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Efficient, crosswise catalytic promiscuity among enzymes that catalyze phosphoryl transfer ☆

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ABSTRACT

The observation that one enzyme can accelerate several chemically distinct reactions was at one time surprising because the enormous efficiency of catalysis was often seen as inextricably linked to specialization for one reaction. Originally underreported, and considered a quirk rather than a fundamental property, enzyme promiscuity is now understood to be important as a springboard for adaptive evolution. Owing to the large number of promiscuous enzymes that have been identified over the last decade, and the increased appreciation for promiscuity's evolutionary importance, the focus of research has shifted to developing a better understanding of the mechanistic basis for promiscuity and the origins of tolerant or restrictive specificity. We review the evidence for widespread crosswise promiscuity amongst enzymes that catalyze phosphoryl transfer, including several members of the alkaline phosphatase superfamily, where large rate accelerations between 10^6 and 10^{17} are observed for both native and multiple promiscuous reactions. This article is part of a Special Issue entitled: Chemistry and mechanism of phosphatases, diesterases and triesterases.

1. Introduction

Phosphoryl transfer plays a crucial role in many fundamentally important cellular processes, from simple metabolism, to cell signalling, nucleic acid replication and repair, and gene expression. The inherent stability of the phosphate linkage [1] makes it the ideal functional group for the storage of precious genetic information in DNA and RNA, but this stability also means that cleavage of phosphate esters is extremely thermodynamically and kinetically challenging and these reactions are exceedingly slow. Half-lives for the uncatalyzed hydrolyses of un-activated phosphate monoesters and diesters have been estimated to be 10^{12} years and 10^7 years respectively [2,3]. The hydrolysis of sulfate esters, which is chemically related to phosphoryl transfer, is another important biochemical transformation [4] for which uncatalyzed rates are also prohibitively slow. The spontaneous hydrolysis of un-activated sulfates is estimated to have a half-life of 10^{18} years [5,6]. To counter the intrinsically slow rates of phosphate and sulfate hydrolysis, many enzymes have evolved to accelerate these reactions and allow them to occur on biologically relevant timescales. The rate accelerations afforded by phosphatases and sulfatases, reaching as high as 10^{27}-fold [7], are among the most impressive enzymatic rate accelerations known [8].

The enormous rate enhancements provided by phosphatases are made even more impressive by the recent observations of catalytic promiscuity among these enzymes. *Catalytic promiscuity*, first described by Jensen [9] and later elaborated by O'Brien and Herschlag [10], refers to the ability of an enzyme to catalyzes several chemically distinct reactions involving different types of bond making and breaking events (and hence reactions proceeding through different transition states). Despite the superficial similarities of the hydrolysis of phosphate mono-, di-, and triesters (Fig. 1), these substrate classes undergo hydrolytic cleavage through very different transition states in solution (Fig. 1), characterized by different degrees of bond making, bond breaking, and charge distributions. For example, the hydrolysis of phosphate and sulfate monoesters is generally thought to proceed through a so-called dissociative transition state, with a large degree of charge build-up on the leaving group and little nucleophile participation, while phosphate diesters react via a more synchronous transition state with more advanced bonding to the nucleophile and less charge accumulation on the leaving group. Phosphate triesters hydrolyze through associative transition states with significant nucleophile addition and little leaving group departure (see More O'Ferrall-Jencks diagram, Fig. 2) [7,11]. Evidence for these mechanistic alternatives for the solution reactions are based on entropies of activation, isotope effects, and linear free energy relationships [7,8]. If catalysis is based on extremely tight recognition of a transition state, then these differences should matter. The fact that single enzymes can efficiently catalyze several different kinetically demanding reactions is at once impressive and puzzling since the factors making the enzyme proficient for one reaction would not be expected to do the same for the other

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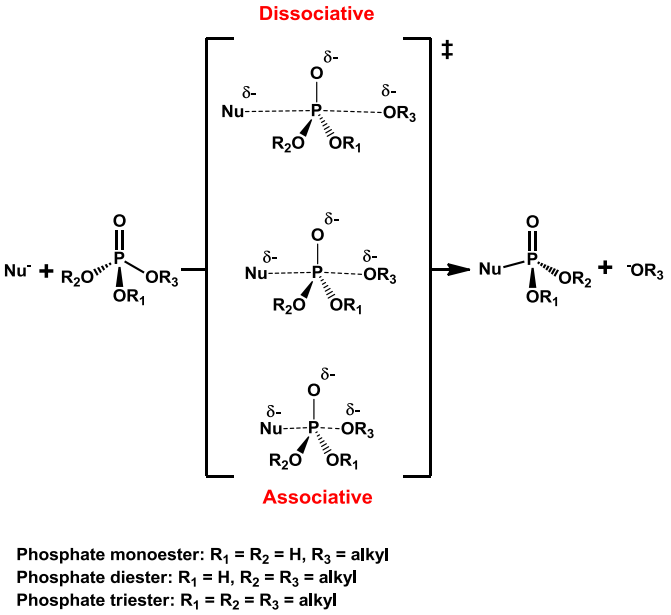

Fig. 1. The hydrolysis of phosphate mono-, di-, and triesters. The nature of associative ('tight') and dissociative ('loose') transition states for a concerted reaction are shown. (Note: at biologically relevant pH, phosphate monoesters exist as the dianon, and phosphate diesters as the monoanion.)

reactions with different ground and transition state charges and different steric demands.

The catalytic promiscuity observed among phosphatases and sulfatases challenges the notion that an enzyme is specifically optimized for its native substrate and that enzyme catalysis originates from an active site that perfectly accommodates the transition state for just one native reaction. Table 1 summarizes the observed promiscuous activities of a variety of phosphatases. In most cases the promiscuous activities are several orders of magnitude lower than the native ones but the rate enhancements remain substantial, sometimes reaching as high as 10^{18}-fold [12]. To rule out the possibility that promiscuous activities are the result of contaminants, control experiments are often conducted to show that all of the activities co-purify during protein purification, share common pH-rate behavior, and can all be destroyed by the same active site mutations [12–15].

Several of the enzymes listed in Table 1 are members of the alkaline phosphatase superfamily [17], a group of enzymes which are related on the basis of structure and mechanism (although they share only limited sequence homology). This superfamily includes alkaline phosphatase (AP) [18], nucleotide pyrophosphatase/ phosphodiesterase (NPP) [19], phosphonate monoester hydrolase (PMH) [20], and aryl sulfatase (AS) [21]. Catalytically promiscuous members of enzyme superfamilies often exhibit reciprocal and crosswise promiscuity, where the promiscuous activity of one member is the native function of another (Fig. 3) [12].

The reciprocal promiscuity among structurally related enzymes, such as those of the AP superfamily may suggest close evolutionary relationships based on the ability to provide a head start for adaptation of a new function. Catalytic promiscuity allows the beneficial contribution of the new activity to be gradually phased in during evolution. The original function would be preserved, while new activities emerged. Such repurposing of an existing enzyme is an evolutionary shortcut: [22] the enzyme *structure* does not have to be evolved, but merely adapted, and catalytic functionality (such as a metal ion cofactor) needs only to be retuned rather than generated. The combination of low sequence homology and high structural homology among these enzymes suggests that evolutionary pressure is borne on function (and, indirectly, structure), but not on amino acid composition.

Enzymes harboring high adaptive potential are generalists with multiple, albeit low, activities so that relatively few mutations are required during an adaptive walk, [23] without exceeding the mutational load that the protein structure can bear [24]. Several directed evolution studies have confirmed that promiscuous activities can be significantly enhanced by a small number of mutations [25–28]. By analogy, these observations suggest that the evolution within the AP superfamily should also be facilitated by the many promiscuous starting points. Indeed, evolution of a phosphonate hydrolase [29] and a phosphodiesterase [30] from the sulfate hydrolase PAS (which provides multiple promiscuous starting points) has been possible.

Table 1 contains several unexpected results. In some cases, the k_{cat}/K_M for one enzyme's promiscuous activity is larger than that for

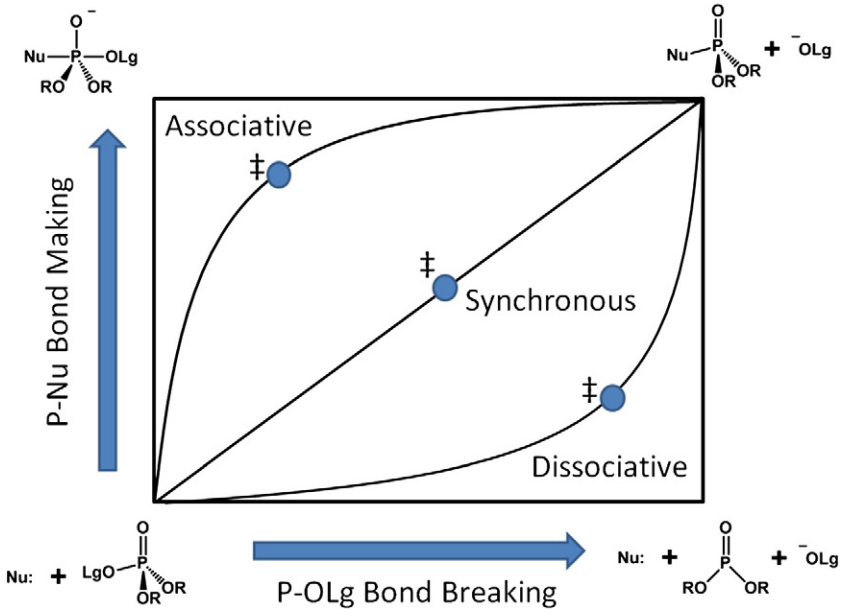

Fig. 2. A More O'Ferrall-Jencks diagram showing the continuum of possible transition states for phosphate ester hydrolysis. Phosphate triesters react through associative transition states (top left corner), phosphate diesters and phosphonate monoesters through synchronous transition states, and phosphate and sulfate monoesters through dissociative transition states (bottom right corner).

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