CATALYSIS AND LOW TEMPERATURE: MOLECULAR ADAPTATIONS

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Summary

Psychrophilic organisms have successfully colonized cold environments and are able to grow efficiently at subzero temperatures. At the enzymatic level, such organisms have to cope with the reduction of chemical reaction rates induced by low temperatures in order to maintain adequate metabolic fluxes. Cold-active enzymes produced by

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psychrophilic organisms display a high catalytic efficiency associated however with low thermal stability. These properties are beginning to become understood thanks to recent advances using X-ray crystallography, protein engineering and biophysical methods. Nevertheless, the rules governing protein adaptation to cold appear to be relatively diverse. Psychrophilic enzymes are not only of extraordinary interest at the fundamental level to understand the structure-function relationships but also to investigate the general problem of protein folding. Increasing our knowledge in these fields will clearly require further multidisciplinary approaches.

1. The Psychrophilic Context

It is generally assumed that extremophiles are localized in very peculiar and specific niches and therefore are scarce. In the case of psychrophiles, it should be realized that the main part of Earth is exposed to low temperatures if one considers the vast extent of permanently cold environments such as the Antarctic continent, the Arctic ice floes, and oceans surrounding them, the mountain and glacier regions including their caves or buried cracks, and last but not least, the deep-sea waters covering three-quarters of the planet surface. Moreover, when talking about psychrophiles, most people intuitively think of polar microorganisms. If a psychrophile is defined as an organism living permanently at temperatures close to the freezing point of water in thermal equilibrium with the medium, such definition includes *de facto* a large range of species: yeast which have the natural propensity to grow well in low temperature environments, algae which are responsible for the green to red color of the ice floe lower layer and of some snow fields, marine invertebrates that can develop at near-freezing temperatures and polar fish, which are the biggest psychrophiles that enjoy swimming between the ice needles. Of course, bacteria represent the largest psychrophilic community, and in fact bacterial life has been found to exist in the most amazing locations such as super-cooled cloud droplets in high altitude, or maybe below 3 500 m of ice in the Antarctic subglacial Lake Vostok (although this has been contested), just to mention those having temperatures well below 0 °C. These examples underline that psychrophiles are numerous, taxonomically diverse, and have a widespread distribution.

Most psychrophiles do not simply endure such extreme conditions but instead colonize, grow well, and reproduce successfully in these environments. In addition, they frequently possess a character of “irreversible adaptation” in the sense that low temperatures are not only the optimal conditions for growth but are also mandatory for sustained cell metabolism. For instance, polar fish do not survive slightly positive temperatures and psychrophilic microbial isolates, which have a larger temperature span, sometimes do not grow above 10 °C but in any case at 37 °C. Such deep adaptation of course requires a vast array of metabolic and structural adjustments at nearly all organization levels of the cell, most of them being discussed in this series. However, for enzymologists, the striking aspect of life at low temperatures is certainly the pronounced temperature dependence of most biochemical reactions. Low temperatures slow down and strongly inhibit chemical reaction rates catalyzed by enzymes, which are the elementary components of any cell metabolism. Nevertheless, some psychrophilic bacteria grown in a rich medium at 4 °C have doubling times close to that of *Escherichia coli* at 37 °C, in some instance yielding huge cell density, and the oxygen consumption of polar fish near 0 °C, reflecting the metabolic activity, is only

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slightly lower than that of temperate species. Considering these organisms perfectly evolving in near-freezing conditions, it has to be concluded that powerful mechanisms of thermal compensation are at work at the enzyme level. In addition, it should be also realized that activity at low temperature is a strong selection parameter at the evolutionary time scale. Accordingly, thermal compensation should have affected most, if not all, enzyme activities because a single "noncold adapted" enzyme is sufficient to impair the function of a given metabolic pathway and to restrain metabolic fluxes at levels incompatible with the cellular requirements.

The effect of temperature on chemical reaction rates is described in basic terms by the Arrhenius law relating the exponential rise of the reaction rate *k* to the temperature increase.

$$k = A e^{-Ea/RT} \quad (1)$$

where *A* is the pre-exponential term (related to steric factors and molecular collision frequency), E_a is the activation energy, *R* is the gas constant, and *T* is the absolute temperature. Accordingly, for a biochemical reaction catalyzed by an enzyme from a mesophile (a bacterium or a warm-blooded vertebrate), a drop in temperature from 37 °C to 0 °C results in a 20 to 80 times lower activity, and sometimes more. This is the main factor preventing the growth of nonadapted organisms, even the simplest microbial forms, at low temperatures.

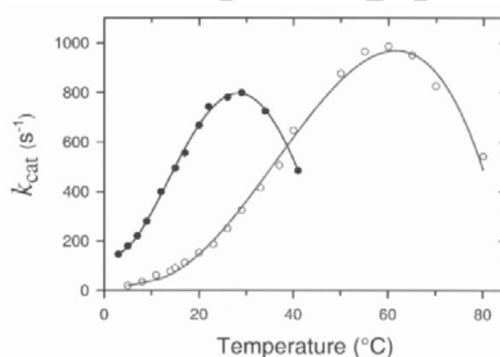

Figure 1. Effect of temperature on the activity of psychrophilic (•) and mesophilic (o) enzymes. This classical experiment, here on α-amylases, illustrates the four main adaptive traits of psychrophilic enzymes.

The effect of temperature on the activity of psychrophilic and mesophilic enzymes is illustrated in Figure 1. For both enzymes, three regions of the bell-shaped plot can be roughly delineated as the temperature is increased. The first region corresponds to the exponential rise of the activity with temperature. Thermal activation in this domain of the plot obeys the Arrhenius equation and these experimental data points can be used to calculate the thermodynamic parameters of activation. The second region spans from the inflexion point in the exponential rise to the activity maximum. In this domain, thermally induced alterations of the enzyme catalyst itself compete with thermal activation of the reaction. Active site distortion or excess of vibrational motion at the reactive side chains are examples of factors affecting here the enzyme activity. Finally,

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the third region corresponds to the activity decay at high temperature, when enzyme inactivation dominates as a result of unfolding or aggregation. The comparison of the effect of temperature on the activity of psychrophilic and mesophilic enzymes in Figure 1 reveals four basic features which will form the body of the next sections.

- In order to compensate for the slow reaction rates at low temperatures, psychrophiles synthesize enzymes having an up to tenfold higher specific activity in this temperature range. This is in fact the main physiological adaptation to cold at the enzyme level.
- The effect of temperature on psychrophilic enzymes is less pronounced. For instance, increasing the temperature by 10 °C produces a 1.6-times higher activity for the cold-active enzyme illustrated (this is the so-called Q_{10} value) compared with a 2.3-times higher reaction rate for the mesophilic enzyme. This is the consequence of smaller values of the activation energy E_a for cold-adapted enzymes, which render chemical reactions relatively less temperature-dependent.
- The temperature for apparent maximal activity for cold-active enzymes is shifted towards low temperatures, reflecting the weak stability of these proteins and their unfolding and inactivation at moderate temperatures.
- Finally, the adaptation to cold is not perfect. It can be seen in Figure 1 that the specific activity of the psychrophilic enzymes at low temperatures, although very high, remains generally lower than that of the mesophilic enzymes at 37 °C.

A recurrent aspect of the molecular adaptations to catalysis at low temperatures is the lack of dogma: each enzyme analyzed so far adopts its own strategy to adjust the catalytic parameters via discrete structural modifications but all strategies converge to the same goal—to achieve a sustainable enzyme activity in cold environments.

2. Kinetic Optimization of Cold-Active Enzymes

2.1. Cold-Active Enzymes

Both k_{cat} and K_m are fundamental kinetic parameters characterizing an enzymatic reaction. As mentioned, improving the turnover number k_{cat} offsets the inhibitory effect of low temperatures on reaction rates and therefore provides adequate raw metabolic activity to the growing organism. This strategy is not specific to psychrophilic organisms but is also demonstrated by enzymes from ectothermic species when compared to homologues from warm-blooded animals. Adaptation in k_{cat} values of psychrophilic enzymes represents an extreme trend of thermal compensation strategies. The turnover number k_{cat} reflects the catalytic potential of enzyme-substrate complexes at saturating substrate concentration. However, in the case of regulatory enzymes that catalyze their reaction at substrate concentrations close to the K_m value, the specificity constant k_{cat}/K_m ,or catalytic efficiency, is generally a better indication of catalytic evolution than k_{cat} alone. Optimization of k_{cat} is presumably the only relevant parameter for enzymes buried in the substrate, such as digestive enzymes or enzymes secreted by microorganisms growing within organic debris used as substrate source. Alternatively, extracellular enzymes from marine or freshwater microbes should have highly optimized K_m values if they are secreted in the substrate-poor liquid medium, whereas regulatory intracellular enzymes should optimize the k_{cat}/K_m ratio. These examples

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illustrate that it is often difficult to decide which parameter is to be optimized in the context of cold adaptation. Inspection of the available data for cold-adapted enzymes indicates that they optimize the k_{cat}/K_m ratio by increasing k_{cat}, decreasing K_m or by changes in both k_{cat} and K_m. Even from the limited set of data, it appears that optimization of the catalytic parameters is reached via various ways and this should be related to the enzyme targeting (intra- or extracellular), its physical state (soluble, cell-bond), its physiological function and the nature, solubility, or availability of substrates. A possible bias introduced in the current view of kinetic optimization of cold-active enzymes may be found in the use of chromogenic substrates. Small synthetic substrates are easy to handle and provide more reliable results but may have, and in some cases do have distinct binding modes and requirements when compared to natural substrates, especially if the reaction temperature is varied. Kinetic determinations using natural substrates is therefore highly desirable but is technically complex for hydrolases or depolymerases for instance, acting on large macromolecular substrates such as polypeptides, polysaccharides, or lipids. Some sophisticated techniques such as microcalorimetry are currently developed and will certainly provide reliable data in future works. Another bias arises from the fact that the Michaelis parameter can be a complex function of individual kinetic constants. Nevertheless, the K_m values recorded for most cold-active enzymes studied so far may be regarded as the overall dissociation constant for all the enzyme-bound species and reflect the substrate binding affinity (even if K_m does not match rigorously the dissociation constant for ES).

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Biographical Sketches

Georges Feller was born in Arlon, Belgium, in February 1958. He obtained a PhD in Biomedical Sciences from the University of Liège in 1987. He is now Senior Lecturer for the Department of Life Sciences, attached to the Laboratory of Biochemistry, University of Liège, Belgium.

Georges Feller's research activities started with the study of metabolic and structural adaptations of the Antarctic icefish to the lack of hemoglobin, focused on the heart as an unusual model of hypoxia tolerance. This work, involving three Antarctic expeditions, prompted him to use bacteria as a source of cold-active enzymes. The research activities of the Laboratory of Biochemistry is now mainly devoted to the molecular adaptations of these psychrophilic enzymes to catalysis at low temperatures and to the biotechnological potential of these enzymes and of the parent microorganisms.

Charles Gerday received a BSc degree in chemistry in 1961 from the University of Liège in Belgium and was appointed research assistant in the Department of Biochemistry, Faculty of Medicine in the same university. In 1964, he became lecturer at the Department of Biochemistry of the University of Montreal (Canada) and got a PhD degree in 1967 with a thesis focusing on the synthesis of tritium labeled optically active amino acids. Back in Belgium in 1967, he was appointed successively lecturer and senior lecturer in the Department of General Biology at the University of Liège in which his research explored the regulation of muscle contraction by calcium-binding proteins. In 1972, he was a postdoctoral research fellow in the Laboratory of Molecular Biophysics at the University of Oxford (UK). From 1981, his research activities were recentered on the molecular adaptation of enzymes produced by cold-adapted organisms such as fish and bacteria originating from the Antarctic. He participated in several expeditions in the Antarctic. In 1988, he was appointed Professor of Biochemistry and Head of the Laboratory of Biochemistry at the University of Liège. He is the author of 160 original publications and is chairman of the Belgian Biophysical Society since 1994.

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