Two Heme-binding Domains of Heme-regulated Eukaryotic Initiation Factor-2α Kinase

N TERMINUS AND KINASE INSERTION*

(Received for publication, September 27, 1999, and in revised form, December 1, 1999)

Maryam Rafie-Kolpin‡‡, Peter J. Chefalo‡‡, Zareena Hussain‡, Joyce Hahn‡, Sheri Uma‖, Robert L. Matts‖, and Jane-Jane Chen‡‡‡

From the ¥Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139 and the †Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, Oklahoma 74078

In heme deficiency, protein synthesis in reticulocytes is inhibited by activation of heme-regulated α-subunit of eukaryotic initiation factor-2α (eIF-2α) kinase (HRI). Previous studies indicate that HRI contains two distinct heme-binding sites per HRI monomer. To study the role of the N terminus in the heme regulation of HRI, two N-terminally truncated mutants, Met2 and Met3 (deletion of the first 103 and 130 amino acids, respectively), were prepared. Met2 and Met3 underwent autophosphorylation and phosphorylated eIF-2α with a specific activity of approximately 50% of that of the wild type HRI. These mutants were significantly less sensitive to heme regulation both in vitro and in vivo. In addition, the heme contents of purified Met2 and Met3 HRI were less than 5% of that of the wild type HRI. These results indicated that the N terminus was important but was not the only domain involved in the heme-binding and heme regulation of HRI. Heme binding of the individual HRI domains showed that both N terminus and kinase insertion were able to bind hemin, whereas the C terminus and the catalytic domains were not. Thus, both the N terminus and the kinase insertion, which are unique to HRI, are involved in the heme binding and the heme regulation of HRI.

Initiation of protein synthesis in reticulocytes is inhibited during heme deficiency as a result of the activation of heme-regulated eIF-2α kinase (HRI).1 The activation of HRI is accompanied by its autophosphorylation and the phosphorylation of eIF-2α at serine 51 (reviewed in Refs. 1 and 2). eIF-2α is the eukaryotic translational initiation factor that binds GTP and Met-tRNAf and is necessary for the formation of the 43 S preinitiation complex. The recycling of eIF-2α requires the exchange of GDP for GTP. Under physiological conditions, the affinity of eIF-2α for GDP is much higher than for GTP. This exchange reaction is catalyzed by eIF-2B, which is rate-limiting and which is present at 15–20% of the amount of eIF-2α in reticulocytes. The phosphorylation of eIF-2α by HRI renders eIF-2B nonfunctional, resulting in the shut off of protein synthesis (reviewed in Ref. 3).

HRI belongs to the family of the eIF-2α kinases, which also includes double-stranded RNA (dsRNA)-dependent protein kinase (PKR) (4), GCN2 (5–7), PIPK in malaria parasite (8), and endoplasmic reticulum-resident kinase (PERK), which is highly expressed in pancreas (9, 10). However, the regulatory mechanisms of these eIF-2α kinases are very different. HRI is regulated by heme (1), whereas PKR, GCN2, and PERK are regulated by dsRNA (11), amino acid starvation conditions (12), and stress signal from endoplasmic reticulum (9), respectively. Alignment of HRI with the other eIF-2α kinases indicates the presence of three unique regions in HRI, the N terminus (amino acids 1–160), the kinase insertion (KI) region (amino acids 236–380), and the C-terminus (amino acids 507–626) (10, 11, 13, 14). These unique regions may be involved in the regulation of HRI by heme.

The two dsRNA binding domains of PKR are located in the first 171 amino acids (2, 15–18). Recently, it has been shown that the KI region of PKR is required for its autokinase, eIF-2α kinase activities (19, 20), and substrate binding (20). Taylor et al. (21) have identified a cluster of amino acids important for autophosphorylation between the dsRNA binding motif and the first kinase domain of the PKR. Activation of PKR by dsRNA is achieved by its autophosphorylation (21). Furthermore, mutation of Thr-446 and Thr-451 in the PKR activation loop to Ala impaired the kinase activity of this protein (22). The exact mechanism of activation of PKR is not known, but it may be brought about by dimerization or conformational changes (2).

The involvement of the N-terminal 184 residues in the dimerization of PKR has been demonstrated (23–27). Patel and Sen (28) have shown recently that the hydrophobic residues present in the N-terminal domain of PKR are required for dimerization and protein-protein interaction between PKR monomers.

The GCN2 protein contains a domain in the C terminus that is closely related in sequence to histidyl-tRNA synthase. This domain is essential for the activation of the protein kinase domain when uncharged tRNA accumulates due to amino acid starvation (14, 30). Romano et al. (22) have demonstrated that...
autophosphorylation of Thr-882 and Thr-887, located in the activation loop of GCN2, is important for the kinase activity of GCN2. The C terminus of GCN2 expressed in Escherichia coli has been shown to bind yeast tRNA (31). The importance of dimerization in the activation and catalytic function of GCN2 has been demonstrated (30, 32). Qiu et al. (33) have provided evidence indicating that the protein kinase domain and the C terminus are required for dimerization of GCN2.

HRI has been expressed in insect SF9 cells using the baculovirus expression system. This recombinant HRI is an active autokinase and eIF-2α kinase and is regulated by heme. HRI expressed in insect cells can inhibit the protein synthesis in SF9 cells (34). Baculovirus-expressed wild type (Wt) HRI and the inactive K199R mutant, in which the conserved Lys-199 in the catalytic domain II is changed to Arg, have been purified to homogeneity. Purified HRI is a homodimer and a hemoprotein containing 50 mM KCl, 20 mM Tris-HCl, pH 7.8, 0.1 mM EDTA, and 10% glycerol and was dialyzed against 2 liters of this buffer overnight (13).

Met2 and Met3 HRI, were active kinases. Met2 and Met3 HRI homogeneity. Purified HRI is a homodimer and a hemoprotein where the kinase catalytic domains and the C terminus were not. These results show that the N terminus and the KI domain of HRI are involved in the homodimerization and heme regulation of HRI.

EXPERIMENTAL PROCEDURES

Preparation of the Constructs—The Wt HRI was previously cloned in the TMV-SP64 plasmid in which a unique Nco I site at the first ATG of HRI cDNA was engineered (13). Similarly, a Nco I site was engineered in Met2 and Met3 HRI constructs at the initiation codon. The introduction of the Nco I site resulted in the change of the second amino acid from Arg to Gly for Met3 construct, whereas no change was made in Met2 construct. The Met2 and Met3 HRI were prepared by site-directed mutagenesis and recombinant polymerase chain reaction (PCR) technique (36). PCR products were digested with Nco I and Kpn I and subcloned to TMV-HRI plasmid.

Expression and Purification of Recombinant Wild Type and N-Terminally Truncated Mutant HRI—The plasmid containing the Met2 or Met3 HRI was digested with BglII and EcoRI and ligated into the polylinker region of the pV5/2 baculovirus recombination vector. The recombinant HRI baculovirus were generated as described previously (34, 35). The postriboosomal supernatants was subjected to 50% ammonium sulfate precipitation. The pellet was dissolved in a low salt buffer containing 50 mM KCl, 20 mM Tris-HCl, pH 7.8, 0.1 mM EDTA, and 10% glycerol and was dialyzed against 2 liters of this buffer overnight (13). Wt and mutant HRI proteins were purified by immunoaffinity column chromatography, which was prepared by using anti-HRI monoclonal antibody mABF as described previously (35). The antigenic determinants of mABF is localized in the KI region; therefore, the Wt and the N-terminally truncated mutants could purify similarly.

Protein Kinase Assays and Western Blot Analysis—The autokinase and eIF-2α kinase activities of Wt, Met2 and Met3 HRI were determined by protein kinase assays as described previously (37). HRI proteins were incubated with various concentrations of heme (0–5 μM) at 30 °C for 5 min. Protein kinase assays were initiated by the addition of 5 μCi of [γ-32P]ATP (3000 Ci/mmol), 50 μM ATP, and 100 ng of eIF-2α, and the reactions were proceeded for 5 min at 30 °C. Reactions were terminated by the addition of SDS-sample buffer containing 1.25% β-mercaptoethanol. The phosphorylated polypeptides were separated on 10% SDS-polyacrylamide gels, stained with Coomassie Brilliant Blue G-250. The extents of eIF-2α phosphorylation were visualized by autoradiography and quantitated by scintillation counting of the gel slices corresponding to eIF-2α. Protein kinase assays of the S10 crude extract of each HRI protein in the absence of purified eIF-2α was also performed and was used for background subtraction. Western blot analysis of HRI and various domains was performed, using anti-HRI mABF monoclonal antibody (38) or anti-His6 monoclonal antibody (Amersham Pharmacia Biotech), as described previously (40).

RESULTS

Expression and Characterization of the N-Terminally Truncated HRI—To determine the role of the N terminus in the heme regulation of HRI, two N-terminal deletion mutants were prepared by site directed mutagenesis and recombinant PCR as described under “Experimental Procedures.” These two mutants are schematically illustrated in Fig. 1. Met2 HRI began at the second methionine, and the first 103 amino acids were deleted. Met3 HRI began at the third methionine, and the first 130 amino acids were deleted. Wild type and mutant HRI were expressed in SF9 cells using the baculovirus expression system. The expressions of the Wt, Met2, and Met3 HRI proteins were examined by Western blot analysis using anti-HRI monoclonal antibody (Fig. 2A). Wt HRI migrated at about 90 kDa in the

2 P. J. Chefalo and J.-J. Chen, unpublished observations.
SDS-polyacrylamide gels (Fig. 2A, lane 1). As expected, Met2 and Met3 HRI were smaller than the Wt HRI and migrated at about 67 kDa in SDS-polyacrylamide gels (Fig. 2A, lanes 2 and 3). The levels of expression of Met2 and Met3 HRI were comparable to that of the Wt HRI (Fig. 2A).

The autokinase and eIF-2α kinase activities of these mutants in Sf9 cell extracts and in purified proteins were examined. Both Met2 and Met3 HRI were active eIF-2α kinases and autokinases (Fig. 2B and D). The autophosphorylations of Wt, Met2 and Met3 HRI were more evident when larger amounts of HRI proteins were used (Fig. 2D). To determine the specific activities of these mutants for eIF-2α, the protein kinase assays were done quantitatively by using limiting amounts of Wt, Met2, and Met3 HRI. Thus, the autophosphorylation was not as obvious in these experiments (Fig. 2B). Under these conditions, the rate of eIF-2α phosphorylation was linear for the first 10 min, as shown in Fig. 2C, and is also linear with the amount of HRI used (data not shown). The specific activities of Wt, Met2, and Met3 HRI, were determined from Fig. 2B to be 5.64, 3.49, and 2.72 pmol of eIF-2α phosphorylated per ng of HRI per 5 min at 30 °C, respectively. We have consistently observed that the specific activities of Met2 and Met3 HRI were about 50% of that of the Wt HRI. These results suggest that the N-terminal 103 amino acids may be important for achieving the high specific eIF-2α kinase activity but may not be essential for kinase activity of HRI.

The Heme Responsiveness of Wt, Met2, and Met3 HRI—To determine the role of the N terminus in the heme regulation of HRI, the inhibitions of eIF-2α kinase activities of the Wt, Met2, and Met3 HRI by heme were compared. Incubation of HRI proteins in S10 extracts with hemin (0–5 μM) reduced the eIF-2α kinase activities of the Wt, Met2, and Met3 HRI. However, the eIF-2α kinase activity of Wt HRI was always more sensitive to inhibition by heme than that of Met2 or Met3 HRI (Fig. 3). Furthermore, the extent of the inhibition of Met2 and Met3 HRI by heme was less than that of Wt HRI at all hemin concentrations tested.

Previously, it was shown that baculovirus expressed HRI could act as an inhibitor of protein synthesis and shut off its own synthesis. The addition of hemin to culture medium has been shown to increase the expression of Wt HRI, presumably by inhibiting its activity in insect cells and thus allowing a higher level of HRI protein expression (34). Therefore, the increase in the expression level of Wt or mutant HRI in response to exogenous heme was used as a criterion for the heme responsiveness of HRI in vivo. In the presence of 5 μM hemin,
the expression level of Wt HRI increased to 3 times the control value. In contrast, no significant increase in the expression level of Met2 or Met3 HRI was observed when heme was added to the culture media (Table I). These results indicated that Met2 and Met3 HRI were less heme-responsive than the Wt HRI in vivo. It is to be noted that the Sf9 cells are very sensitive to heme and that heme concentrations higher than 5 μM result in cell death (34). Therefore, it is not possible to examine heme response at concentrations greater than 5 μM in Sf9 cells.

Using synchronized pulse-chase translations, we have shown previously that newly synthesized HRI can be transformed into a stable and active heme-regulated eIF-2α kinase by autophosphorylation in RRL (40). The transformed HRI exhibits a slower electrophoretic mobility on SDS-PAGE. This transformation of HRI is inhibited by heme and can be used as another criterion for heme regulation of this protein under erythroid environment. As shown in Fig. 4, [35S]Met2 HRI, like [35S]-labeled Wt HRI, underwent transformation with the characteristic mobility shift on SDS-PAGE when incubated in heme-deficient RRL. The transformation of Met2 HRI was inhibited by heme as was that of Wt HRI. However, the extent of Met2 HRI transformation at 2.5 μM heme was greater than that of the Wt HRI (42.8 versus 26.4%) as quantitated by the intensities of the two forms of HRI. Transformation of Met3 HRI was similar to that of Met2 HRI (data not shown), indicating that these HRI mutants were less heme-responsive in RRL. Collectively, the results in Figs. 3 and 4 and Table I indicate that Met2 and Met3 HRI were less heme-responsive in vitro, in RRL, and in intact Sf9 cells.

Heme Contents in Purified Wt, Met2, and Met3 HRI—Recently, we have reported that HRI purifies as a hemoprotein with a characteristic absorption peak of the Soret band at 424 nm (35). To determine whether Met2 and Met3 HRI were hemoproteins, Wt and mutant HRI were expressed in the Sf9 cells supplemented with 5 μM heme and were immunopurified as described under “Experimental Procedures.” The UV-visible spectra of equal amounts (80 μg/ml) of Wt, Met2, and Met3 HRI and nonhemoprotein controls were obtained by scanning from 250 to 500 nm (Fig. 5). Both Wt and K199R HRI displayed the characteristic spectra of the hemoproteins, with the absorption peak of the Soret band at 424 nm. In contrast, Met2 and Met3 displayed very little absorption at 424 nm. The nonhemoproteins, such as glyceraldehyde dehydrogenase and pyruvate kinase, had no detectable Soret band. These results demonstrated that the N-terminal 103 amino acids of HRI were required for stable heme binding to HRI.

When a higher concentration of Met2 HRI (250 μg/ml) was used, a small absorption peak of Soret band at 424 nm was observed (Fig. 6). Scanning of Met3 HRI (80 μg/ml) from 300 to 500 nm also indicated the presence of a small peak of Soret band at 424 nm (Fig. 6). However, the absorption peaks of these two mutants were less than 5% of the Wt and K199R HRI absorptions at 424 nm. When exogenous hemin was added to purified Met2 and Met3 HRI (60 μg/ml), these mutants, like Wt HRI, could bind heme and showed the characteristic Soret band at 420 nm (Fig. 7). These results suggested that other regions of HRI might also be responsible for heme binding, because Met2 and Met3 HRI were not devoid of heme and could bind heme in vitro. This interpretation is consistent with the results of Figs. 3 and 4 that Met2 and Met3 HRI were still heme-responsive in intact Sf9 cells.
characteristic Soret band at 414 nm (Fig. 8), indicating that the N terminus by itself could bind heme. In addition, when this region was expressed in the presence of 5 \( \mu M \) hemin, it purified as a hemoprotein with a Soret band at 415 nm and visible pink color (data not shown). We have also expressed the mouse HRI N-terminal domain (aa 1–138), which is highly conserved among HRI from different species, except for the first 5–15 amino acids. Similar to rabbit N-terminal domain, the mouse N-terminal domain was also capable of binding heme and displayed the characteristic Soret band (data not shown).

The second domain consisted of amino acids 141–231 and included mostly the first lobe of kinase domain; it is referred to as the kinase I domain. When hemin was added to this polypeptide, it did not bind heme, and the characteristic Soret band was not observed (Fig. 8). This result indicated that the kinase I domain could not bind heme.

The third domain consisted of aa 219–420, which contained the entire rabbit KI sequence unique to HRI and included one putative heme regulatory motif (42). Unfortunately, rabbit KI, expressed as a His-tagged fusion protein, was insoluble. Therefore, the UV-visible spectrum of rabbit KI could not be obtained. However, when part of the rabbit KI domain (aa 301–420) was expressed, it was soluble. It bound hemin and displayed the characteristic Soret band of hemoproteins at 414 nm (Fig. 8). To determine the heme binding of the KI domain, the entire KI domain of mouse HRI (aa 241–406) was expressed as a His-tagged fusion protein. This mouse KI was soluble and was purified as described under "Experimental Procedures." When hemin was added to this polypeptide, it could bind heme and displayed the characteristic Soret band at 415 nm (Fig. 8). These results demonstrated that the KI domain could bind heme. In contrast to the N-terminal domain, when mouse KI was expressed in the presence of 5 \( \mu M \) hemin, it did not purify as a hemoprotein (data not shown). This observation suggested that KI might be the heme-binding site responsible for the dynamic heme regulation of HRI.

The fourth domain consisted of amino acids 421–540 and included the second kinase lobe, referred to herein as kinase II. The fifth domain was composed of amino acids 541–626, which are unique to HRI and well conserved among HRI from different species. Neither the kinase II domain nor the C terminus could bind heme, because the characteristic Soret band of hemoproteins was not observed in either case (Fig. 8).

As shown in Fig. 8, heme could bind only to certain domains of HRI. The presence of the His tag did not affect the heme binding of these domains. Similar results were obtained when the His tags of domains of HRI were cleaved by TEV protease (data not shown). In addition, when KI was purified from inclusion body under denatured conditions with subsequent attempts of renaturation by the stepwise removal of denaturant, it did not bind heme. Thus, heme did not bind nonspecifically to proteins under our assay conditions. These observation further strengthen the validity of the heme binding assay using difference spectroscopy. The results from the heme binding study of the domains of HRI demonstrated that the N terminus...
samples in the left panels, 260–500 nm. Right panels, the UV-visible spectra of Wt (A), Met2 (B), and Met3 (C) after mixing with 2 μM hemin were scanned between 260–500 nm. Low salt buffer with 2 mM DTT was used as blank for the samples in the left panels, and low salt buffer with 2 mM DTT and 2 μM hemin was used as blank for the samples in the right panels.

and KI could bind heme, whereas kinase I, kinase II, and the C terminus could not.

**DISCUSSION**

In this report, we have investigated the role of individual domains of HRI in its heme binding and heme regulation. We showed that the N-terminal deletion mutants of HRI, Met2 and Met3, were less sensitive to heme regulation than the Wt HRI (Table I and Figs. 3 and 4) and that the N-terminal domain of HRI could bind heme (Fig. 8). In addition, the heme contents of the purified Met2 and Met3 HRI were much less than that of the Wt HRI (Fig. 6). However, both Met2 and Met3 HRI could still bind exogenous heme (Fig. 7). These results indicated that the N-terminal domain was required but was not the only region involved in the heme binding and heme regulation of HRI. These observations are in agreement with our recent finding that HRI contains two heme-binding sites per monomer (35). The binding of heme to the first site is stable and copurifies with HRI to homogeneity. In contrast, the heme binding to the second site is dynamic and may be involved in rapid down-regulation of HRI by heme (35). Because deletion of the N-terminal domain of HRI resulted in nearly complete loss of the heme content of purified Met2 and Met3 HRI (Fig. 6), the N terminus is most likely the first and the stable heme-binding site.

Recently, we have found that there is a significant structural similarity between N-terminal amino acids 11–118 of HRI and amino acids 16–120 of mammalian α-globin with His-83 in the HRI N terminus as the predicted proximal heme ligand. Furthermore, secondary structure prediction of HRI indicates that the N terminus of HRI has mainly a helical structure and can be modeled to fit the structure of α-globin (41). In hemoglobin, heme is held by hydrophobic interactions of amino acid residues brought together by the tertiary structure of the protein. The stable heme binding to the N terminus of HRI, like that of hemoglobin, may also be facilitated by the helical structure and hydrophobic interactions.

The importance of heme binding in the folding of α-globin has been demonstrated by Komar et al. (43). The binding of heme to the N-terminal region of HRI may be required for proper folding and stability of full-length protein in reticulocytes. Furthermore, the N terminus may facilitate the heme regulation of the second site because, despite the presence of a second heme-binding site, Met2 and Met3 HRI were less responsive to heme regulation in vivo and in vitro (Fig. 3 and Table I).

Rabbit KI domain (aa 219–420) expressed as His-tagged fusion protein was not soluble, and therefore, the heme binding of this domain could not be determined. To overcome this problem, we expressed the KI region of mouse HRI, which has about 60% overall homology to the KI domain of rabbit HRI. The mouse KI was soluble, could bind heme, and displayed the characteristic Soret band of hemoproteins (Fig. 8). In addition, a portion of the KI (aa 301–420) of rabbit HRI is sufficient for heme binding (Fig. 8). Unlike the N terminus, the KI did not purify as a hemoprotein when it was expressed in E. coli in the presence of heme. These results supported our hypothesis that the KI is the second heme-binding site and that the heme binding to KI is dynamic and reversible. We have shown previously that heme inhibits ATP binding to HRI and kinase activities of HRI (44). Binding of hemo to the KI region might block the ATP binding site and thereby inhibit the kinase activities of HRI.

Little is known about the proteins or the motifs that bind heme reversibly, as is the case in the second heme-binding site...
of HRI. We found three examples of proteins involved in the reversible heme binding in the literature. The first is a histidine-proline-rich serum glycoprotein, which has been shown to bind heme (45–47). The exact physiological function of this protein is still unknown. A recent study suggests that the histidine-proline-rich serum glycoprotein functions as a plasma pH sensor that binds to glycosaminoglycans only when the pH decreases or when the local free metal concentration increases (48). The second example is hemopexin a plasma protein that transports heme to liver. Mutations at His-127 or at His-56 and His-127 have been shown to reduce the affinity of this protein for heme, indicating that these His residues were the heme-iron coordinating residues of hemapexin (49). The third example is histidine-rich protein HRP II, III, and IV from the malaria parasite *Plasmodium falciparum* (50). HRPs are found in the digestive vacuoles of this parasite, where hemoglobin degradation and heme polymerization to crystalline hemozoin occur. HRPs have been shown to bind heme and catalyze its polymerization. Among the 23 His residues in rabbit HRI, 10 are in the KI sequence and are clustered together (aa 226–243 and 366–376). All of these His residues except His-243 and His-366 are conserved among human, rat, rabbit, and mouse HRI. The importance of His residues in the reversible heme binding of HRI is currently under investigation.

In addition to hemoglobin, myoglobin, and cytochromes, the structures of several hemoproteins, such as FixL (51, 52) and nitric-oxide synthase (53, 54), have recently been solved. Of structures of several hemoproteins, such as FixL (51, 52) and nitric-oxide synthase (53, 54), have recently been solved. Of structures of several hemoproteins, such as FixL (51, 52) and nitric-oxide synthase (53, 54), have recently been solved. Of structures of several hemoproteins, such as FixL (51, 52) and nitric-oxide synthase (53, 54), have recently been solved. Of structures of several hemoproteins, such as FixL (51, 52) and nitric-oxide synthase (53, 54), have recently been solved.
5178  Heme-binding Domains of HRI

chem. 267, 498–506
42. Zhang, L., and Guarente, L. (1995) EMBO J. 14, 313–320
43. Komar, A. A., Kommer, A., Krasheninnikov, I. A., and Spirin, A. S. (1997) J. Biol. Chem. 272, 10646–10651
44. Chen, J.-J., Pal, J. K., Petryshyn, R., Kus, I., Yang, J. M., Throop, M. S., Gehrke, L., London, I. M. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 315–319
45. Morgan, W. T. (1985) Biochemistry 24, 1496–1501
46. Tsutsui, K., and Mueller, G. C. (1982) J. Biol. Chem. 257, 3925–3931
47. Katagiri, M., Tsutsui, K., Yamano, T., Shimonishi, Y., and Ishibashi, F. (1987) Biochem. Biophys. Res. Commun. 149, 1070–1076
48. Borza, D.-B., and Morgan, W. T. (1998) J. Biol. Chem. 273, 5493–5499
49. Satoh, T., Satoh, H., Iwahara, S.-I., Hrkal, Z., Peyton, D. H., and Muller-oberhard, U. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8423–8427
50. Sullivan, A. D., Ittarat, I., and Meshnick, S. R. (1996) Parasitology 112, 285–294
51. Gong, W., Hao, B., Mansy, M. S., Gonzalez, G., Gilles-Gonzalez, M. A., and Chan, M. K. (1998) Proc. Natl. Acad. Sci. 95, 15177–15182
52. Miyatake, H., Kanai, M., Adachi, S., Nakamura, H., Tamura, K., Tanida, H., Tsuchiya, T., Iizuka, T., and Shiro, Y. (1999) Acta Crystallogr. D Biol. Crystallogr. 55, 1215–1218
53. Crane, B. R., Arvai, A. S., Gachhui, R., Wu, C., Ghosh, D. K., Getzoff, E. D., Stuehr, D. J., and Tainer, J. A. (1997) Science 278, 425–431
54. Raman, C. S., Li, H., Martasek, P., Kral, V., Masters, B. S. S., and Poulos, T. L. (1998) Cell 95, 939–950
55. Arnoux, P., Huser, R., Izadie, N., Lecrousey, A., Delepierre, M., Wandersman, C., and Cejzek, M. (1999) Nat. Struct. Biol. 6, 516–520
56. Lathrop, J. T., and Timko, M. P. (1993) Science 259, 522–525
57. Dumont, M. E., Ernst, J. F., Hampsey, D. M., and Sherman, P. (1987) EMBO J. 6, 235–241
58. McCoubrey, W. K., Jr., Huang, T. J., and Maines, M. D. (1997) J. Biol. Chem. 272, 12568–12574
Two Heme-binding Domains of Heme-regulated Eukaryotic Initiation Factor-2α Kinase: N TERMINUS AND KINASE INSERTION
Maryam Rafie-Kolpin, Peter J. Chefalo, Zareena Hussain, Joyce Hahn, Sheri Uma, Robert L. Matts and Jane-Jane Chen

J. Biol. Chem. 2000, 275:5171-5178.
doi: 10.1074/jbc.275.7.5171

Access the most updated version of this article at http://www.jbc.org/content/275/7/5171

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 55 references, 34 of which can be accessed free at http://www.jbc.org/content/275/7/5171.full.html#ref-list-1