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Mini Review

Biocatalysts: Beautiful creatures

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ABSTRACT

The chemical industry has come under increasing pressure to make chemical production more eco-friendly and independent to fossil resources. The development of industrial processes based on micro-organisms can especially help to eliminate the use or the generation of hazardous substances and can support the transition from dependence on fossil resources towards real sustainable and eco-safety industrial processes. The biocatalysts are the best solution given by nature that can be used to improve some biotechnological applications. In this research review, we report some peculiar properties of biocatalysts, implicated in a range of metabolic pathways and biotechnological tools.

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1. Introduction

Catalysis is a process that increases the speed with which a reaction reaches equilibrium. Since the reaction rate depends on the free energy of activation, a catalyst causes the decrease of the energy barrier and accelerates the catalytic stage [1]. Enzymes are molecules which can reduce the activation energy of reaction and thus accelerate the biochemical reactions that take place in the cell. Therefore, the functionality of a cell, its reproduction requires that all reactions occur in coordinated and controlled synergy. The enzymes are thus key players in life [2].

2. Biocatalysts: activity assay and classification

2.1. Enzymatic activity

The catalytic activity of an enzyme is measured by determining the increase in the reaction rate under defined conditions, the difference between the turnover of the catalyzed reaction and uncatalyzed reaction in a specific time interval. Normally, reaction rates are expressed as the change in product concentration per unit of time. Since the enzymatic activity is independent of the volume, the unit used for enzymes is usually turnover per unit time, expressed in katal (kat, mol s^{-1}). However, the international unit U is still more commonly used (μmol turnover min^{-1}; 1 U = 16.7 nkat).

2.2. Enzyme classes

More than 2000 enzymes are currently known. A system of classification has been developed that takes into account both their reaction and substrate specificity. Each enzyme is entered in the enzyme catalog with a four-digit enzyme commission number (EC number). The first digit indicates membership of one of the six major classes. The next two indicate subclasses and subsubclasses. The last digit indicates where the enzyme belongs in the subsubclass. For example, lactate dehydrogenase has the EC number 1.1.1.27 (class 1, oxidoreductases; subclass 1.1, CHOH group as electron donor; subsubclass 1.1.1, NAD (P^+) as electron acceptor). Enzymes with similar reaction specificities are grouped into each of the six major classes: (1) the oxidoreductases catalyze the transfer of reducing equivalents from one redox system to another; (2) the transferases catalyze the transfer of other groups from one molecule to another. Oxidoreductases and transferases generally require coenzymes; (3) the hydrolases are also involved in group transfer, but the acceptor is always a water molecule; (4) the Lyases (also named as "synthases") catalyze reactions involving either the cleavage or formation of chemical bonds, with double bonds either arising or disappearing; (5) the isomerases move groups within a molecule, without changing the gross composition of the substrate and (6) the ligases (also named synthetases) are energy-dependent and are therefore always coupled to the hydrolysis of nucleoside triphosphates. They catalyze the ligation reactions.

The enzymes thus have characteristics that make them vital molecules within the living world. Indeed, two closely related properties make powerful enzymes: specificity of substrate

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binding associated with the optimal arrangement of their catalytic site. The optimal arrangement of the provision of liaison groups and catalytic groups is the result of centuries of evolution. Those biocatalysts operate on a variety of mechanisms that fall into six categories [2]: (1) acid–base catalysis; (2) covalent catalysis; (3) metal ion catalysis; (4) electrostatic catalysis; (5) catalysis by proximity effect and orientation and (6) catalysis by preferential binding to the complex of the transition state.

3. The cellulose hydrolysis requires a multienzymatic system: cellulases

Cellulose represents the most abundant carbohydrate substance in nature. It is a bio-polymer of glucose units related by β1, 4 glucosidic linkages [3]. Its degradation requires a multi-enzymatic system composed of three enzymes: the endoglucanases which randomly attack cellulose in amorphous zones releasing cellooligomers; the cellobiohydrolases which liberate cellobiose from reducing and none reducing ends and finally the β-glucosidases which hydrolyze cellobiose and other cellooligosaccharides into glucose units. β-glucosidases (BGL) are present in all living organisms (bacteria, archaea, and eukarya) and perform wide range of functions [4]. In bacteria and fungi, β-glucosidases play an important role in cellulose degradation, that is, cellobiohydrolases and endoglucanases catalyze the cellulose hydrolysis to produce cellobiose and short cellooligosaccharides, which are ultimately hydrolyzed to glucose by β-glucosidase [4,5]. In the process of saccharification of lignocellulosic biomass, cellooligosaccharides and cellobiose are often produced by the limited hydrolysis of cellulose, leading to inefficient ethanol fermentation. The hydrolytic product, glucose, is the best substrate for ethanol production.

In addition, β-glucosidases have potential roles in various fields such as food industries, pharmacology, cosmetic, and valorisation of some products, owing the properties of this enzyme to convert and to synthesize bio-molecules of high added value [4].

Therefore, many trials have been carried to produce highly efficient BGL and more precisely "cellobiase". Most BGLs have a lower affinity and conversion efficiency for cellobiose [6]. *In vivo*, the last one represents the physiological substrate of the most β-glucosidases. However, *in vitro*, this ability becomes a non apparent criterion; the ability of β-glucosidase to split cellobiose gives him the denomination of cellobiase, if not, it is named aryl/alkyl β-glucosidase [6].

The cellulasic systems are generally inducible and repressible. Cellulose and their derivatives (CMC, HEC) represent their most inductors. In addition, there are other inductors like disaccharides (sophorose, gentiobiose, lactose) and monosaccharides (xylose). However, glucose constitutes the most popular repressor of hydrolases, especially cellulases [5].

Like various biocatalysts, the inhibition of β-glucosidases has been the subject of several studies [7]. We mentioned the role played by the hydroxyl groups of glucose in β-glucosidase inhibition. We have demonstrated the importance of the distribution of these hydroxyls, not only in the inhibition efficiency but also in the inhibition severity. The type, the nature of the linkage and the state of cyclization of the sugar influence the presence and the levels of inhibition of those biocatalysts [7].

According to the thermodynamics of inhibitor binding, the increased affinity for the transition state over the ground state by an enzyme is primarily derived from enthalpy. Therefore, it could be argued that inhibitors which truly mimic the transition state should also bind with large enthalpic contributions. Measurements were made using various strategies and methods such as ITC, which gives a direct read-out on the affinity of the inhibitor, stoichiometry of binding between inhibitor and enzyme and the enthalpic contribution to binding, from which the Gibbs free energy and entropy can be calculated. All of the inhibitors examined in this case displayed a negative and therefore favorable enthalpic contribution to binding [1].

4. The cellobiose dehydrogenases (CDH)

CDH is an extracellular redox enzyme produced by various wood degrading fungi and ascomycetes fungi. It catalyzes the oxidation of cellobiose, higher water soluble cellodextrins, lactose and mannobiose to their corresponding lactones. Efficient and complete degradation of woody plant cell walls is generally ascribed to certain basidiomycetes collectively referred to as white rot fungi [8]. Commonly associated with woody debris and forest litter, these fungi can depolymerize, degrade, and fully mineralize all cell wall polymers, including cellulose, hemicelluloses and the normally rather recalcitrant polymer lignin. Such plant cell wall deconstruction requires complex extracellular oxidative and hydrolytic systems. Mechanistic aspects of the degradative processes remain uncertain, but the field has attracted interest because woody feedstocks are increasingly viewed as potential sources for high-value low-molecular-weight products. The involvement of low-molecular-weight, diffusible oxidants, especially hydroxyl radicals, has long been suspected.

Repression and inhibition of biocatalysts can be affected by other factors. In this case, we demonstrated that production of β-glucosidase (bglG) is negatively affected by CDH. Indeed, on cellobiose-based medium, bglG time course production decreases drastically. This effect is the consequence of the release in the culture medium of a secondary metabolite (Δ-gluconolactone) that is known for its inhibitory effect on β-glucosidase activity. So we can say that inhibition of β-glucosidases and enzymes in general may be influenced by the presence of an enzyme or a metabolite secreted during kinetics production.

5. Bifunctionality's concept of some enzymes: evolutive aspect and necessity to modulate metabolic pathways

5.1. Fructose-1,6-bisphosphate aldolase/phosphatase

Some enzymes are referred to as being bifunctional; they consist of either two distinct catalytic domains or a single domain that displays promiscuous substrate specificity. Thus, one enzyme active site is generally responsible for one biochemical reaction. In contrast to this conventional concept, archaeal fructose-1,6-bisphosphate (FBP) aldolase/phosphatase (FBPA/P) consists of a single catalytic domain, but catalyzes two chemically distinct reactions of gluconeogenesis: (1) the reversible aldol condensation of dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (GA3P) to FBP; (2) the dephosphorylation of FBP to fructose-6-phosphate (F6P). Thus, FBPA/P is fundamentally different from ordinary enzymes whose active sites are responsible for a specific reaction. However, the molecular mechanism by which FBPA/P achieves its unusual bifunctionality remains unknown. Here we report the crystal structure of FBPA/P at 1.5-Å resolution in the aldolase form, where a critical lysine residue forms a Schiff base with DHAP. A structural comparison of the aldolase form with a previously determined phosphatase form revealed a dramatic conformational change in the active site, demonstrating that FBPA/P metamorphoses its active-site architecture to exhibit dual activities. Thus, these results expand the conventional concept that one enzyme catalyzes one biochemical reaction [9].

This concept of bifunctionality observed with some enzymes is also implicated with the hemicellulases such as xylanases. These categories of enzymes follow various bifunctionality aspects.

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