

P012 The role of NapD in the maturation of NapA
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Many bacterial species have genes encoding a periplasmic nitrate reductase (Nap). To date, all *nap* operons contain three common genes, *napD*, *napA* and *napB*. The *Paracoccus pantotrophus nap* operon contains two other genes, *napE* and *napC*, and the Nap system is synthesised during aerobic growth to facilitate disposal of excess reducing equivalents during oxidative metabolism of reduced carbon substrates. Previous biochemical studies have established roles for NapA, NapB and NapC in electron transfer and catalysis in the periplasm. However NapD, which is predicted to be a cytoplasmic protein, has never been characterised biochemically from any organism. In the present study, we show that *napD* and *napA* mutants of *P. pantotrophus* exhibit similar physiological and biochemical phenotypes. NapD was purified as a recombinant 6-His-tagged protein and characterised by UV-visible and CD spectroscopy. This showed that the protein was cytoplasmic, did not purify with a chromophore bound and was around 36% α -helical. Protein-protein interaction studies were conducted using affinity chromatography and SPR. These techniques showed that NapD could form a complex with NapA. The similar phenotype of *napD* and *napA* mutants demonstration of complex formation is discussed in the context of a role for NapD in maturation of NapA in the cytoplasm prior to export to the periplasm.