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Polyphosphate kinases: the perfect enzymes for phosphorylation?

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PURPOSE OF THE ABSTRACT
Polyphosphate kinases of the family 2 (PPK2) have become popular biocatalysts for the regeneration of adenosine 5´-triphosphate (ATP), as well as the biocatalytic production of nucleotides. Based on phylogenetic analyses PPK2s have been assigned to three different classes I, II, and III that catalyse the phosphorylation of ADP, AMP, and both nucleotides, respectively. We used combinations of these enzymes successfully for the phosphorylation of nucleotides, e.g. in biomimetic systems for the regeneration of cofactors such as S adenosylmethionine and coenzyme A; as well as for the production of nucleotide triphosphates (NTPs) from non-physiological nucleosides. By analysing three-dimensional structures from all classes of PPKs and a range of biochemical assays using physiological and non-physiological substrates, we are working on the elucidation of the PPK2s' molecular mechanism and substrate discrimination. This includes the analysis of polyP molecules with varying chain lengths and nucleoside derivatives such as adenosine 5´-tetra- und pentaphosphate.
Inorganic polyphosphate (polyP) is an abundant, inexpensive and stable phosphate donor, and PPK2s can be used for the phosphorylation of AMP as well as ADP. Nevertheless, during our efforts to optimise the PPK2-catalysed reactions for the biocatalytic production of NTPs, we could never reach full conversion to the corresponding NTP. An investigation of the thermodynamic equilibrium of these enzymes in comparison with family-1 PPKs and literature data from other kinases shows that the thermodynamic equilibrium of the PPK reaction is located less far on the NTP side than it is the case for other kinases; this can be traced back to the phosphate transfer potential of the corresponding phosphate donors. The results raise the question how suitable PPKs are for biocatalytic applications, including syntheses of NTPs and NTP regeneration systems.

FIGURES

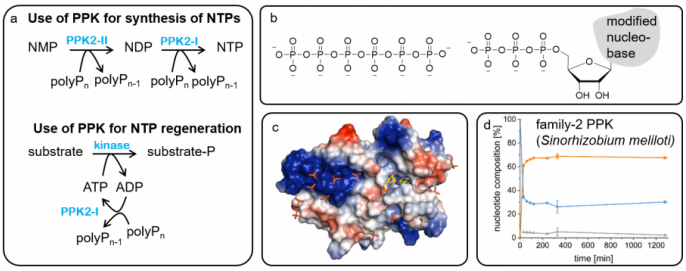

FIGURE 1

Family-2 polyphosphate kinases.

a: Use of PPK2s for eiter biocatalytic synthesis of NTPs or ATP regeneration coupled to another kinase reaction; b: chemical structures of polyP and an NTP.; c: three-dimensional structure of a class II PPK2 with co-crystallised substrate; d: thermodynami

FIGURE 2

KEYWORDS

BIBLIOGRAPHY