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Effect of crowding by Dextrans in enzymatic reactions

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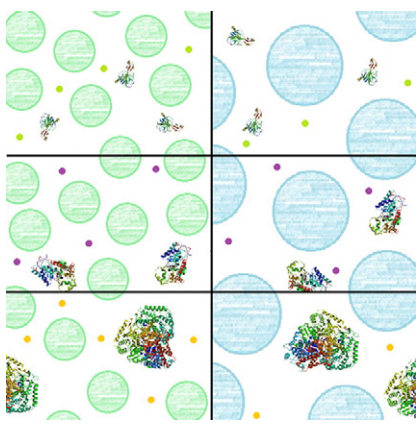
HIGHLIGHTS

- We studied and compared the velocity rates of three reactions in different crowded media.
- The crowded media is produced by Dextran of different concentrations and sizes.
- The volume occupied by the crowding agent plays an important role on tiny enzymes reactions.
- The rate of the reactions of large enzymes depends on both the occupied volume and dimension of the crowding agent.

GRAPHICAL ABSTRACT



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ABSTRACT

The interior of the living cell is highly concentrated and structured with molecules that have different shapes and sizes. Almost all experimental biochemical data have been obtained working in dilute solutions, situations which do not reflect the in vivo conditions. The consequences of such crowding upon enzymatic reactions remain unclear. In this paper, we have studied and compared the initial velocity of the hydrolysis of N-succinyl-L-phenyl-Ala-p-nitroanilide catalyzed by alpha-chymotrypsin, the oxidation of ABTS by H_2O_2 catalyzed by HRP and the oxidation of NADH in presence of pyruvate catalyzed by LDH. These reactions were chosen as model enzymatic processes occurring in different in vitro crowded media. The systems crowding has been built by introducing Dextran of several concentrations and sizes. Our results indicate that the volume occupied by the crowding agent, but not its size, plays an important role on the initial velocity of reactions involving tiny enzymes. However, the enzyme size is another important factor influencing the velocity of the reactions of large enzymes occurring in Dextran crowded media. In this situation, the reaction initial velocity depends on both occupied volume and dimension of the crowding agent that is present in the reaction media.

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1. Introduction

Molecular crowding is by now an extensively studied issue in the micro world of cells. The intracellular fluid is a complex matrix in which a dense mixture of macromolecules and solutes is present up to a large percentage (40%) of total cellular volume [1]. Molecular crowding is one of the cell features that accounts for the distinct way biochemical reactions progress in vivo than in laboratory assays. Well-mixed dilute solutions, with less than 1 mg/mL macromolecules content, represent the typical environment for in vitro experiments [2]. Various crowding effects have been put into evidence over the last decades related to changes of diffusion rate [3–9], protein folding, self-association and protein binding enhancement [10–23], enzymatic activity alteration [24–27] and reaction kinetics modification [28–30].

In recent years, the effects of crowding on enzyme catalysis have been explored by different works, excellently depicted by Zhou et al. [9] and Noris and Malys [24]. Most of them indicate that the effect of excluded volume due to the presence of crowding agent is the major player in modulating enzymatic behavior. From the very first study on enzymatic reactions in crowded media developed by Laurent in 1971 [31], the presence of macromolecules had been revealed to produce a moderate decrease in the apparent Michaelis–Menten (MM) constant, K_m. Some years later, Minton and Wilf [10], studying the enzymatic processes of glyceraldehyde-3-phosphate dehydrogenase, predicted that the initial velocity of an enzyme-catalyzed reaction will decrease if the concentration or the size of the crowding agent is increased. In other words, the excluded volume produces a decrease of both MM constant, K_m, and catalytic constant, k_{cat}, when the enzymatic reaction follows the Michaelis–Menten mechanism. However, most subsequent studies reported that a high concentration of neutral polymers only had a moderate influence on enzyme reactions. Briefly, a slight decrease of K_m is frequently found, regardless of the properties of the crowding agent [32–37]. However, the effect of the crowding agent on k_{cat} is diverse: in some cases, k_{cat} increases [24,32,36–39], whereas in other cases it decreases [10,34,35,39]. It should be noted that these quantitative studies used different polymers as crowding agents but only of a fixed size (usually small). The effect of the crowding agent size and shape on enzymatic reactions has been also analyzed [40]: large obstacles with irregular shape reduce the reaction velocity, but large and compact obstacles have minor effects on it. In their work, Homchaudhuri and coworkers [40] studied the hydrolysis of p-nitrophenyl phosphate catalyzed by alkaline phosphase as model reaction and macromolecular crowding was mimicked using inert polymers such as Dextrans and Ficolls of molecular weights ranging from 15 to 500 kDa. They results revealed a steeper decrease of the reaction velocity as a function of fractional volume occupancy with larger Dextrans compared with smaller Dextrans. In the presence of 20% Dextran (w/w), a typical concentration of macromolecules inside the cytoplasm, the reaction rates were 2-folds slowed by smaller Dextrans and between 5- or 7-folds by larger Dextrans. Ficolls of similar size to Dextran had a comparatively smaller influence on the reaction rates (2-folds). In other words, the extent of the crowding effect may strongly depend on both concentration and relative size of the crowding species.

Therefore, our working group has investigated the influence degree of both factors on the initial velocity of different enzymatic processes. First, we studied the crowding effect of Dextrans of various molecular weights on the reaction initial velocity of the hydrolysis of N-succinyl-L-phenyl-Ala-p-nitroanilide catalyzed by alpha-chymotrypsin [41]. Our results pointed out that the volume occupied by Dextran, independent of its size, had an important role on the initial velocity of this reaction. A v_{max} decay and a K_m increase were obtained when bigger Dextran concentration was used in the sample. The rise of K_m could be attributed to a slower diffusion of the protein [42] due to the presence of crowding, whereas the decrease in v_{max} could be explained by the effect of mixed inhibition by product, which is enhanced in crowded media.

Second, we have studied the kinetics of the oxidation of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) by hydrogen peroxidase (H_2O_2) catalyzed by horseradish peroxidase (HRP) as a model enzymatic reaction occurring in different in vitro crowded media [43]. The crowding was generated by Dextran of various concentrations and dimensions. Our results revealed that the reaction initial velocity was also significantly influenced by the crowding agent excluded volume, regardless of its size. Both v_{max} and K_m decayed along with the growth of obstacle concentration. Concerning this particular case, the presented data suggest an activation control of the enzymatic reaction in the studied system. In other words, the catalytic constant (k_{cat}) brings a significant contribution as a result of the environmental surroundings influence. This contribution may be the consequence of one of the following aspects or their additive effect: the rise of the ratio value between the activity coefficients of natural enzyme and enzyme–substrate complex due to the presence of crowding agents; the enhancement of water chemical activity favored by highly crowded solution; and the crowding-induced conformational change of the enzyme active site.

Besides the hydrolysis of N-succinyl-L-phenyl-Ala-p-nitroanilide catalyzed by alpha-chymotrypsin and the oxidation of ABTS by H_2O_2 catalyzed by HRP, we have also studied the oxidation of NADH in the presence of pyruvate catalyzed by lactate dehydrogenase (LDH) [Balcells et al., unpublished work]. When choosing these particular reactions as model processes, several reasons have been put forth: first, they are well-known reactions; second, these reactions can be easily monitored by UV-spectroscopy; and third, all reactions are accompanied by a minimal change in the excluded volume. In fact, the substrate and product molecules of each investigated system are tiny compared to the size of involved proteins and crowding agent. Hence, the effect of both molecules (i.e. substrate and product) on the excluded volume can be neglected. As a result, the effect of macromolecular crowding on these particular reactions can be mainly interpreted in terms of the crowding agent presence.

Moreover, the usage of these specific proteins in our investigation also presents some advantages: on the one hand, the absence of known interactions with Dextran and on the other hand, the size of the proteins (alpha-chymotrypsin of Mw = 25 kDa, HRP of Mw = 42 kDa and LDH of Mw = 140 kDa). The protein dimensions are within the size range of the crowding agent (Fig. 1).

The ideal crowding agents must provide nature-like microenvironments. The best approach to mimic macromolecular crowding would be using cell extracts [1]. However, the heterogeneity of chemical, geometrical, and physical properties of cell extracts makes difficult the experimental data collection and their interpretation. Therefore, a variety of purified macromolecules were used as crowding agents in most experimental studies of crowding effects [9,25]. Among these purified macromolecules, Dextran is one of the most commonly used since it is inert, water-soluble and resembles more closely the types of macromolecules encountered in the natural state of the cell [9]. It is also readily available in various sizes and large quantities [44]. Dextran is a very flexible polymer, represented by a random coil with spherical shape in aqueous solution, especially when present in high concentration [45]. Due to these reasons, Dextrans with molecular weight ranging from 5 to 410 kDa are used to generate the crowding complexity.

Based on the previous findings, the present paper aims at assessing and comparing, in the interest of completeness, the way in which the kinetics of three enzyme-catalyzed reactions is modulated while they are carried out in vitro, under similar crowding conditions. The systems are crowded with Dextran of various sizes and concentrations. The comparative analysis proposed here tries to emphasize the crowding induced effects on enzymatic reaction initial velocities in systems targeting the complexity of natural cellular media.

2. Materials and methods

Alpha-chymotrypsin (E.C. 3.4.21.1) from bovine pancreas type II (60 U mg^{-1}), Peroxidase (E.C. 1.11.1.7) from horseradish