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NO-Induced Activation Mechanism of the Heme-Regulated eIF2α Kinase

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Nitric oxide (NO) has been recognized as a key signaling agent in a variety of physiological processes.[1] In addition to its crucial regulatory roles in the circulatory, and central, peripheral, and enteric nervous systems, NO has now been suggested to be an effector molecule for protein synthesis in a number of cell lines.[1] Exposure of cells to high levels of NO stimulates the phosphorylation of the α-subunit of eukaryotic initiation factor 2 (eIF2α), leading to suppression of the protein synthesis.[2] Although the eIF2α kinase that mediates the NO-induced increase in eIF2α phosphorylation has not been definitively identified, recent evidence suggests that the increase in eIF2α phosphorylation is mediated by the heme-regulated eIF2α kinase (HRI).[2] However, the molecular mechanism by which the NO-induced activation of HRI occurs has yet to be characterized.

Recent biochemical studies of HRI found in rabbit reticulocyte lysate revealed that NO can bind to the heme iron in the N-terminal heme binding domain (NT-HBD) of HRI and activate its kinase activity, while the binding of carbon monoxide (CO) to the heme iron suppressed its kinase activity.[2] The competitive inhibition by CO in the NO-induced activation confirms that the binding of NO to the heme iron, not the modification of cysteine by NO, is responsible for the activation.[2] Preliminary spectroscopic studies allow us to speculate that the binding of NO to the heme iron preferentially leads to cleavage of the bond between the heme iron and axial ligand from the protein moiety, as found for guanylate cyclase (sGC).[3] This bond cleavage was hypothesized to be the primary trigger for the activation of the kinase activity of HRI. However, the changes in the heme environment that occur upon the NO binding and the coordination structure of the NO-ligated heme still remain to be determined under more defined conditions, as the initial spectroscopic studies were not carried out under anaerobic conditions. In this communication, we have utilized resonance Raman and EPR spectroscopies to clarify the heme environmental structure within the NT-HBD in the presence and absence of NO, and we discuss the discrimination between NO and CO in the activation mechanism of HRI.

As previously reported, the NT-HBD can be purified as a hemoprotein[4] with a characteristic Soret band at 414 nm,[2] typical of six-coordinate hemoproteins. The resonance Raman spectra of the isolated NT-HBD also confirmed the six-coordinate heme in the ferric and ferrous states. In the ferric state (Figure 1, upper trace), the spin state markers, ν_3, ν_2, and ν_{10}, which are sensitive to the spin state of the heme iron,[5] were observed at 1508, 1580, and 1634 cm^{-1}, respectively. These positions correspond to a ferric low spin heme,[5] implying that the heme iron of the ferric NT-HBD is in the six-coordinate low spin state.

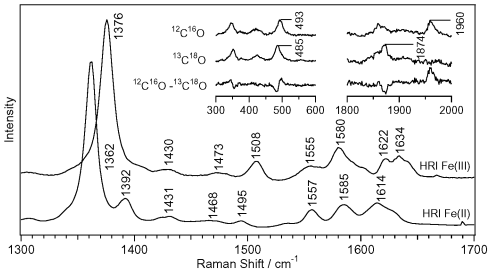

Figure 1. Resonance Raman spectra of the NT-HBD. High-frequency region of the ferric (top) and ferrous (bottom) NT-HBD. Low- and high-frequency regions of the CO-bound form are shown in the inset. The spectra were obtained by excitation with 413.1 nm light from a Kr^+ laser (Spectra Physics, model 2016). The sample concentration is about 50 μM in 20 mM Tris-Cl, pH 7.5, at room temperature.

The g values from the EPR spectrum of the ferric NT-HBD (g = 3.05, 2.20, and 1.46, figure not shown) also indicate that the low spin species is the major component of the ferric NT-HBD and are similar to those of bis-histidine-ligated ferric bovine liver cytochrome b_5 (g = 3.03, 2.23, and 1.43).[6] In the ferrous state (Figure 1, lower trace), two lines in the ν_3 region, 1495 and 1468 cm^{-1}, were detected. Although the appearance of the ν_3 band at 1468 cm^{-1} is suggestive of a five-coordinate heme,[5] the signal intensity of the band at 1468 cm^{-1} is weak, and the other coordination marker bands, ν_2 and ν_{10}, appeared at 1585 and 1614 cm^{-1}, both of which are indicative of a six-coordinate low spin heme.[5] The coordination structure of the NT-HBD is, therefore, predominantly in the low spin state with two axial ligands. From the expected globin fold of NT-HBD,[2] the heme axial ligands are likely to be His83 and another residue distinct from histidine.[2]

To identify the axial ligands of the NT-HBD, the resonance Raman spectrum of the CO adduct was measured. Previous studies have shown that the frequencies of the ν(FeC−O) and ν(Fe−CO) bands for heme-CO complexes are inversely correlated and depend on the σ donor strength of the trans ligand, which enables us to estimate the axial ligand trans to CO.[5] These two Raman bands of the CO adduct were identified by the isotope shifts for the ^{13}CO isotopomer as displayed in the inset of Figure 1. In the high-frequency region, one isotope-sensitive band assignable to the ν(FeC−O) band[7] appeared at 1960 cm^{-1}. Another isotope-shifted band derived from the ν(Fe−CO) mode[7] was detected at 493 cm^{-1} in the low-frequency region. The correlation of these two Raman bands falls on the line of the neutral ligand-ligated hemoprotein.[7]

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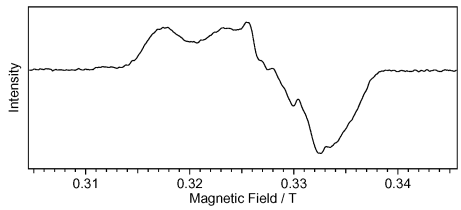

Figure 2. EPR spectrum of the ferrous-NO complex: EPR spectra were measured on a Varian E-12 spectrometer (X-band; 9.22 GHz) at 35 K. The sample concentration was 500 μM in 20 mM Tris-Cl at pH 7.5.

Together with the sequence alignment with globin,[2] we can conclude that the axial ligand of the CO-ligated NT-HBD is histidine.

Because the CO-adduct of sGC also gives the frequencies of the ν(Fe−CO) and ν(Fe−CO) bands on the same line as that of the NT-HBD,[8] it can be supposed that the coordination structure of the axial histidine side in the NT-HBD is not that much different from that of sGC. Surprisingly, however, the structural changes induced by the binding of NO to the heme iron in the NT-HBD are quite different from those of sGC. As shown in Figure 2, the EPR spectrum of the NO-bound NT-HBD shows several derivative-shaped lines around $g = 2.00$ and rhombic symmetry ($g_1 = 2.08$, $g_2 = 2.00$, $g_3 = 1.98$), which is one of the well-documented signature patterns of a six-coordinate NO complex with histidine as the axial ligand trans to NO.[9] The six-coordinate NO complex is stable for more than 30 min.[4] On the other hand, addition of NO to sGC results in a spectrum with three prominent lines, a signature pattern of five-coordinate ferrous heme-NO complexes; the breakage of the iron−histidyl bond is considered to be the primary trigger for the activation of sGC.[3] Thus, in sharp contrast to sGC, the retention of the iron−histidyl bond in the NO adduct of the NT-HBD implies that formation of the five-coordinate NO complex via cleavage of the iron−histidyl bond is *not* necessary for the activation of HRI.

Another possible trigger for the activation of HRI would be the dissociation of the axial ligand trans to histidine. The dissociation of the axial ligand trans to histidine has been proposed to play a crucial role in the activation of CooA.[10] As previously reported, however, replacement of the axial ligand trans to the axial histidine by CO does not activate its kinase activity.[2] It is also unlikely that CO displaces the different axial ligand from NO as proposed in cytochrome c',[11] because the NO and CO adducts of the NT-HBD have histidine as the axial ligand, and the sequence alignment with globin shows no histidine residues in the region corresponding to the distal histidine side of globin.[2] Thus, the NO-induced dissociation of the axial ligand is not sufficient to induce the activation of HRI.

It should be noted here that the geometry of the heme-ligated state is significantly different between CO and NO. The bond angles for Fe−N−O are in the range from 112 to 147° in most NO-ligated hemoproteins, while the bond angles for Fe−C−O are much larger (155−180°), and the axis of the C−O bond is almost perpendicular to the heme plane.[12,13] Because of this difference in geometry, it is likely that interactions between ligated NO and surrounding amino acid residues are different from those in the CO complex. Therefore, we hypothesize that some specific interactions responsible for activation of HRI would be preferentially formed in the HRI-NO complex, but that the different geometry of CO ligated to the NT-HBD of HRI would inhibit the formation of these interactions. Although we cannot currently identify the amino acid residues involved in these specific interactions, it can be concluded that the molecular mechanism of the NO-induced activation of HRI is different from that in sGC and that the interactions between a ligated gaseous molecule and amino acid residues are suggested to play a primary role in regulating HRI.

In summary, the present spectroscopic data clearly show that the NT-HBD of HRI has a six-coordinate low spin heme, in which one of the axial ligands is histidine. The binding of NO, but not CO, to the heme iron in HRI's NT-HBD initiates the activation of its catalytic activity as found for sGC. However, unlike sGC, the binding of NO to HRI's NT-HBD does not disrupt the iron−histidyl bond, and eventual structural rearrangements in the axial histidine side do not appear to be crucial for HRI's activation. Instead of the cleavage of the iron−histidyl bond, essential roles of the interactions between liganded NO and the surrounding amino acid residues are proposed. To further confirm our hypothesis, several mutations in the ligand-binding site are now in progress.

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(13) We tried to detect the resonance Raman bands of ν(FeN−O) and ν(Fe−NO) for the ferrous-NO NT-HBD, but ligated NO is photolabile, and the NO-dissociated ferrous NT-HBD was rapidly oxidized. The spectrum after the measurement showed a mixture of the ferrous and ferric forms. Measurements of the infrared spectrum of the ferrous-NO NT-HBD have not yet been successful due to the instability of the protein at high concentration.

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