Novel variant of the metal free class Ie ribonucleotide reductase

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Class I ribonucleotide reductases catalyse the reduction of ribonucleotides to deoxyribonucleotides under aerobic conditions. A smaller subunit R2 generates and stores a radical, that is shuttled to a larger, catalytic subunit R1 when required for catalysis, where it plays a key role in the substrate reduction. Different subclasses of class I RNRs utilise different metal cofactors for the radical generation in R2; class Ib uses a di-manganese metal centre[1]. Recently, a new subclass, class Ie, has been described that does not require a metal cofactor for radical generation[2]. This class evolved from class Ib and three of the highly conserved metal-binding ligands are lost. Important human pathogens like *Streptococcus pyogenes* and *Mycoplasma pneumoniae* contain a class Ie RNR. Two different variants of R2e are known, the evolutionary older variant has three glutamates substituted by glutamine, serine and lysine (EEE -> QSK). This version evolved further with glutamine substituted by valine and serine substituted by proline (QSK -> VPK). To date, the focus of research has been on the VPK version of R2e. The active $R2e_{VPK}$ contains a tyrosine post-translationally *meta*-hydroxylated to a 3,4-dihydroxyphenylalanine or DOPA, which is the residue that harbours the radical[3]. The formation of the DOPA is dependent on the coexpression of a small flavoprotein NrdI, whose presence is also required for the radical generation in R2b.

Here, the QSK version of class Ie RNR, the evolutionary link between class Ib and class Ie_{VPK} is characterised for the first time. The taxonomic distribution of the two R2e versions is analysed, which both form a distinct clade derived from R2b. It is demonstrated that the $R2_{QSK}$ of the human pathogen *Gardnerella vaginalis* also modifies the active-site adjacent tyrosine to a DOPA like in $R2e_{VPK}$ using LC-MS. The amount of protein modified is shown to be dependent on coexpression with the other proteins encoded in the RNR operon, NrdI, R1 and NrdH, with most DOPA formed when the whole operon is expressed at once. The first structures of an $R2e_{QSK}$ protein in the unmodified and modified states are solved and compared with available structures of $R2e_{VPK}$. The structures and spectroscopic investigation confirm the absence of a di-nuclear metal site. Finally, *in vitro* activity of the class Ie_{QSK} RNR system is demonstrated, which requires the presence of the DOPA modification and NrdI, suggesting that as in $R2e_{VPK}$ a radical can be formed without metals bound in the active site.

1. John, J. *et al.* Redox-controlled reorganization and flavin strain within the ribonucleotide reductase R2b–NrdI complex monitored by serial femtosecond crystallography. *eLife* **11**, (2022).

2. Srinivas, V. *et al.* Metal-free ribonucleotide reduction powered by a DOPA radical in Mycoplasma pathogens. *Nature* **563**, 416–420 (2018).

3. Lebrette, H. *et al.* Structure of a ribonucleotide reductase R2 protein radical. *Science* **382**, 109–113 (2023).