Identification of residues indirectly involved in cation coordination by HUH endonucleases

Lorena González-Montes and Gabriel Moncalián
Molecular Biology Department and
Institute of Biomedicine and Biotechnology of Cantabria, University of Cantabria, Spain

Background

HUH endonucleases rely on a divalent metal ion to perform different site-specific DNA processing reactions in biological processes like plasmid replication, transposition or bacterial conjugation. They all have an HUH motif (U=hydrophobic residue) and a Y motif (one or two catalytic Tyrs). A catalytic Tyr creates a covalent 5′-phosphothioate intermediate and a free 3′OH at the cleavage site. This 3′-OH primes replication or acts as nuclease for strand transfer. The metal is coordinated by the two HUH His and a third polar residue (Glu, Asp, His or Gin) being Mg2+ and Mn2+ the physiological cofactors. TrwC is one of the most studied HUH endonucleases at a biochemical and structural level. Its function is to transfer a single-stranded DNA (ssDNA) plasmid copy from one cell to another at conjugation process by nicking at the nic site of the plasmid origin of transfer (oriT), guiding the copy to the recipient cell and catalyzing there the recircularization of the transferred ssDNA plasmid.

Hypothesis

All HUH endonucleases superfamily members have conserved HUH and Y catalytic domains (orange arrows). However, they have different metal specificities. We propose that metal affinity depends on the different character of the residues surrounding the amino acids directly involved in metal coordination at the active site, which are conserved within each relaxase family (blue arrows). Polar residues are found in the relaxases binding Mg2+ or Mn2+ such as TrwC and Moba. Hydrophobic residues are present in relaxases only binding Mn2+ such as NES. These secondary residues could modify cation specificity by affecting histidine tautomeration. In this work we have changed the protein metal specificity by mutating one of these polar amino acids (T87I in TrwC) by a non polar residue.

Methodology

Results and Discussion

Mg2+ does not allow TrwC T87I cleavage of nic-containing oligonucleotides

Effect of different cations in the oligonucleotide cleavage by TrwC WT and TrwC T87I at different cofactor concentrations. Mean and standard deviation of three independent experiments.

TrwC T87I structure shows a slight change in the H163 orientation

It is proposed that T87I by hydrogen bond formation plays an important role in the orientation of one of the histidines (His163) that coordinate the divalent cation.

Conclusions and Future Research

We have proved that a charged amino acid not directly interacting with the metal cofactor is involved in the orientation of the catalytic histidines in TrwC. This residue is therefore indirectly involved in the metal coordination and specificity. Thus, we think that by mutating the equivalent hydrophobic amino acid for a polar residue on an HUH endonuclease only able to bind Mn2+, the protein could recover the ability to use Mg2+ too. But this hypothesis has still to be proven. These findings open a new path to protein engineering for cofactor specificity modification and de novo protein design for different purposes.

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References