

Case 42

Mechanism of inhibition of protein-tyrosine phosphatases by vanadate and pervanadate

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Focus concept

Protein tyrosine phosphatases (PTPs) are investigated because of their ability to reverse the effects of protein tyrosine kinases (PTKs). Inhibitors of the PTP investigated in this study have insulin-mimetic effects and have potential in the treatment of diabetes.

Prerequisites

- Enzyme kinetics and mechanisms of inhibition
- Lineweaver-Burk analysis
- Enzyme catalytic mechanisms

Background

Protein tyrosine kinase enzymes are a family of enzyme activated by extracellular signaling molecules such as growth factors. Once activated, they autophosphorylate specific tyrosines which serve as docking sites for intracellular proteins which then put into motion a cascade of intracellular reactions that lead to changes in cellular growth and differentiation. Although not a growth factor, activation of the insulin receptor occurs via a similar mechanism.

Protein-tyrosine phosphatase enzymes have the ability to reverse the effects of the activators of the protein tyrosine kinases because the phosphatase enzyme catalyzes the hydrolysis of phosphate from the phosphotyrosine. Thus they are also important in cellular regulation and there is a delicate balance in the cell between activation of protein tyrosine kinases and protein tyrosine phosphatases (see Figure 42.1). Inhibitors of the protein tyrosine phosphatases have the effect of potentiating the action of the kinases. Inhibitors of protein tyrosine phosphatase PTP1B acts as insulin mimetics because they potentiate the activity of the insulin receptor, a protein tyrosine kinase. In clinical trials, inhibitors of PTP1B have been shown to be effective at treating diabetes.

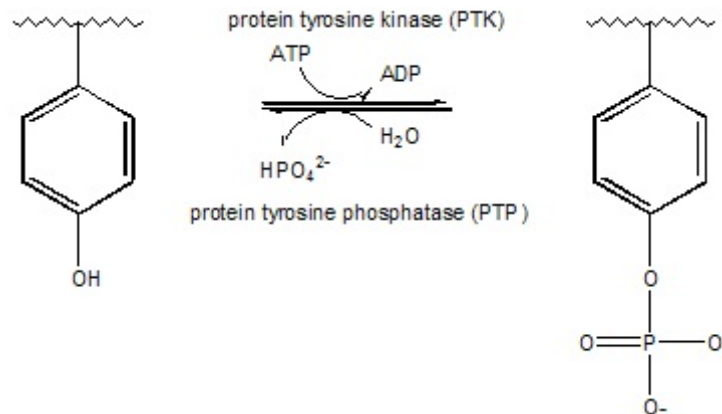


Figure 42.1: Opposite actions of protein tyrosine kinases (forward reaction) and protein tyrosine phosphatases (reverse reaction).

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As mentioned above, protein tyrosine phosphatases catalyze the hydrolysis of phosphate groups from specific phosphotyrosyl residues. PTP enzymes have an essential cysteine in the catalytic site of the enzyme that is essential for activity. During the process of the removal of the phosphate, a thiol-phosphate group is formed. Oxidation of the essential cysteine renders the enzyme inactive; therefore during the purification process, dithiothreitol (DTT), a reducing agent, is added to buffers to keep the essential cysteine in its reduced form.

In this study, the investigators studied the ability of vanadate and pervanadate to inhibit PTP1B. For the enzymatic assays, they used an artificial substrate, fluorescein diphosphate (FDP). Removal of a phosphate group from FDP by the PTP1B produces the product fluorescein monophosphate (FMP), which absorbs light at 450 nm. The reaction is shown in Figure 42.2. The structure of vanadate, and its comparison to phosphate, is shown in Figure 42.3.

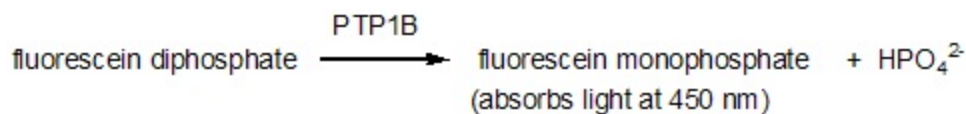
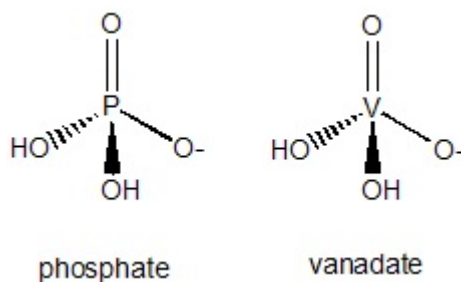


Figure 42.2: PTP1B-catalyzed hydrolysis of phosphate from FDP to produce FMP; formation of FMP can be monitored spectroscopically at 450 nm.



The investigators measured the activity of the PTP1B enzyme in the presence and in the absence of 4.0 μM vanadate. The data are shown in Table 42.1.

Table 42.1: Hydrolysis of FDP by PTP1B in the presence and the absence of vanadate (from Huyer, G., et al., 1997).

[FDP], μM	velocity, nM/s (w/o vanadate)	velocity, nM/s (w/ vanadate)
6.67	5.7	0.71
10	8.3	1.06
20	12.5	2.04
40	16.7	3.70
100	22.2	8.00
200	25.4	12.5

The investigators also studied the ability of pervanadate to inhibit PTP1B activity. “Pervanadate” is the name given to a family of compounds that are formed when vanadate reacts with hydrogen peroxide. The structure of one of the complexes formed is shown in Figure 42.4.

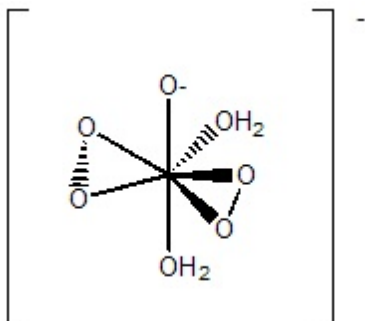


Figure 42.4: Structure of the diperoxo complex of pervanadate.

The investigators did a side-by-side comparison of the ability of vanadate and pervanadate to inhibit the activity of PTP1B. They added enzyme and substrate, then added inhibitor. They found that the inhibition by vanadate could be reversed if EDTA¹ was added, but the inhibition by pervanadate was only partially reversed. In addition, the investigators isolated the PTP1B enzyme after pervanadate was added and subjected the enzyme to analysis by mass spectrometry. They found that the mass of PTP1B increased by 48 g/mol (the mass of three oxygen atoms) in the presence of vanadate.

Questions

1. Construct a Lineweaver-Burk plot using the data provided in Table 42.1. Calculate K_M and V_{max} for PTP1B in the absence and in the presence of vanadate.
2. What kind of inhibitor is vanadate? Explain, using the data in the Lineweaver-Burk plot. Calculate α or α' (or both), whatever is appropriate, then determine K_i or K_i' (or both, again, whatever is appropriate). Using the information in the background and in the Lineweaver-Burk plot, explain how vanadate inhibits the activity of PTP1B.
3. An alternative way to calculate K_i for an inhibitor is to measure the velocity of the enzyme-catalyzed reaction in the presence of increasing amounts of inhibitor and a constant amount of substrate. These data are shown in Table 42.2 for a substrate concentration of 6.67 μM . For each concentration of inhibitor, calculate α is calculated, then plot α vs. $[I]$. Determine K_i from the slope of the plot.

¹EDTA is a chelating agent that binds both vanadate and pervanadate.

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Table 42.2: Velocity of the PTP1B reaction in the presence of varying amounts of the inhibitor vanadate (from Huyer, G., et al., 1997).

[vanadate], μM	velocity, nM/s
0.0	5.70
0.20	3.83
0.40	3.07
0.70	2.35
1.0	2.04
2.0	1.18
4.0	0.71

4. Does pervanadate inhibit PTP1B via the same mechanism as vanadate? Using the information in the background explain how pervanadate might inhibit PTP1B.

Reference

Huyer, G., Liu, S., Kelly, J., Moffat, J., Payette, P., Kennedy, B., Tsaprailis, G., Gressner, M. J., and Ramachandran, C. (1997) *J. Biol. Chem.* **272**, pp. 843-851.