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MC02 OA

A novel α -N-acetylgalactosaminidase family with a NAD $^+$ dependent catalytic mechanism suitable for enzymatic removal of blood group A antigens

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Keywords: Blood group conversion; glycosidase mechanism; convergent evolution

Enzymatic removal of blood group A and B antigens to develop universal red blood cells was a pioneering vision originally proposed 25 years ago. Processes for group B red cells have previously been developed and feasibility of the approach demonstrated in clinical trials. A major obstacle has been the lack of acetylgalactosaminidases for efficient removal of A antigens. We describe a novel family of prokaryotic α -Nacetylgalactosaminidases with a unique NAD+-dependent catalytic mechanism deduced from x-ray crystallography [1]. Using a recombinant protein from this gene family, an efficient process for enzymatic removal of A antigens from blood group A red cells was developed. The digestion process is performed at neutral pH within 60 min and uses 50 mg enzyme/unit blood. This process holds promise for finally achieving the goal of universal red blood cells with significant improvements in safety and cost-effectiveness for transfusion medicine. The properties of family GH109 α -N-acetylgalactosaminidases with neutral pH optimum and favorable substrate specificity for branched blood group A structures are unique and distinctly different from those of other families containing α -N-acetylgalactosaminidases. The three-dimensional structure of α -N-acetylgalactosaminidase is unique amongst glycosidases structurally elucidated so far, but exhibits similarity to oxidoreductases, and its proposed catalytic mechanism involving NAD⁺ is only the second example of a NAD⁺ based glycolytic mechanism. Thus, the novel glycosidase family GH109 considerably expands our knowledge of the diversity of glycosidase folds and mechanisms

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Restriction endonucleases that resemble a component of the bacterial DNA repair machinery Matthias Bochtler^{a,b}, Monika Sokolowska^{a,b}, Magdalena Kaus-Drobek^{a,b}, Honorata Czapinska^{a,b}, Roman Szczepanowski^{a,b}, Gintautas Tamulaitis^c, Virginijus Siksnys^c, a International Institute of Molecular and Cell Biology, Trojdena 4, 02-109 Warsaw, Poland b MPI-CBG, Pfotenhauerstr. 108, 01309 Dresden, Germany c Institute of Biotechnology, Graiciuno 8, LT-02241, Vilius, Lithuania E-mail: MBochtler@iimcb.gov.pl

Keywords: DNA; restriction endonuclease; repair

It has long been known that most type II restriction endonucleases share a conserved core fold and similar active sites. The same core folding motif is also present in the MutH protein, a component of the bacterial DNA mismatch repair machinery. In contrast to type II restriction endonucleases, which assemble into functional dimers to make a double strand break, MutH (/GATC, "/" indicates the cleavage site) is a monomer and nicks hemimethylated DNA. We have shown that restriction endonucleases BcnI(CC/SGG, S stands for C or G) and MvaI (CC/WGG, W stands for A or T), which catalyze double-strand breaks in pseudopalindromic DNA, are also monomers that recognize their pseudosymmetric targets asymmetrically. The crystallographic results demonstrate that BcnI and MvaI resemble MutH more closely than other restriction endonucleases and establish a close link between some restriction enzymes and the DNA repair machinery [1], [2].

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