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Thermostable alkaline phosphatase in bacteria and archaea at a glance

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Abstract

Alkaline Phosphatase (AP) is one of the most ubiquitous enzymes for the dephosphorylation of nucleic acids in molecular biology; as reporter enzymes for secreted proteins; for colorimetric immunoassays; and as an indicator of activity in research and diagnostic kits. Today, there are continuing efforts suggesting the possibility of producing unique AP from thermophilic bacteria and archaeal cells. As AP is found in a few members of thermophiles, it is also anticipated that it will be detected in their siblings, yet the reason behind the variation in their AP activities is ambiguous. This mini review provides a comprehensive survey of the bacterial and archaeal alkaline phosphatases with particular emphasis on the thermostable APs from the members of thermophiles and their activity variation.

Introduction

Phosphatases (orthophosphoric monoester phosphohydrolase, EC.3.1.3.1), are defined as enzymes which hydrolyze esters of phosphoric acid; and are classified (according to their pH optima) as acid phosphatases or alkaline phosphatases [1] and [2]. Alkaline phosphatases (APs), catalyze the release of inorganic phosphate (Pi) from several phosphorylated compounds and are classically described as homodimeric nonspecific metalloenzymes which catalyze phosphomonoesterase reactions [3] and [4]. APs are found in all life forms and are involved in numerous and essential biological processes. They are linked with transport mechanisms in mammals [1], and have their optimum reaction in an alkaline condition, usually in the pH range of 8-9 [5]. Regardless of their sources, APs have the same catalytic characteristics; however, they are varied in their physiochemical properties. Figure 1 shows the overview of ALP roles and functions [Taken from http://www.ebi.ac.uk/QuickGO/ and modified by the author].

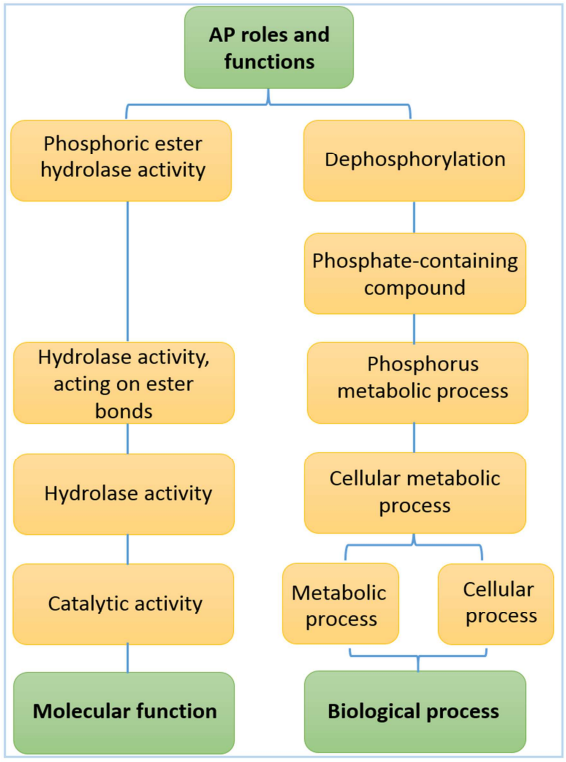
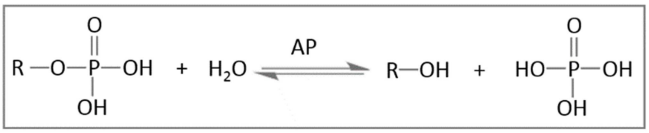
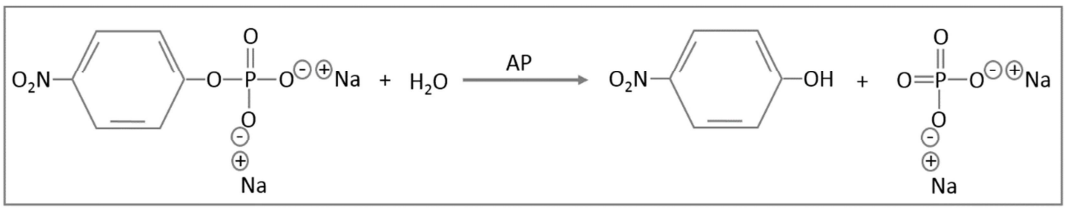


Figure 1. Overview of the roles and functions of AP.

They hydrolyze organic phosphate esters to liberate inorganic phosphate (Pi) even at the serum pH of 7.4 although their optimum pH is around 9.0 (depicted as shown in Scheme 1) [4] and [5]. AP catalyzes the hydrolysis of the colorless organic phosphate ester substrate, p-nitrophenylphosphate, to the yellow-colored product, p-nitrophenol, and phosphate at pH 10.3 (summarized as shown in Scheme 2):



Scheme 1. Alkaline phosphatase reaction. AP hydrolyzes organic phosphate esters and liberates inorganic phosphate.



Scheme 2. Alkaline phosphatase chemical assay. AP hydrolyzes phosphate group in the substrate p-nitrophenylphosphate (colorless), producing yellow-colored product p-nitrophenol.

Inorganic phosphate (Pi) is the primary source of phosphorus for microbes, and it is generated in the periplasmic space from various organophosphate and phosphate sources by dephosphorylating enzymes such as acid and alkaline phosphatases [1] and [6]. In prokaryotes, APs play very significant roles in the phosphate metabolism under inorganic phosphate limitations although their physiological functions are not well understood. The commercial source of APs is predominantly from mammalian tissues with the highest concentrations found in the liver, biliary tract epithelium and bone. AP is also found in raw milk and used as

an indicator for the efficiency of pasteurization in which the enzyme is inactivated by the combination of time and temperature applied during pasteurization [7]. The commercial application of mammalian AP is restricted by the enzyme's instability at elevated temperatures and hence, the microbial APs are potential alternative sources industrially. APs represent a large research field as they are good models to study metal ion-dependent catalysis [8] and are used in several application fields such as molecular biology [9] and immunology [10].

Bacterial alkaline phosphatase has been studied widely since the 1960s. Biosynthesis, structure, and catalytic properties of *E.coli* AP have been the attraction of many researchers [11], [12], [13] and [14]. AP from *E. coli* was found to be similar to the human enzyme and to have similar catalytic properties, such as forming the same phosphoryl intermediate as the intestinal enzyme. Scientists have extensively studied AP from *E. coli,* and detailed information on the structure and function of the enzyme are available [15] and [16]. The most commercially available isolated and purified APs are from calf intestine as well as from E.coli and are widely used in molecular biology [9]. However, APs from these sources have constraints such as low specific activity [17], therefore restricting its potential and rendering it of less use. Thus, seeking new AP-producing bacteria of considerable merits of AP that have the propensity to work at high temperatures is in need and could be an alternative to the commercial AP. Quite a number of researches on the biophysical and biochemical properties of AP, especially, in clinical chemistry and molecular biology applications have been accomplished. However, from the perspectives of microbiologists, there is not much literature available on APs. The aim of this minireview is to register and conduct a comprehensive survey of the bacterial and archaeal alkaline phosphatases with particular emphasis on the thermostable APs from the members of thermophiles and their activity variation.

This review is the first attempt is made to compile all AP-producing microbes, in particular, bacteria and archaea. Further, this work highlights the necessity for finding the appropriate AP-producing hyperthermophiles in order to gain additional insights into the variation and function of APs. Readers will appreciate the functional significance of AP regardless of the wide variation in APs'activitiesas well as what sort of genomic modifications among thermophiles were involved in creating the variation in AP thermostability. In addition to understanding the differentiation in the activities of APs in cells, finding new APs in hyperthermophiles may provide information about the mechanisms that created the disparity between bacterial and archaeal APs.

Bacterial Sources of Alkaline Phosphatases

In various commercial processes, much attention has been paid to the use of APs from thermophile origins. Although some thermostable APs appear to be interesting for understanding life at high temperatures, unique APs exhibiting this property have also been investigated for industrial processes [8]. In addition, the high thermal stability of the AP might permit its exploitation in numerous biotechnological applications; for instance in molecular cloning, as second antibody-enzyme conjugate [18] and most recently, in one-step competitive immunoassays [19]. Moreover, the current potential for this enzyme lies in the design of biosensors [20], degradation of certain nerve agents and pesticides [21] and detoxification of heavy metal waste streams [22]. AP is ubiquitous in nature and is found in organisms stemming from all three life domains. AP can be produced by numerous bacterial strains with their greatest abundance of data being obtained from *E. coli* and *Bacillus subtilis* [11] and [23]. AP exists in the periplasmic space of *E. coli* as a dimer of identical subunits each containing 429 amino acids [15]. The enzyme may be secreted into the medium or may be loosely or tightly cell-bound; the proportions of each depending on the strain, medium composition, and cultivation conditions. Nevertheless, extracellular production of APs has several advantages over secretion into the periplasm. Besides, extracellular production does not require outer membrane disruption, and therefore, it avoids intracellular proteolysis by periplasmic proteases and allows continuous production of APs. Hamzah and Hassan (2005), reported that AP produced from a local isolate of *Bacillus stearothermophilus* using solid-state fermentation system [24].Bacterial sources of AP including cyanobacteria, mutant strains, and thermophiles are compiled in this mini-review *(Supplementary Table 1)*.

Three main alkaline phosphatases PhoA, PhoX, or PhoD families are found in prokaryotes, and they have been characterized and are found to be functionally equivalent. Its activity was stimulated by Ca^{2+} and was optimal at pH 9-11 [2], [4], [25] and [26]. PhoD and PhoX are monomeric enzymes with promiscuous behavior and able to hydrolyze both phosphomonoesters and phosphodiesters. Noticeably, the three types of APs share weak sequence homology with each other and exhibit different metal ion requirements for their active sites, different subcellular localizations, and various substrate preferences [27].Horizontal gene transfer (HGT) was suggested as a mechanism for PhoX and PhoD among different marine bacteria [2], whereas gene duplication was proposed for the PhoA [28].

Hyperthermophiles as Promising Candidates for AP

The discovery of an AP with selective advantages such as high catalytic efficiency and stability provides excellent opportunities in a wide range of applications. Intriguingly, thermophilic organisms are found to be effective for their unique characteristics and stability. However, some thermophiles such as *Geobacillus thermodenitrificans* and *Meiothermus ruber* provide a moderately thermostable AP [29] and [30]. Unexpectedly, a recent report showed that mesophilic *Bacillus licheniformis* has relatively thermostable AP with its optimum activity at 50°C [31]. APs applications in biotechnology, clinical medicine, and molecular biology sometimes have limitations due to heat-lability and might be subjected to a significant effect on the restoration of the enzymatic activity [32]. Remarkably, several thermophiles such as *Thermus* species show considerable differences in their APs activities and they are promising candidates for thermostable AP production [33], yet some thermostable APs-producing strains exhibit variations in their thermostability and tolerance to alkaline pH *(Table 1)*.

Table 1. Optimum temperature and pH value of AP in thermophilic bacteria.

Microorganism	Tm[a] (°C)	pH[b]	Reference
Geobacillus stearothermophilus	60-70	9.0	***[1]***
Geobacillus caldoxylosilyticus	50	9.5	***[2]***
Geobacillus thermodenitrificans	65	9.0	***[3]***
Meiothermus ruber	60-65	11.0	***[4]***
Thermus aquaticus	75-80	9.2	***[5]***
Thermus caldophilus	80	11.0-11.5	***[6]***
Thermus thermophilus	80	10.2	***[7]***
*Thermus yunnanensis*sp. nov.	70-80	8.0-10.0	***[8]***

[a]Optimum temperature of AP.
[b]Optimum pH value of AP.

Hyperthermophiles are already being used as a reliable and effective source of many enzymes for medicinal and industrial applications [34], [35], [36], [37] and [38]. Consequently, these amazing microorganisms produce unique proteins that function under extreme conditions, comparable to those that are produced by both mesophiles and thermophiles. Therefore studies focusing on the discovery of new AP enzymes from hyperthermophilic archaea and bacteria might be the most suitable practice that may positively influence their activities for improved biological processes. Moreover, analyzing APs differences in members of hyperthermophiles will shed significant light on its mechanism of action. The finding of an AP that possesses variation in its catalytic function will help elucidate how these enzymes have evolved in their hosts. Interestingly, recent studies showed that analysis of catalytic promiscuity provided mechanistic and evolutionary insights of AP superfamily [39] and [40]. Archaeal sources of AP including members of hyperthermophiles are also summarized in this mini-review *(Supplementary Table 2)*.

Specific conserved regions in AP DNA or amino acids might make it suitable for differentiation of closely related species. One example is a sequence of the active site, which might be considered as a barcode for differentiation among thermophiles [41]. Interestingly, hyperthermophilic *Thermotoga* species might develop a possible evolutionary history of AP genomic region. *Thermotoga maritima* as one of the

hyperthermophile shows the highest percentage (24%) of gene sequences that are most analogous to archaeal genes. Eighty-one archaeal-like genes are clustered in 15 regions of the *T. maritima* genome that range in size from 4 to 20 kb. Additionally, the order of many of the clustered sequence regions is largely syntenic between *T. maritima* and some archaeal members, suggesting that these genes presumably were introduced to the *Thermotoga* lineage prior to speciation through a horizontal gene transfer mechanism [42] and [43].
To better understand the size discrepancies and other differences among the hyperthermophilic archaeal and hyperthermophilic bacteria, there are several instances in which tRNAs have been duplicated, yet the same active site residues exist and are found to be syntenic. The conservation of these tRNAs suggests that they are essential, yet it is surprising that synteny has been maintained considering a large number of apparent indels that have occurred among the AP ORFs *(Supplementary Figure1)*. Further comparative studies could provide clues to the mechanism by which genes can be gained and evolved while conserving the number and order of active site tRNAs.

Conclusions

To summarize, phosphatases are widely distributed among living organisms and excreted by many bacteria. Advances in development and applications of AP has proven to be a beneficial factor in molecular biology, immunoassays, and as an indicator of several bioprocesses. This work has provided exemplary report on the bacterial and archaeal sources of alkaline phosphatases. The above has further demonstrated that AP from hyperthermophiles is preferable in the field of applied microbiology and biotechnology. The productivity of the enzyme from hyperthermophiles sources is eminently worthwhile due to the thermostability and unique properties of the enzymes. Although AP sequences from a few members of hyperthermophilic archaea such as *Methanocaldococcus infernus* (Accession WP_013099938), *Thermococcus litoralis* (Accession WP_004070020), *Thermococcus barophilus* (Accession WP_056934741), *Thermococcus eurythermalis* (Accession AIU69308), *Thermococcus paralvinellae* (Accession WP_042682540) and hyperthermophilic bacteria *Thermotoga petrophila* (Accession NC_009486), have recently been made available in NCBI, availability of more hyperthermophiles means that detailed mechanistic investigations into APs and possibilities for understanding their roles in cells are feasible. Moreover, analyses of AP DNAs and amino acids provide significant information regarding phylogenetic relationships of hyperthermophiles. It is important that researchers focus attention on providing valuable information that will revolutionize the current knowledge on the evolution of hyperthermophiles.

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