg^{2+}, 0.24 μg of guanylate cyclase, and 0.01–1 mM GTP. Reaction volumes were 0.2 ml or 1 ml in the absence or presence, respectively, of protoporphyrin IX.

min per mg of protein (basal activity) and from 2 to 8 μmol/min per mg of protein (protoporphyrin IX-activated), depending upon the preparation of guanylate cyclase used in a given experiment.

Inhibition of Guanylate Cyclase Activity by Hematin. Hematin inhibited basal guanylate cyclase activity in a noncompetitive manner when either MgGTP (Fig. 3) or MnGTP (not shown) was used as substrate. The K_m remained constant at 100 μM, whereas the V_{max} was decreased by 10 μM hematin. Replots of slopes and intercepts versus hematin concentrations yielded K_i values of 2.8 μM and 8.3 μM in the presence of MgGTP and MnGTP, respectively. In contrast to basal guanylate cyclase activity, when the enzyme was activated by 1 μM protoporphyrin IX, 10 μM hematin decreased the V_{max} and increased the K_m from 55 to 100 μM, the latter value being similar to that for the inactivated enzyme. Thus, the inhibition by 10 μM hematin was still noncompetitive with respect to GTP in the presence of 1 μM protoporphyrin IX. However, preliminary data indicate that hematin concentrations below 1.5 μM competitively inhibited protoporphyrin IX without altering basal guanylate cyclase activity. The decreased V_{max} and increased K_m observed with larger hematin concentrations in the presence of protoporphyrin IX are attributed to a secondary binding interaction of hematin with the enzyme and correlate with the noncompetitive inhibition with respect to GTP for unactivated enzyme.

Interaction Between Protoporphyrin IX and Guanylate Cyclase. By using either MgGTP or MnGTP as substrate, guanylate cyclase was activated by protoporphyrin IX over a range of 0.1 nM to 10 μM. The apparent K_a for protoporphyrin IX in the presence of saturating concentrations of MgGTP and MnGTP was 23 nM (range, 15–25 nM) and 50 nM (range, 45–55 nM), respectively (Fig. 4). Assuming a guanylate cyclase M_r of 150,000 (19, 20, 25), the catalytic turnover rate (mol of GTP per mol of enzyme per sec) was 0.15–0.35 and 0.6–1.3 in the presence of Mg^{2+} and Mn^{2+}, respectively. Saturating concentrations of protoporphyrin IX stimulated the turnover rate 30- to 40-fold in the presence of MgGTP to values of 4.6–13.4

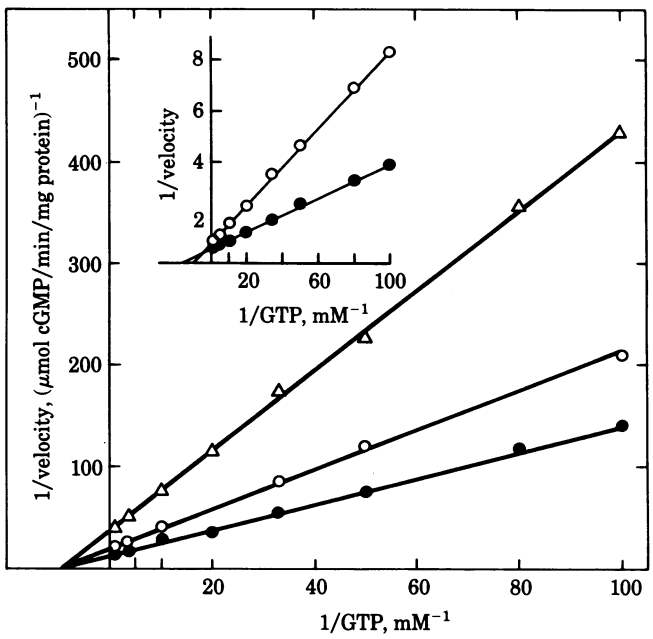

FIG. 3. Effect of hematin on basal and protoporphyrin IX-activated guanylate cyclase in the presence of MgGTP. Enzymatic reactions were conducted for 10 min at 37°C in the absence (●) or presence of 3 μM (○) and 10 μM (△) hematin in 40 mM Et_3N·HCl (pH 7.4) containing 2 mM dithiothreitol, 3 mM Mg^{2+}, 0.32 μg of guanylate cyclase, and 0.01–1 mM GTP. (*Inset*) Protoporphyrin IX (1 μM) in the absence (●) and presence (○) of 10 μM hematin. Reaction volumes were 0.2 ml or 1 ml in the absence or presence, respectively, of protoporphyrin IX.

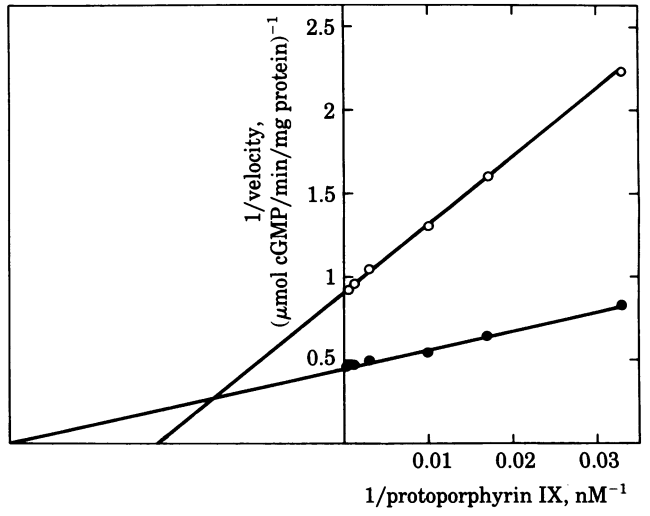

FIG. 4. Effect of protoporphyrin IX concentration on guanylate cyclase activity in the presence of MgGTP (●) and MnGTP (○). Enzymatic reactions (0.2 ml) were conducted for 10 min at 37°C in 40 mM Et_3N·HCl (pH 7.4) containing 2 mM dithiothreitol, 0.3 mM GTP, 0.24 μg of guanylate cyclase, 3 mM Mg^{2+} or 3 mM Mn^{2+}, and 0.03–10 μM protoporphyrin IX.

DISCUSSION

The present study shows that protoporphyrin IX is a potent activator, whereas heme and hematin are inhibitors of soluble guanylate cyclase. The natural occurrence of these substances in mammalian cells at concentrations similar to those used in this study (26, 27) suggests that protoporphyrin IX and heme could play biologic roles in altering guanylate cyclase activity. Bovine soluble guanylate cyclase purified recently by Gerzer *et al.* (19), like the enzyme purified in the present study, also contained heme. Although heme is well known to serve as a prosthetic group for various enzymes and other proteins, the role of heme in the expression of basal guanylate cyclase activity is unclear. Heme apparently is not required for basal enzyme activity (17) and, in fact, inhibits basal enzyme activity at concentrations of 3–10 μM. On the other hand, experimental evidence suggests that heme is required for activation of guanylate cyclase by nitric oxide and nitroso compounds (17). However, any physiological significance of guanylate cyclase activation by nitric oxide and related chemicals remains to be established.

Protoporphyrin IX is a naturally occurring substance in mammals and is the immediate precursor to heme, which is formed enzymatically by the insertion of iron. Thus, the incorporation of iron into protoporphyrin IX converts the latter from a potent activator (K_a, 15–25 nM) to an inhibitor (K_i, 3.7 μM) of guanylate cyclase. Slight alterations in the chemical structure of protoporphyrin IX (uroporphyrin I, coproporphyrin I, and dimethyl ester of protoporphyrin IX) and heme (biliverdin and bilirubin) resulted in compounds that failed to alter guanylate cyclase activity. However, hematoporphyrin IX activated guanylate cyclase, although it was slightly less potent than protoporphyrin IX. Hematoporphyrin IX, which does not occur naturally in mammals, differs from protoporphyrin IX in having two hydroxyethyl groups in place of vinyl groups. Apparently, this minor structural modification still permits binding to the enzyme associated with activation. Although the physiological significance of the present observations is not yet appreciated, the natural occurrence of protoporphyrin IX and the extremely low concentrations (0.1–1 nM) required to activate guanylate cyclase indicate that additional studies are warranted.

The magnitude of regulation of guanylate cyclase may center around the metal cofactor. In the presence of Mg^{2+}, the physiologically relevant metal, guanylate cyclase is activated 30- to 40-fold by protoporphyrin IX or nitric oxide. Because the major effect of these activators on guanylate cyclase appears to be on the maximal velocity rather than on the binding constant or K_m for GTP, the activators may facilitate catalysis by stabilization of the transition state of the enzyme–substrate complex. However, alternative explanations are possible. Although protoporphyrin IX does not eliminate the enzyme's requirement for free metal, preliminary evidence suggests that a lowered free metal requirement is a component of the activation mechanism. Because all known functions of protoporphyrin IX are expressed as a metalloporphyrin, and because divalent metals readily form complexes with protoporphyrin IX (28), it is plausible that a direct binding interaction between protoporphyrin IX and metal could contribute to the mechanism of guanylate cyclase activation.

The mechanism of guanylate cyclase activation by nitric oxide is unknown. Considerable controversy exists as to whether or not heme is required for enzyme activation by nitric oxide (17, 18, 21, 24). However, evidence exists to suggest that heme is required to observe guanylate cyclase activation by nitric oxide and that nitrosyl-heme is the species responsible for enzyme activation (17). Accordingly, one possible mechanism by which nitric oxide activates guanylate cyclase is that the nitrosyl-heme complex, which may bind near or at the catalytic site of guanylate cyclase (18), could possess a structure with the metal out of the plane of the porphyrin ring, thereby resembling protoporphyrin IX. Experimental evidence from electron paramagnetic resonance studies indicates that the binding of nitric oxide to heme iron results in considerable stress and weakening of coordinate bonds holding iron within the plane of the porphyrin ring (29–31). Studies are in progress to compare and contrast the characteristics of guanylate cyclase activation by protoporphyrin IX and nitric oxide and the inhibitory effect of heme on the activation process.

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1. Kimura, H., Mittal, C. K. & Murad, F. (1975) *J. Biol. Chem.* **250,** 8016–8022.
2. DeRubertis, F. R. & Craven, P. A. (1976) *Science* **193,** 897–899.
3. DeRubertis, F. R. & Craven, P. A. (1977) *J. Biol. Chem.* **252,** 5804–5814.
4. Arnold, W. P., Mittal, C. K., Katsuki, S. & Murad, F. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3203–3207.
5. Kimura, H., Mittal, C. K. & Murad, F. (1975) *Nature (London)* **257,** 700–702.
6. Diamond, J. & Blisard, K. S. (1976) *Mol. Pharmacol.* **12,** 688–692.
7. Schultz, K. D., Schultz, K. & Schultz, G. (1977) *Nature (London)* **265,** 750–751.
8. Bohme, E., Graf, H. & Schultz, G. (1979) *Adv. Cyclic Nucleotide Res.* **9,** 131–143.
9. Katsuki, S. & Murad, F. (1977) *Mol. Pharmacol.* **13,** 330–341.
10. Kukovetz, W. R., Holzmann, S., Wurm, A. & Poch, B. (1979) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **310,** 129–138.
11. Mellion, B. T., Ignarro, L. J., Ohlstein, E. H., Pontecorvo, E. G., Hyman, A. L. & Kadowitz, P. J. (1981) *Blood* **57,** 946–955.
12. Ignarro, L. J., Lippton, H., Edwards, J. C., Baricos, W. H., Hyman, A. L., Kadowitz, P. J. & Gruetter, C. A. (1981) *J. Pharmacol. Exp. Ther.* **218,** 739–749.
13. Gruetter, C. A., Gruetter, D. Y., Lyon, J. E., Kadowitz, P. J. & Ignarro, L. J. (1981) *J. Pharmacol. Exp. Ther.* **219,** 181–186.
14. Pfleiderer, T. (1972) *Acta Univ. Carol. Med.* **53,** 247–250.
15. Saxon, A. & Kattlove, H. E. (1976) *Blood* **47,** 957–961.
16. Schafer, A. I., Alexander, R. W. & Handin, R. I. (1980) *Blood* **55,** 649–654.
17. Craven, P. A. & DeRubertis, F. R. (1978) *J. Biol. Chem.* **253,** 8433–8443.
18. Ignarro, L. J., Kadowitz, P. J. & Baricos, W. H. (1981) *Arch. Biochem. Biophys.* **208,** 75–86.
19. Gerzer, R., Hofmann, F. & Schultz, G. (1981) *Eur. J. Biochem.* **116,** 479–486.
20. Garbers, D. L. (1979) *J. Biol. Chem.* **254,** 240–243.
21. Ignarro, L. J., Barry, B. K., Gruetter, D. Y., Ohlstein, E. H., Gruetter, C. A., Kadowitz, P. J. & Baricos, W. H. (1981) *Biochim. Biophys. Acta* **673,** 394–407.
22. Gerzer, R., Bohme, E., Hofmann, F. & Schultz, G. (1981) *FEBS Lett.* **132,** 71–74.
23. Gruetter, C. A., Barry, B. K., McNamara, D. B., Gruetter, D. Y., Kadowitz, P. J. & Ignarro, L. J. (1979) *J. Cyclic Nucleotide Res.* **5,** 211–224.
24. Braughler, J. M., Mittal, C. K. & Murad, F. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 219–222.
25. Murad, F., Lewicki, J. A., Brandwein, H. J., Mittal, C. K. & Waldman, S. A. (1981) *Adv. Cyclic Nucleotide Res.* **14,** 229–239.
26. Moore, M. R. & Goldberg, A. (1974) in *Iron in Biochemistry and Medicine,* eds. Jacobs, A. & Worwood, M. (Academic, New York), pp. 115–144.
27. Drabkin, D. L. (1978) in *The Porphyrins,* ed. Dolphin, D. (Academic, New York), Vol. 1, pp. 29–83.
28. Buchler, J. W. (1978) in *The Porphyrins,* ed. Dolphin, D. (Academic, New York), Vol. 1, pp. 389–483.
29. Kon, H. & Kataoka, N. (1969) *Biochemistry* **8,** 4757–4762.
30. John, M. E. & Waterman, M. R. (1980) *J. Biol. Chem.* **255,** 4501–4506.
31. Morse, R. H. & Chan, S. I. (1980) *J. Biol. Chem.* **255,** 7876–7882.