

which could act as potential templates are fairly common. This reversible dendritic encapsulation will be of particular use when the template is a functional molecule derivatized with NH_3^+ groups. The function will then be dendritically modified (or shielded) until such a point as K^+ ions are added, when controlled release of the functional template would be achieved. Investigations of the reversible encapsulation and controlled release of functional template molecules are currently in progress.

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Phosphite Dehydrogenase: A Versatile Cofactor-Regeneration Enzyme**

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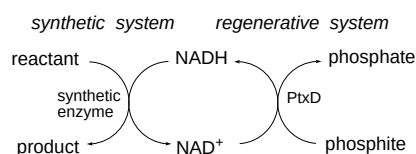
The potential of enzyme-catalyzed transformations in organic synthesis is well recognized.^[1] Oxidoreductases make up an important class of enzymes as they can perform highly stereoselective reductions of a variety of functional groups.^[2] A drawback of the nicotinamide-dependent oxidoreductases is the prohibitively high expense of the cofactor for stoichiometric use. This impediment has been overcome by using a second enzyme system that can continuously replenish a catalytic amount of the active form of the cofactor.^[3] Cofactor regeneration also simplifies product isolation and can influence the position of the equilibrium of the synthetic enzyme system, that is, the regeneration system may drive the reaction to completion when product formation would be unfavorable in its absence. The efficiency of a regeneration system is determined by the expense and stability of the regeneration enzyme and its substrate, the ease of product purification, the kinetic parameters of the regeneration enzyme (k_{cat} , K_M), and the thermodynamic driving force.

We recently described the unusual enzyme phosphite dehydrogenase (PtxD).^[4,5] This protein catalyzes the oxidation of phosphite to phosphate with the concomitant reduction of NAD^+ to NADH. The enzyme can also use NADP^+ as the oxidant, albeit less effectively.^[4] The equilibrium constant for the oxidation of phosphite by NAD^+ can be estimated as 10^{11} by using the reported redox potentials at pH 7.0 for

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NAD^+/NADH (-0.32 V)^[6] and phosphite/phosphate (-0.65 V)^[7]. For comparison, the equilibrium constant for NAD^+ reduction by the industrially used formate dehydrogenase (FDH) from *Candida boidinii* is 7×10^5 .^[8] The k_{cat} of recombinant phosphite dehydrogenase (7.3 s^{-1})^[4] is slightly higher than that of FDH (2.5 s^{-1}),^[9] and the protein has a K_{m} of $\sim 50\text{ }\mu\text{M}$ for both NAD^+ and phosphite,^[4] assuring use of the cofactor in low concentrations. Moreover, phosphite is inexpensive and a phosphite buffer is simply converted into a phosphate buffer during the reaction.^[10] Importantly, it has been shown previously that phosphate does not inhibit PtxD, even at high concentrations, and that the enzyme displays a broad pH-rate maximum.^[4] Collectively, these properties imply that the enzyme has great potential as an NADH-regenerating system (Scheme 1).



Scheme 1. Schematic representation of the use of phosphite dehydrogenase (PtxD) as an NADH-regeneration enzyme.

The time course of the reduction of pyruvate to L-lactate by L-lactate dehydrogenase (LLDH) in the presence of NAD^+ (0.05 %) and PtxD (0.002 %) at relatively high concentrations of pyruvate (200 mM) and phosphite (500 mM) is shown in Figure 1. During the process, the rate of the synthetic system (LLDH) did not decrease over a time span of more than 70 h. At this timepoint, the substrate was completely converted into product, resulting in total turnover numbers (TTNs) of 2000 for the cofactor and 5×10^4 for phosphite dehydrogenase. The apparent robustness was not anticipated as it has been

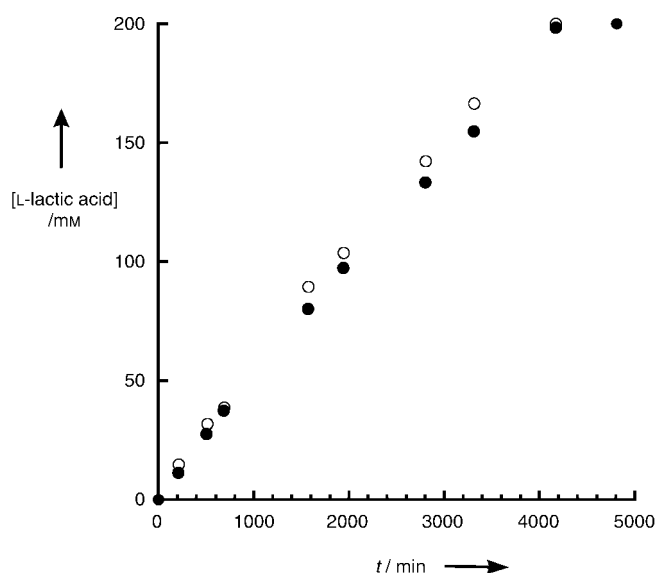
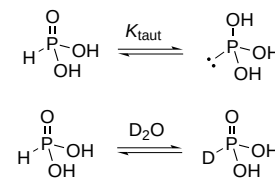


Figure 1. Time dependence of the production of L-lactate in a reaction mixture that contains phosphite (500 mM), pyruvate (200 mM), NAD^+ (0.1 mM), commercial LLDH (0.25 U mL^{-1}), and MBP-PtxD ($3\text{ }\mu\text{M}$). The progress of the reaction was monitored by ^1H NMR (pyruvate) and ^{31}P NMR spectroscopy (phosphite).

reported that among various buffers, phosphate is the most effective in accelerating the decomposition of NADH ($t_{1/2}$ 27 h at 0.1 M).^[11] It appears that in this system, however, the buffer-catalyzed breakdown of the cofactor is not a limiting factor. The regeneration enzyme consisted of phosphite dehydrogenase fused at its N-terminus to maltose-binding protein (MBP) (obtained on a large scale from a 30-L fermentation of *E. coli* that contained a pMAL-derived expression vector). A one-step purification protocol using an amylose affinity column yielded $\sim 70\text{ mg}$ of purified protein per liter of culture.^[12]

A convenient feature of the use of phosphite as the reductant involves the ease by which the reaction progress can be followed. A sensitive colorimetric assay allows the quantification of the phosphate produced,^[4] or alternatively, the diagnostic doublet of phosphite in the ^{31}P NMR spectrum can be monitored ($\delta = 3.58\text{ ppm}$, $J_{\text{P-H}} = 581\text{ Hz}$). The latter method is noninvasive and the amounts of both phosphite remaining and phosphate produced can be determined. After establishing that PtxD can be used for NADH regeneration with LLDH, a series of other dehydrogenases were investigated. PtxD was utilized in transformations catalyzed by D-lactate dehydrogenase (DLDH), horse liver alcohol dehydrogenase (HLADH), and malate dehydrogenase (MDH). In the case of HLADH, trifluoroacetaldehyde monohydrate was used as its nonphysiological substrate.^[13] For all four synthetic enzyme systems, TTNs of 10^2 – 10^3 were observed for NAD^+ and TTNs for PtxD ranged from 10^4 to 10^5 .^[14]

Perhaps the most valuable aspect of using phosphite dehydrogenase as a cofactor-regeneration system is the ease by which it can be used for the preparation of deuterated products. Phosphite exists in two different equilibrating isomers, of which the tetracoordinated tautomer **1** is favored by a factor of 10^{21} at pH 7.0. At pH 2, however, K_{eq} for phosphorous acid is increased to 10^{-10} .^[15] Hence, equilibration of phosphorous acid in D_2O followed by lyophilization provides facile access to labeled substrate (Scheme 2).^[16] The rate of exchange is slow at pH 2 ($t_{1/2} = 500\text{ min}$), and becomes essentially negligible at pH 7.^[17] Therefore, deuterium-labeled phosphite can be used for the in situ preparation of NAD^2H and thereby for the enzymatic preparation of labeled compounds.^[18] We have previously shown that PtxD displays only a moderate primary kinetic isotope effect (KIE) on k_{cat} (2.0) and $k_{\text{cat}}/K_{\text{m,phosphite}}$ (1.8),^[5] and hence the use of labeled substrate should not prohibit cofactor regeneration. Indeed, (*S*)-2-[D]lactate was obtained in high isotopic purity ($> 98\%$) when LLDH and PtxD were used in the presence of [D]phosphite. This methodology allows a low-cost alternative to existing methods for the enzymatic preparation of labeled compounds.^[19]



Scheme 2. The equilibrium between the two tautomeric forms of phosphorous acid can be exploited for the production of deuterium-labeled phosphite.

In summary, we report the use of a new NADH-regeneration system based on phosphite dehydrogenase. This enzyme has much potential owing to a larger thermodynamic driving force for NADH production than any of the previously

reported regeneration systems. The products can be readily separated from the phosphate by-product, and this technology can be conveniently and economically adapted for the production of deuterium-labeled products.

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Guest Preorganization: An Alternative “Bioinspired” Paradigm in Host–Guest Chemistry**

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The tris(pyrazolyl)borate ligands pioneered by Trofimenko in the late 1960s, have arguably become the preeminent platform for metal binding in coordination, organometallic, and bioinorganic chemistries.^[1] Indeed, complexes of this ligand with almost every metal in the periodic table are known.^[1] Modifications to the parent ligand over the last few decades have been primarily directed towards the synthesis of highly sterically congested analogues that have allowed the stabilization of species not previously accessible.^[2–5] Another possible application for this anionic, three-fold-symmetric class of molecule, namely as a “host” to something other than a metal ion, seems to have been largely unexplored. Recently, by using ester- or amide-containing R groups at the 3-position of the pyrazole ring to provide an additional set of sites for secondary interactions, we have begun to explore the host–guest chemistry of the tris(pyrazolyl)borates. Whilst trying to use various transamidation reactions to convert 3-ester-substituted Tp (tris(pyrazolyl)borates) ligands into 3-amido-substituted ligands we isolated complexes of the general stoichiometry $[\text{Tp}^*][\text{NH}_3\text{R}]$, where $\text{Tp}^* = \text{tris}(3\text{-carboxyethyl-5-methylpyrazolyl})\text{borate}$. That these species were not simple salts but true host–guest complexes was revealed by an X-ray crystal structure of the *tert*-butylammonium analogue, **1**.^[6] The structure of **1** (Figure 1) reveals the expected complementarity of fit between the anionic tris(pyrazolyl)borate and the cationic three-fold symmetric protonated amine which is lodged in the “metal-binding” cavity of the former. In addition to the electrostatic attraction, the protonated amine group makes three strong, bifurcated hydrogen bonds with the pyrazole nitrogen atoms and the ester carbonyl groups. The hydrogen atoms of the ammonium ion, which were all easily located and refined in the structure, are at an average of 2.16(6) Å from the pyrazole nitrogen atoms and 2.54(16) Å from the carbonyl oxygen atoms, with 71.6(26)° N–H⋯O angles. Two sharp N–H proton resonances

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