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Characteristics of Cold Adapted Enzyme and Its Comparison with Mesophilic and Thermophilic Counterpart

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Abstract

The large proportion of earth's biosphere (>70%) has cold environments in the form of ocean depths, glaciers, polar and alpine regions; and are dominated by psychrophiles. The ability of psychrophiles to proliferate in cold habitat is due to its unique capacity to transcribe, translate and synthesize cold-adapted enzymes which catalyses biochemical reactions at low temperature. The most of psychrophilic enzymes optimize their high activity at low temperature at expense of substrate affinity and reduction in free energy barrier of the transition state. Psychrophilic enzyme has optimum activity relatively at lower temperature than mesophilic and thermophilic counterpart. We have observed that there is no definite relation between K_m value of psychrophilic, mesophilic and thermophilic enzymes. It has also been observed that psychrophilic enzymes denatured at relatively faster and at lower temperature than mesophilic and thermophilic counterpart. In this review, attempts have been made to compile up to date advances in the field of psychrophilic enzymes and compare its characteristics with mesophilic and thermophilic counterpart.

Keywords: Psychrophilic; Cold denaturation; Psychro-tolerant bacterium; Cold-shock proteins; Psychrophilic enzymes

Introduction

Microorganisms are cosmopolitan in nature and have adapted to different environmental conditions. Psychrophiles occupy a natural cold habitat in the form of ocean depths, glaciers, polar and alpine regions as well as manmade habitats in the form of refrigeration and freezer systems. The ability of psychrophiles to proliferate in cold habitat is due to its unique feature of cell membrane in terms of lipid constituents, transport of substrates across membrane, ability to rapidly synthesize cold shock proteins and cryoprotectants, to transcribe, translate and synthesize cold-adapted enzymes which catalyses biochemical reactions at low temperature. The psychrophilic enzymes have an increased structural flexibility which results in reduced activation energies and high catalytic efficiency [1]. It has been established that organisms that live in permanently cold environment harbour enzymes and proteins that function effectively in cold [2].

The high diversity among microbial psychrophilic enzymes, high yield, immense stability, high catalytic activity and economic feasibility highlighted its biotechnological potential and industrial applications [3]. The continue efforts are being made to identify a novel psychrophilic alkaline protease to replace mesophilic alkaline protease in detergent industries. The use of psychrophilic alkaline protease in detergent would be energy savings, reduce expensive heating steps and reduce adverse chemical reactions with cloth fibres at high temperatures. The use of cold active alkaline protease in detergent not only remove heat input in washing machine but also enable cleaning of cloth in bath tub. In this review attempts are being made to compare the characteristics of psychrophilic enzymes with its mesophilic and thermophilic homologs.

Cold adapted enzymes

The cold adapted enzymes have been evolved in psychrophilic bacteria as a strategy for low temperature adaptation [4-6]. The cold adapted enzymes are being characterized on the basis of their catalytic activity with respect to temperature and these enzymes exhibit optimum activity at <200°C [5,7-9]. A cold-adapted halophilic proteases has been isolated from deep sea psychrotolerant bacterium *Pseudoalteromonas* sp. SM9913. Previously, we have reported a cold active enzyme, t-RNA modification GTPase from psychrophilic *Pseudomonas syringae* Lz4W which has optimum activity around 12°C to 15°C [10]. A list of few cold adapted enzymes along with its mesophilic and thermophilic counterpart; and their optimum activities are represented in Table 1. Now, attempts are being made to identify psychrophilic alkaline protease which would have a potential application in detergent industries. The mesophilic enzymes are most common and are known to have optimum activity at 30°C to 40°C [11,12]. In contrast to psychrophilic enzymes and mesophilic enzymes, thermophilic enzymes generally have optimum activity around 45°C to 85°C [13,14]. The characteristics of cold-adapted enzymes are as follows:

1. They have optimum activity at around 4°C to 20°C.

2. They exhibit optimum activity at lower temperature than mesophilic and thermophilic counterpart.

3. They get denatured at relatively low temperature than mesophilic and thermophilic counterpart.

4. They have much lower stability at 37°C than mesophilic and thermophilic enzymes.

5. Enhance catalytic activity of cold adapted enzymes at low temperature is either due to increase K_{cat} or decreasing K_m or by changing both parameters.

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S.N.	Protein	Psychrophilic Organism (°C)	Mesophilic Organism (°C)	Thermophilic Organism (°C)	References
1.	t-RNA modification GTPase (TrmE)	12-15	37	70	[4]
2.	DNA ligase	4	30	45	[27]
3.	α-Amylase	4	20-30	72	[10,26]
4.	Lactate dehydrogenase	3-8	25-30	91	[12,13]
5.	Ornithine transcarbamylase	5	37	55	[31]
6.	Glucose 6-phasphate dehydrogenase	5	45-50	92	[28]
7.	Aspartate aminotransferase	7	35	60-85	[29, 30]
8.	Glutamate dehydrogenase	14	40	60	[8]
9.	Phosphoglycerate kinase	25	40	76	[15]
10.	Alkaline phosphatase	25	35	65	[9,11,14]

Table 1: Optimum activity for enzyme homolog.

S.N.	Protein	Psychrophilic Organism (K_m, μM)	Mesophilic Organism (K_m, μM)	Thermophilic Organism (K_m, μM)	References
1.	t-RNA modification GTPase (TrmE)	888.2	378.0	833.0	[4]
2.	DNA ligase	0.165	0.702	0.236	[27]
3.	α-Amylase	234	223	260	[10,26]
4.	Lactate Dehydrogenase	200	400	0.000027	[12,13]
5.	Ornithine transcarbamylase	1780	2400	100	[31]
6.	Glucose 6-phasphate dehydrogenase	20	19.4	110	[28]
7.	Aspartate aminotransferase	5820	21040	5000	[29,30]
8.	Glutamate dehydrogenase	2000	1390	1930	[8]
9.	Phosphoglycerate Kinase	370	800	1900	[15]
10.	Alkaline phosphatase	1020	2500	3040	[9,11,14]

Table 2: Comparison of K_m value for psychrophilic, mesophilic and thermophilic enzymes.

Comparison of K_m value of enzymes of psychrophilic, mesophilic and thermophilic origin

The types of interaction involved in enzyme-substrate complex formation play a major role in the variation of K_m value of cold-adapted enzymes. Temperature is one of the most important factor that governed the enzyme-substrate complex formation. Increase in temperature result in decrease in strength of enzyme-substrate complex interactions. It has been reported that most of cold-adapted enzymes have higher K_m than its mesophilic or thermophilic counterpart. We have analyzed K_m value of different enzymes homolog from psychrophilic, mesophilic and thermophilic microorganisms. The results indicate that there is no definite correlation exists between K_m value of enzymes homolog from psychrophilic, mesophilic and thermophilic origin. The comparative list of K_m value of 10 different psychrophilic, mesophilic and thermophilic enzymes are represented in Table 2. The K_m value of different psychrophilic enzyme may be higher or lower than its mesophilic or thermophilic counterpart. The reported K_m value for psychrophilic t-RNA modification GTPase is higher than its mesophilic or thermophilic homolog [4]. Whereas, reported K_m value for psychrophilic phosphoglycerate kinase and alkaline phosphatase is much lower than its mesophilic or thermophilic homolog [4,11,14,15]. The psychrophilic chitobiase isolated from an Antarctic marine bacterium has 25-fold lower K_m value than its homolog isolated from a mesophile [16].

Cold denaturation

The psychrophilic enzymes are prone to denature faster and at relatively lower temperature than its mesophilic and thermophilic counterpart. It has already been established that folding and refolding of cold-damaged proteins is crucial during cold adaptation. The low temperature exposure of bacteria induces preferential synthesis of some proteins known as cold-shock proteins (Csps) to regulate various metabolic and cellular processes at low temperature [17]. The cold-shock proteins are molecular chaperones (caseinolytic proteases, trigger factor, GroEL, DnaK and GroES) and prevent miss folding and aggregate formation of protein during cold shock [17-19]. The different molecular chaperones have already been implicated in cold adaptation in *Escherichia coli* [20-23]. The molecular chaperones prevent aggregate formation by binding to exposed hydrophobic region of unfolded polypeptide. Molecular chaperones interact and assist co-translational protein folding of nascent polypeptide [24,25]. It has already been reported that when molecular chaperone such as *cpn60* (encode GroEL) and *cpn10* (encode GroES) of Antarctic bacterium *Oleispira antarctica* are being expressed in trans in *Escherichia coli*. Then, *Escherichia coli* have achieved the ability to grow at 4°C (normally does not grow below 10°C), demonstrating the importance of chaperone-mediated proteins folding during growth at low temperature [10,26-31].

Conclusion

The psychrophilic microorganisms have abilities to grow and reproduce at low temperatures (0°C or sub-zero temperature). These psychrophiles produce cold-adapted enzymes, optimally active at relatively lower temperature than mesophilic and thermophilic counterpart. These microorganisms compensate the loss of enzyme activity at low temperature by decreasing free energy barrier of transition state. The cold-adapted enzymes optimize their high enzymatic activity at lower temperature than mesophilic and thermophilic counterpart. In evolutionary process, psychrophilic enzymes have acquired minor variation in amino acid sequence of their enzymes to achieve higher flexibility at low temperature than their mesophilic and thermophilic counterparts. Thus, cold adapted enzymes provide a strategy to psychrophilic microorganism for cold adaption at low temperature.

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