In vitro selection of deoxyribozymes active with Cd^{2+} ions resulting in variants of DNAzyme 8-17.

Autorzy

Aleksandra Kasprowicz

Kamila Stokowa-Sołtys

Jan Wrzesiński

Małgorzata Jeżowska-Bojczuk

Jerzy Ciesiołka

Rok wydania

2015

Czasopismo

Dalton Transactions

Numer woluminu

44

Strony

8138-8149

DOI

10.1039/C5DT00187K

Kolekcja

Naukowa

Język

Angielski

Typ publikacji

Artykuł

Streszczenie

In vitro selection was performed to search for RNA-cleaving DNAzymes catalytically active with Cd^{2+} ions from the oligonucleotide combinatorial library with a 23-nucleotide random region. All the selected, catalytically active variants turned out to belong to the 8–17 type DNAzyme. Three DNAzymes were prepared in shortened, *cis*-acting versions which were subjected to a detailed study of the kinetic properties and metal ion preferences. Although the selection protocol was designed for Cd^{2+}-dependent DNAzymes, the variants showed broader metal ion specificity. They preferred Cd^{2+} but were also active with Mn^{2+} and Zn^{2+}, suggesting that binding of the catalytic ion does not require an extremely specific coordination pattern. The unexpected decrease of the catalytic activity of the variants along with the temperature increase suggested that some changes occurred in their structures or the rate-limiting step of the reaction was changed. Two elements of the catalytic core of DNAzyme 1/VIIWS, the nucleotide at position 12 and the three-base-pair hairpin motif, were mutated. The presence of a purine residue at position 12 was crucial for the catalytic activity but the changes at that position had a relatively small influence on the metal ion preferences of this variant. The middle base pair of the three-base-pair hairpin was changed from A–T to C–G interaction. The catalytic activity of the mutated variant was increased with Zn^{2+}, decreased with Mn^{2+}, and was not changed in the presence of Cd^{2+} ions. Clearly, this base pair was important for defining the metal ion preferences of the DNAzyme 1/VIIWS.

Adres publiczny

http://dx.doi.org/ 10.1039/C5DT00187K

Strona internetowa wydawcy

https://www.rsc.org/