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Gram-scale Enzymatic Synthesis of Phosphorylated Sugars

ATP-independent enzymatic phosphorylation relies on the transphosphorylation activity of acid phosphatases. Rapid product formation and high yields, stable enzymes, mild conditions and facile reaction setup feature the formation of monophosphorylated primary and secondary alcohols. We have developed a handful of techniques to overcome the limitations set by product hydrolysis and high amount of inorganic monophosphate by-product formation. The protocols allow the multiple gram-scale synthesis of high value-added phosphorylated mono- and oligosaccharides being of importance in metabolic and drug research.

Introduction
Synthesis of valuable phosphate (mono)esters, e.g. sugar phosphates, nucleotides, metabolites and prodrugs can be achieved by rather laborious chemical or by mild enzymatic routes. The latter traditionally employs kinases in conjunction with a second enzyme for the recycling of ATP. Kinases, though applied on preparative-scale, are rather substrate-specific and the efficiency of ATP-recycling systems is still a limiting factor.

The ability of phosphatases to perform in the (reverse) transphosphorylation mode employing cheap high-energy phosphate donors (P-donors), e.g. pyrophosphate (PPi), was recognized decades ago. The lack of a recycling system and the rather relaxed substrate spectrum of phosphatases renders them attractive alternatives to kinases and allows the synthesis of a broad range of phosphate monoesters.

However, this method suffers from a major limitation, i.e. the enzyme-catalyzed hydrolysis of the product phosphate ester leading to product depletion and unpredictable reaction times.

Creation of a toolbox
We developed a toolbox consisting of various techniques to overcome the above-mentioned limitation. The enabling technology includes reaction engineering, protein engineering, continuous flow synthesis and phosphate donor engineering (Fig. 1). The protocols available in our laboratory allow the flexible adjustment of reaction conditions to the property of the substrates and/or products and the process parameters. The enzymes are easily accessible, highly productive (TONs in the range of $10^5$) and enable rapid large-scale production of phosphate esters with space-time yields of several hundreds of g/L*h.
Towards sugar-phosphates

The successful establishment of this technology led to the development of a synthetic protocol resulting in regioselectively phosphorylated mono- and oligosaccharides. These compounds are of interest for drug discovery and metabolic investigations, however, their availability is rather limited. The existing chemical routes require the use of toxic activating reagents and multiple protection/deprotection steps rendering the whole process tedious. Our technique enables the isolation of gram amounts of these compounds with remarkable space-time yields employing inexpensive reagents and mild conditions. In addition, application of a novel phosphate donor can reduce the amount of toxic waste by ~75%.

Impact and effects

The developed technology enables the facile synthesis of valuable phosphorylated molecules, for instance, biologically important mono- and oligosaccharides (Fig. 2). Products can be isolated on multiple gram-scale in a short time. This biocatalytic method effectively rivals existing synthetic routes by employing inexpensive and mild reagents and obviates the need for protection chemistry. This, accompanied by the high value of the products, holds out good promises for potential commercial applications.