The coordination and function of the redox centres of the membrane-bound nitrate reductases

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Abstract. Under anaerobic conditions and in the presence of nitrate, the facultative anaerobe Escherichia coli synthesizes an electron-transport chain comprising a primary dehydrogenase and the terminal membrane-bound nitrate reductase A (NarGHI). This review focuses on recent advances obtained on the structure and function of the three protein subunits of membrane-bound nitrate reductases. We discuss a global architecture for the Mo-bisMGD-containing subunit (NarG) and a coordination model for the four [Fe–S] centres of the electron-transfer subunit (NarH) and for the two b-type haems of the anchor subunit NarI.

Key words. Nitrate reductase; molybdenum cofactor; [Fe–S] centres; haems.

Nitrate reduction by Escherichia coli

In order to use nitrate as an electron acceptor, E. coli synthesizes two distinct enzymes: a membrane-bound enzyme (nitrate reductase A, NarGHI) encoded by the narGHJI operon [1] and a soluble periplasmic nitrate reductase (NapAB) encoded by the napFDAGHBC operon [2]. A second membrane-bound nitrate reductase (nitrate reductase Z, NarZHV) encoded by the narZHV operon is biochemically similar to NarGHI [3, 4]. Whereas NarGHI synthesis is induced by nitrate under anaerobic conditions, NarZHV is expressed at a cryptic level and may assist E. coli in the transition from aerobic to anaerobic respiration [3, 5]. Recently, Chang and co-workers [6] have provided evidence for RpoS-mediated regulation of the narZHV operon, supporting a physiological role of this isoenzyme at the onset of the stationary growth phase in rich media. NapAB is mainly expressed in the presence of low concentrations of nitrate under both aerobic and anaerobic conditions, and its expression is suppressed at high nitrate concentrations [7–9]. Conversely, NarGHI is maximally expressed when nitrate concentration is elevated, and under these conditions becomes the predominant enzyme in E. coli. Thus, NapAB and NarGHI seem to function in different ranges of nitrate concentration in a complementary way to support anaerobic respiration on nitrate (see also article by Richardson et al.).

Of the two respiratory nitrate reductases, NarGHI is the more complex. Its general organisation is similar to that of other respiratory enzymes in which intramolecular electron transfer is coupled to the creation of a transmembrane proton electrochemical potential. NarGHI is a heterotrimer comprising a molybdenum cofactor-containing subunit [molybdo-bis(molybdopterin guanine dinucleotide), Mo-bisMGD, NarG, 139 kDa], an [Fe–S] cluster-containing electron-transfer subunit (NarH, 58 kDa) and a haem-containing membrane-anchor subunit (NarI, 26 kDa). These three subunits are arranged in two domains, with the NarG and NarH subunits constituting a cytoplasmic domain and the NarI subunit constituting a membrane-intrinsic domain required for attachment of the NarGH dimer to the cytoplasmic side of the cell membrane.
NarG, the Mo-bisMGD-binding subunit of NarGHI

Towards a global architecture of NarG

The NarG subunit of NarGHI is a member of a superfamily of oxidoreductase subunits with highly conserved organisation and sequence. During the last decade, the crystal structures of five bacterial Mo-bisMGD-containing enzymes have been reported: dimethylsulfoxide reductase from *Rhodobacter sphaeroides* [10] and *Rh. capsulatus* [11], formate dehydrogenase H from *E. coli* [12], trimethylamine N-oxide reductase from *Shewanella massilia* [13] and the periplasmic nitrate reductase, NapA, from *Desulfovibrio desulfuricans* [14]. All are globular proteins and are organised in four αβ domains (I–IV) grouped around the Mo-bisMGD, and a funnel-shaped tunnel leads from the protein surface to the molybdenum atom. The Mo-bisMGD, which is ligated within the interfaces of the four domains through hydrogen bonds, salt bridges and van der Waals interactions, is buried in the centre of the protein and extends through a major portion of the polypeptide. Today, no three-dimensional structure is available for a NarGHI type enzyme. Nevertheless, alignment of amino acid sequences of Mo-bisMGD-containing polypeptides, including NarG, with those of proteins with known structures shows extensive blocks of homology throughout the length of the protein, with the amino-terminal regions being the most highly conserved [15, 16]. The order of these blocks from amino- to carboxyl-terminus is constant and the residues involved in Mo and in Mo-bisMGD ligation are well conserved among these molybdoenzymes [10]. This strongly supports the idea that NarG has the same basic architecture as the other structurally characterised Mo-bisMGD-containing subunits. It is very likely to be organised in four αβ domains grouped around the Mo-bisMGD. In taking the comparison a little further, we can guess that three segments constituted by residues 1–114, 795–823 and