

Nitric Oxide Reductase in the Forms Different from the Resting Form and Reactions with Nitric Oxide

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The respiratory nitric oxide reductase (NOR) catalyzes the two-electron reduction of two NO molecules to N₂O. We have isolated NOR from *Halomonas halodenitrificans* and characterized its Fe centers by using absorption, MCD and EPR spectroscopies (*Biochemistry*, **36**, 13809; *BBRC*, **251**, 248; *JIB*, **83**, 281). Further, we determined amino acid sequences of the smaller subunit NorC containing a low spin heme *c* and the larger subunit NorB containing a low spin heme *b*, a high spin heme *b*₃ and a non-heme Fe, comparing with those of other NOR and terminal oxidases (*BBRC*, **243**, 400). At the resting state, heme *b*₃ and non-heme Fe are antiferromagnetically coupled with a bridged oxo group and EPR-undetectable.

The resting NOR does not react with NO as well as with small exogenous probes for metal center such as CO, CN⁻, N₃⁻ at neutral pHs and absorption, MCD and EPR spectra did not change. However, the characteristic absorption band at 595 nm and the corresponding the S-shaped MCD band due to the coupled heme *b*₃ became considerably weak at pH 5.2-5.5, while the bands due to the low spin heme *c* and heme *b* centers were intact. The EPR signals at *g* = 6.0 and 4.3 due to high spin heme *b*₃ and non-heme Fe, respectively, were very weak at neutral pHs, but became considerably strong at pH 5.2-5.5, although the intensities of the EPR signals due to heme *c* (*g* = 3.59) and heme *b* (*g* = 2.96, 2.26, 1.46) did not change, indicating that the bridged structure Fe(III)-O-Fe(III) was partly lost and/or significantly weakened.

When NO was acted on NOR at pH 5.2-5.5, the absorption band at 595 nm and the corresponding S-shaped MCD band disappeared, while the bands due to heme *c* and heme *b* did not change. In the corresponding EPR spectrum, intensities of the *g* = 6 and 4.3 signal were prominently weakened. However, these signal intensities were restored by evacuation. This reversible NO-binding with NOR at acidic pHs indicates that NO binds with both heme *b*₃ and non-heme Fe in the ferric state, when they are not bridged or weakly bridged. Therefore, it appears that the access of NO to the active center of NOR is not necessarily determined by the oxidation state of Fe centers but by the blocking effect of the bridged oxo group and the strength of the bridged structure.