EXTREMOPHILIC ENZYMES FOR NOVEL SYNTHETIC METHODOLOGIES IN SUSTAINABLE CHEMISTRY

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Abstract
Novel synthetic methodologies using extremophilic enzymes for catalysing O-phosphorylation, water elimination, reduction and hydrolysis reactions will be discussed. The preparation of kinases from extremophiles enabled novel biocatalytic asymmetric phosphorylations of small molecules using the phosphoenolpyruvate-pyruvatekinase-system for ATP cofactor regeneration. The selective enzymatic elimination of water from sugar acids is another area where extremophilic enzymes are of interest and is described in the one-step biocatalytic route to 2-keto-3-deoxy-D-gluconate from D-gluconate using an extremophilic gluonate dehydratase. Finally, novel synthetic methodologies from the classes of biocatalytic reduction and hydrolysis reactions are described.

Keywords: Enzymes, Biotechnologies, Phosphorus, Bacteria, Mediterranean Sea

Resource efficiency combined with selectivity and robustness are common goals for the biosynthetic machinery of extremophiles in nature as well as for sustainable chemical processes in industrial manufacturing. Complete conversion in a reaction step, the waste per reaction step and the number of reaction steps are key factors for reaching these goals. Highly selective, protecting group-free and sustainable processes have the potential to reduce the number of reaction steps and therefore also to improve the efficiency of processes, reagents, and tools. The replacement of stoichiometric by catalytic methods leads to mass and energy savings, selectivity improvements, safety, health and environment benefits. Biocatalytic methods have become well established in a large number of industrial applications and have even become the first choice for certain reaction classes in the process design and the route selection [1]. Extremophilic enzymes from marine environments catalyzing the synthesis of the required molecules of life along the fascinating metabolic pathways under extreme conditions provide a blueprint from nature for synthesis and are of much interest in the development of novel synthetic methodologies towards sustainable chemical processes.

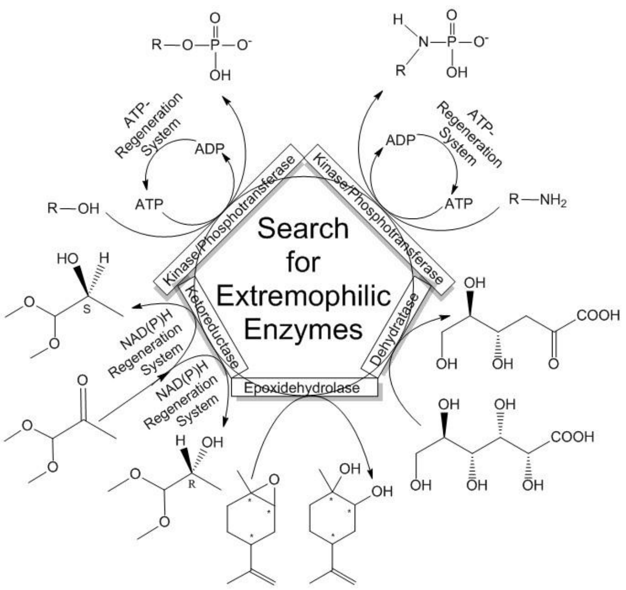

Fig. 1. Novel extremophilic enzymes for catalyzing phosphorylation, water elimination, hydrolysis and reduction reactions

Phosphorus is an important element for life on our planet and occurs in living cells as part of many key biochemical constituents such as nucleic acids, phospholipids, phosphoproteins, carbohydrates or various metabolites, where the element phosphorus is mainly present in the O-P-bonds, but also in N-P-, S-P- or C-P-bonds and needs to be introduced by phosphorylation reactions. Among these the majority are O-phosphorylation reactions, but also N-phosphorylation, S-phosphorylation and C-phosphorylation reactions play a role. As phosphorylation reactions are therefore of major importance in natural biological processes, a large number of enzymes catalyzing the transfer of phosphogroups from one compound to another have been discovered in nature. This biodiversity is also of much interest in synthetic production processes in industry, because classical chemical phosphorylation reactions lack selectivity [2]. A variety of kinases catalyzing O-phosphorylation reactions from extremophiles have been produced and analyzed. These kinases have been sucessfully applied in a number of biocatalytic asymmetric phosphorylations of small molecules like mevalonate [3], glyceraldehyde [4] and glycerate [5] using the phosphoenolpyruvate-pyruvatekinase-system for ATP cofactor regeneration. The selective enzymatic elimination of water from sugar acids is another area where extremo-philic enzymes are of interest, as moving from fossil-based raw materials towards biobased raw materials needs, instead of introducing functional groups into highly reduced raw materials, selective defunctionalisation of highly oxidized raw mate-rials like carbohydrates. The one-step biocatalytic route to 2-keto-3-deoxy-D-gluconate from D-gluconate using an extremophilic gluconate dehydratase offers higher enantiomeric purity, step economy, safety, health and environment benefits [6]. Selective biocatalytic reductions have become an indispensable synthetic methodology as cofactor regeneration is a routine task and novel biocatalytic reductions of keto-groups to (R)- and (S)-hydroxygroups are discussed [7-8]. The large class of extremophilic hydrolases has been well established for hydrolysis as well as acylation. Novel enzymes and applications have been discovered in the case of the epoxidehydrolase-catalyzed resolutions of cis-/trans-mixtures of limonene epoxides and the synthesis of chiral limonene diols [9-10]. In summary, highly efficient and selective extremophilic enzymes have been developed for biocatalytic phosphorylations, dehydrations, reductions, hydrolyses and applied in synthetic methodologies with complete conversions. The rich biodiversity and metabolic capabilities of extremophiles in marine environments has a tremendous potential for novel enzyme developments. Therefore the outlook for extremophilic enzymes from marine environments looks promising if combined with a clear focus on reaction classes where synthetic methodologies need to be developed.

References

1 - Meyer H.P. et al., 2013. *Catal. Sci. Technol.*, 3: 29-40
2 - Gauss D. et al.,2016. in: *Applied Biocatalysis - From Fundamental Science to Industrial Applications* ISBN: 978-3-527-33669-2, Wiley-VCH
3 - Matsumi R. et al., 2014. *RSC Advances* 4: 12989-12994
4 - Gauss D. et al., 2014. *Carbohydrate Research* 389: 18-24
5 - Hardt N. et al., 2016. manuscript in preparation
6 - Matsubara K. et al., 2014. *Journal of Biotechnology* 191: 69-77
7 - R. Wohlgemuth, 2014. in: *Synthetic Methods for Biologically Active Molecules: Exploring the Potential of Bioreductions*, Wiley-VCH
8 - Vogel M.A.K. et al., 2016. Reaction Chemistry & Engineering, DOI:10.1039/C5RE00008B
9 - Ferrandi E.E. et al., 2015. *FEBS Journal* 282:2879-2894
10 - Ferrandi E.E. et al., 2015. ChemCatChem 7:3171-3178