OP-13

CHEMO-ENZYMATIC PREPARATION OF NUCLEOSIDE TRIPHOSPHATES FROM DIPHOSPHATES USING FEEDSTOCK CHEMICALS

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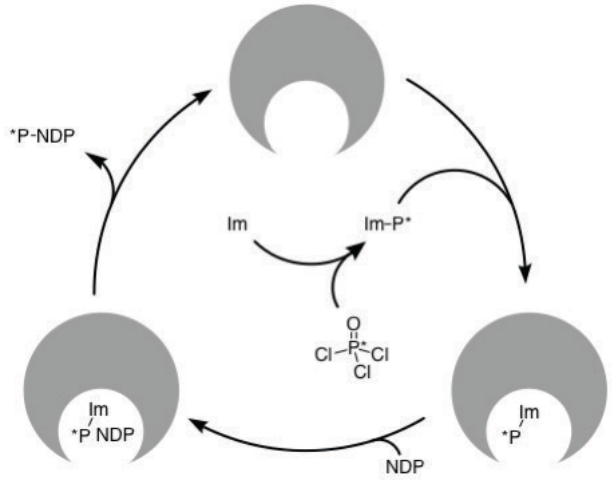

We have used a His→Gly active site mutant of nucleoside diphosphate kinase (NDPK) to prepare nucleoside 5'-triphosphates (NTPs). WT NDPK operates via a ping-pong mechanism where the active site His is phosphorylated by ATP to afford ADP and phosphoryl-enzyme in the 'ping' step. The phosphoryl-enzyme then dispatches the phosphoryl group to a substrate NDP in the 'pong' step. Herschlag and co-workers [1] showed that a His→Gly mutant could successfully *dephosphorylate* ATP using imidazole to 'rescue' the activity that was lost through the mutagenesis of the His residue. In light of the Principle of Microscopic Reversibility, we reasoned that it should be possible to *phosphorylate* NDPs using *N*-phosphoryl-imidazole as the source of the phosphoryl group with His→Gly mutant NDPK as catalyst. We employed our aqueous approach for the phosphorylation of amines to readily and rapidly produce phosphoryl-imidazole from $POCl_3$. We have shown that NTPs are produced rapidly with high conversion levels from these inexpensive precursors. We have shown that mutant NDPK and *N*-phosphoryl-imidazole can be used in combination with another ATP-consuming enzyme, thus illustrating the possibility of using *N*-phosphoryl-imidazole to regenerate (costly) ATP in biocatalytic systems.

REFERENCE

[1] S. J. Admiraal, P. Meyer, B. Schneider, D. Deville-Bonne, J. Janin, D. Herschlag, *Biochemistry* **2001**, *40*(*2*), 403–413. https://doi.org/10.1021/bi002472w