Micelle-induced Release of Haem–NO from Nitric Oxide Complex of Myoglobin

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The cleavage of the haem-proximal histidine bond and concomitant release of haem–NO from MbNO has been achieved at physiological pH by aqueous micelles such as hexadecyltrimethyl ammonium bromide and sodium dodecyl sulfate, and the released haem–NO complex is encapsulated in the hydrophobic micellar cavity; this micellar effect has close similarity with the action of guanyl cyclase on nitrosyl haem proteins.

Interaction of nitric oxide (NO) with haem proteins is currently of considerable interest because of its unique role in neuronal communication processes.[1,2] It has been proposed that the haem complex of NO (haem–NO) activates soluble guanyl cyclase (GC) to produce a secondary neurotransmitter such as cyclic GMP from GTP.[1] Some NO–haem proteins are also known[3,4] to activate GC under physiological conditions, where the haem–NO species has been shown to be transferred from the haem protein to GC. However, the mechanism of release of haem–NO from the haem protein *in vivo* is still not clearly understood.[1]

Recent studies on nitric oxide complexes of several iron(II) haem proteins and model compounds[2,5] have shown that the axial coordination of NO to iron(II) haem apparently weakens the haem-proximal histidine (Fe–N$^{\varepsilon}$) bond and thereby promotes release of haem–NO complex at a pH significantly higher than that required for haem abstraction from the protein in the absence of NO.[2] Thus, in case of Fe^{II}NO-myoglobin (MbNO), the release of haem–NO is essentially complete[6] at pH *ca.* 4 whereas removal of haem from myoglobin in the absence of NO requires the pH to be below *ca.* 3.[2] Similarly, inositol hexaphosphate (IHP) has been shown to cause release of haem–NO from α-subunits of haemoglobins.[7,8]

In the present report we show that cleavage of the haem-proximal histidine bond and subsequent release of haem–NO complex from MbNO can be achieved at physiological pH by aqueous detergent micelles such as hexadecyltrimethylammonium bromide (CTAB) and sodium dodecyl sulphate (SDS). We further show that the resulting haem–NO is encapsulated inside the aqueous micellar cavity.

Horse heart metmyoglobin, CTAB and SDS were obtained from Sigma. MbNO was prepared either by passing purified NO gas through iron(II) myoglobin, or by addition of sodium nitrite and sodium dithionite to a solution of iron(III) myoglobin.[9] CTAB and SDS micelles were prepared at pH *ca.* 7.0 as described earlier.[10–12] All reactions were carried out under anaerobic conditions.

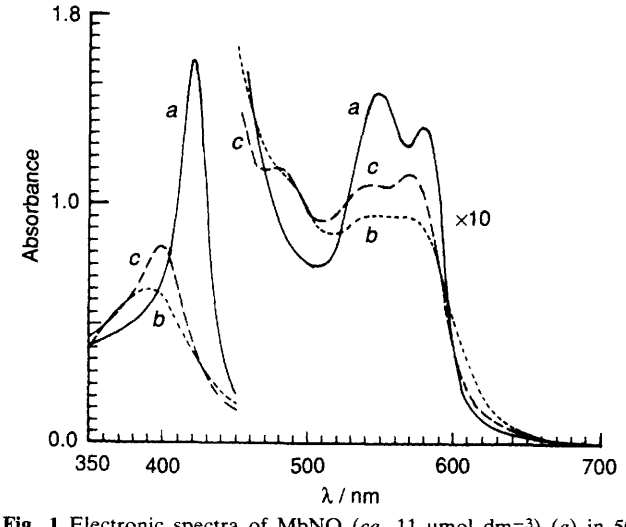

Fig. 1 Electronic spectra of MbNO (*ca.* 11 μmol dm^{-3}) (*a*) in 50 mmol dm^{-3} phosphate buffer, pH 7.0;(*b*) in 50 mmol dm^{-3} acetate buffer, pH 3.9 and (*c*) after addition of CTAB (5% stock) (final concentration, 0.1%) to MbNO in 50 mmol dm^{-3} phosphate, pH 7.0. Further addition of CTAB did not change the nature of the spectrum.

Fig. 1 shows electronic spectra of MbNO (*ca.* 11 μmol dm^{-3}) with and without CTAB at different pH. The optical spectra of MbNO [Fig. 1(*a*)] matches with the previous reports.[13] Lowering of the pH causes reversible spectroscopic changes of the MbNO in solution and cleavage of the haem–NO moiety was found to be complete at pH 3.9 [Fig. 1(*b*)]. The pK_a of this transition was observed to be 4.7.[6] The observed blue shift in the Soret band and an overall broadening of the spectra of this haem–NO species (Fig. 1) might arise because of its aggregation in aqueous solution.[10–12] A similar type of blue-shifted broad Soret band has been observed in the difference spectrum of IHP-treated HbNO.[7]

Addition of CTAB (final concentration = 0.1%) to MbNO at pH 7.0 gives a spectrum [Fig. 1(*c*)] with the Soret band shifted from 419 to 397 nm and a weak absorption band at around 482 nm which is characteristic of five-coordinate Fe^{II} haem–NO species.[14] The spectrum did not change on further addition of CTAB (up to *ca.* 5%). The same electronic spectrum (not shown) was obtained on addition of CTAB to MbNO at pH 3.9, in which the haem–NO moiety had already come out of the protein cavity.[2] Similar results were also obtained when SDS, instead of CTAB, was added to the MbNO solution. Previous studies have shown that aqueous micelles can stabilize monomeric haem complexes encapsulated inside the micellar cavity.[10–12] The spectrum of haem–NO in Fig. 1(*c*) has the signature of monomeric haem–NO encapsulated inside CTAB micelles. This spectrum closely matches with the reported spectrum of five-coordinated Fe^{II} (PPIXDME) (NO) (PPIXDME = protoporphyrin dimethyl ester) complex in benzene.[14] Comparison of circular dichroism spectra (data not shown) showed that the micelle-induced transition of MbNO at pH 7.0 did not affect the helical structure of the protein.

Further to confirm the above observations, five-coordinated Fe^{II} (protoporphyrin IX) (NO) inside CTAB (1–5%) was prepared by passing NO gas for a short time (*ca.* 1 minute) to a sodium dithionite-treated solution of four-coordinated iron(II) haem encapsulated in aqueous CTAB micelles.[12] The electronic spectrum of this model nitrosyl derivative was almost identical to that of CTAB-treated MbNO [Fig. 1(*c*)] and matched closely with that reported in benzene solution.[14]

These observations clearly demonstrate that positively charged CTAB as well as negatively charged SDS micelles can induce the Fe–N$^{\varepsilon}$ bond cleavage in MbNO at pH *ca.* 7. The resulting five-coordinated haem–NO moiety comes out of the protein cavity and is encapsulated inside the aqueous micelles. This observation has close resemblance to the MbNO-induced GC activation process observed under physiological conditions.[3] However, the fact that both positive and negative micelles can cause release of haem–NO from MbNO indicates that the hydrophobic micellar interactions might be an important factor in the abstraction of haem–NO from the protein. We observe that a smaller cationic micelle such as dodecyltrimethyl ammonium bromide (DTAB) is also capable of abstracting haem–NO from MbNO while a neutral micelle, *e.g.* Triton X-100, is unable to do so. This indicates that the presence of charge on the surface of the micelles may also be

necessary for such processes. Previous studies have suggested that to capture the haem–NO group in its hydrophobic cavity, GC may enforce a charge interaction on the vicinal propionate groups of haem–NO.[4]

Received, 6th April 1993; Com. 3/02004E

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