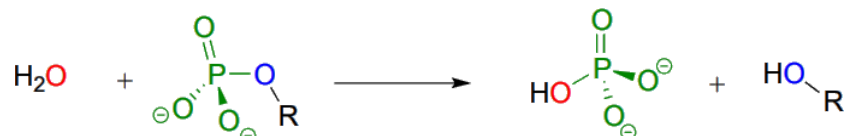


9.7: Hydrolysis of Organic Phosphates

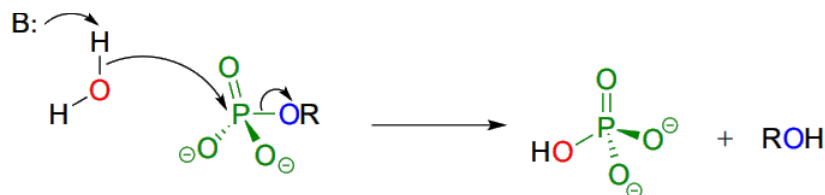
While kinase enzymes catalyze the phosphorylation of organic compounds, enzymes called phosphatases catalyze dephosphorylation reactions. The reactions catalyzed by kinases and phosphatases are not the reverse of one another: kinases irreversibly transfer phosphate groups from ATP (or sometimes other nucleoside triphosphates) to various organic acceptor compounds, while phosphatases transfer phosphate groups from organic compounds to water: these are hydrolysis reactions. Kinase reactions involve an inherently 'uphill' step (phosphorylation of an alcohol, for example) being paid for with an inherently 'downhill' step (cleavage of an anhydride bond in ATP). Phosphatase reactions, on the other hand, are thermodynamically 'downhill', and while they require an enzyme to speed them up, they do not involve 'spending' energy currency the way kinase reactions do.

Phosphatase reaction:

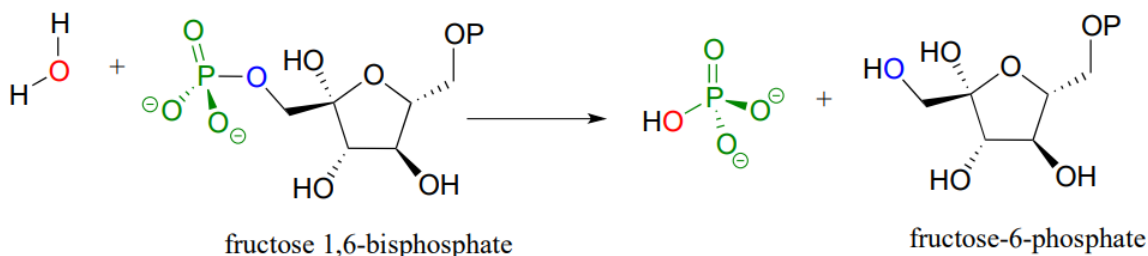


There are two possible general mechanisms for a phosphatase reaction. Some enzymes catalyze direct hydrolysis reactions, in which the phosphate group is removed by direct attack of a water molecule at the phosphate center:

Phosphatase mechanism (direct hydrolysis):



One of the two phosphate groups on fructose 1,6-bisphosphate is hydrolyzed in such a way late in the gluconeogenesis pathway. (Biochemistry 2000, 39, 8565; EC 3.1.3.11)



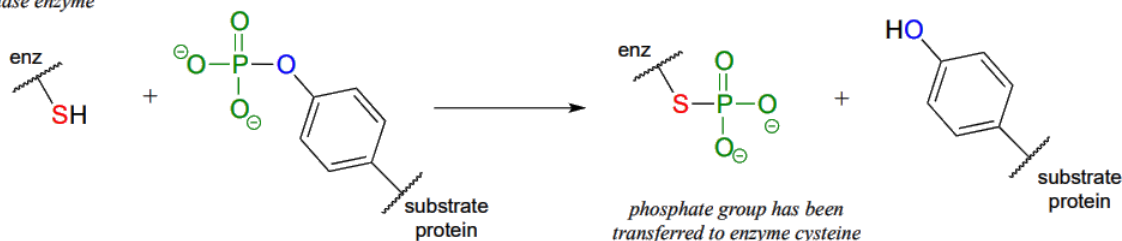
Many phosphatase reactions, however, operate by a slightly more complicated mechanism than what is shown above. In the first phase, a nucleophilic enzyme group (typically a cysteine, aspartate, glutamate, or histidine side chain, designated in the figure below as 'X') attacks the phosphate group. In the second phase, the phosphorylated residue is hydrolyzed. For example, protein tyrosine phosphatase catalyzes the dephosphorylation of phosphotyrosine residues in some proteins - this is the other half of the regulatory 'on-off switch' that we discussed earlier in the context of protein kinases. In the first step, the phosphate group is directly donated to a cysteine side chain in the phosphatase enzyme's active site. In the second step, the phosphocysteine intermediate is cleaved by water to form inorganic phosphate and regenerate the free cysteine in the active site.

Indirect phosphatase reaction:

Step 1:

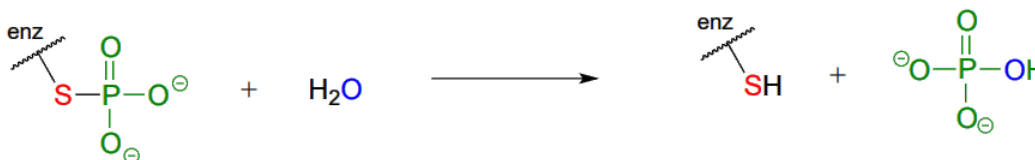
active site cysteine on
phosphatase enzyme

phosphotyrosine on substrate protein



Step 2:

free cysteine is regenerated



Notice that in the end, the phosphate group has still been transferred to a water molecule, albeit indirectly. How would you know, just by looking at the substrate and product of the protein tyrosine phosphatase reaction, that the phosphate is not transferred directly to a water molecule? Simply put, you wouldn't know this information without the benefit of knowledge gained from biochemical experimentation.

? Exercise 9.7.1

If you were to look just at the substrates and products of a phosphatase reaction without knowing anything about the mechanism, it is apparent that a *nucleophilic substitution* mechanism could theoretically account for the products formed. Draw out a hypothetical nucleophilic substitution mechanism for the hydrolysis of a phosphoserine residue and show how researchers, by running the reaction in H₂¹⁸O, (isotopically labeled water), could potentially distinguish between a nucleophilic substitution and phosphate group transfer mechanism by looking at where the ¹⁸O atom ends up in the products.

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