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Effect of partitioning equilibria on the activity of β-galactosidase in heterogeneous media

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Abstract

We had demonstrated that membrane adsorption or penetration differentially modulated β-Galactosidase (β-Gal) activity against soluble substrates (Coll. and Surf., 24, 21, 2002). In a heterogeneous media, not only the enzyme but also the rest of the chemical species taking part in a chemical reaction would eventually interact with the available surfaces. The aim of the present work was to investigate if, in addition to changes in the intrinsic mechanism of the reaction at the lipid–water interface, the kinetics of enzyme-catalyzed reactions could be significantly affected by the partitioning of the substrate (*ortho*-nitro-phenyl galactopyranoside (ONPG)), the product (*ortho*-nitro-phenol (ONP)) and the enzyme (*E. coli* β-Gal) towards the membrane. Multilamellar vesicles of sPC were used as model membranes. Membrane–water partition coefficients ($P_{m/w}$) were determined according to the theory and methodology developed previously (J. Neurosci. Meth. 36, 203, 1991). The values of $P_{m/w}$ obtained ($P_{ONPG}=0$, $P_{ONP}=50$ and $P_{\beta\text{-Gal}}=118$) were applied to a two-compartment model, which assumed a free access of the substrate to the enzyme and a nucleophile-like activatory effect exerted, within the membrane compartment, by the lipid–water interface. This model: (i) reproduced the lipid concentration-dependence we had observed previously in V_{max}, (ii) predicted the values of $k_4=3.54\times10^7\ s^{-1}$ and the extinction coefficient of the aglycone in the membrane phase, 4012 $M^{-1}\ cm^{-1}$, with $p<0.0001$ and $p<0.02$, respectively, as well as for $P_{\beta\text{-Gal}}=117$ (which was poor ($p=0.6716$) but gave a numerical value within the same order of magnitude that the experimental value) and (iii) emphasized the importance of the more efficient reaction mechanism in the membrane phase compared with that in the aqueous phase ($k_4 \gg k_3$).

Keywords: β-galactosidase; O-nitrophenyl-β-D-galactopyranoside; Partition coefficient; Phospholipids vesicles; Enzyme kinetics

1. Introduction

In previous works we demonstrated that beta-Galactosidase from *Escherichia coli* (β-Gal), which is a soluble protein, was able to interact with biomembranes. The type of interaction (adsorption or penetration) depended on the membrane composition, organization and topology, and differentially modulated the activity of the enzyme toward a soluble substrate [1,2]. However, in complex systems like membrane suspensions, not only the enzyme but also the other chemical species participating in the reaction could eventually partition or be adsorbed to the lipid–water interface. Many studies of enzyme kinetics in reverse micellar solutions addressed a similar problem ([3] and Refs. therein). However, in those cases the enzyme was totally associated to the micelles while the substrate was partitioned between the micelles and the external solvent. Our previous findings regarding the modulation of β-Gal [1,2] suggested that in the presence

Abbreviations: *A*, absorbance; *C*, concentration; *D*, dielectric constant; ε, extinction coefficient; f_m, volume fraction of membrane phase; f_w, volume fraction of water phase; MLV, multilamellar vesicle; ONPG, *ortho*-nitro-phenyl-β-D-galactopyranoside; ONP, *ortho*-nitro-phenol; ONPx, *ortho*-nitro-phenoxide; $P_{o/w}$, octanol–water partition coefficient; $P_{m/w}$, membrane–water partition coefficient; rpm, revolutions per minute; sPC, soybean phosphatidylcholine; V_{max}, maximal velocity; λ, wavelength; *X*, volume fraction of water phase trapped within the pellet.

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of lipid–water interfaces, in addition to partition equilibria and diffusional barriers, changes in the intrinsic mechanism of the reaction, would contribute to the modulation of the kinetics of substrate hydrolysis.

In order to get deeply in this problem, in the present work we determined the values of the membrane–water partition coefficients ($P_{m/w}$) of all the chemical species that participate in the reaction of hydrolysis of *o*-nitro-phenyl-β-D-galactopyranoside (ONPG) catalyzed by β-Gal.

The effect of nonzero $P_{m/w}$ values on the reaction kinetics was modeled and the results were compared with our previous experimental results of the hydrolysis of ONPG catalyzed by β-Gal in the presence of phospholipid bilayers [1].

2. Theory

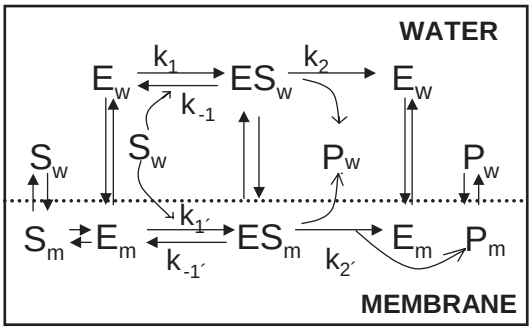
2.1. Modulation of β–gal activity in a heterogeneous system

A theoretical model was developed in order to describe the effects of PC multilamellar vesicles (MLVs) on the values of maximal velocity (V_{max}) of the hydrolysis of *ortho*-nitro phenyl β-D-galactopyranoside (ONPG) to *ortho*-nitro-phenol (ONP), catalyzed by the enzyme β-Gal.

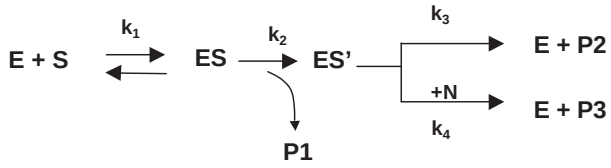
2.1.1. The model

The system consists of two compartments: the membrane phase and the water phase (see Scheme 1). The reaction can occur in both compartments with the following characteristics:

a) The reaction product and the enzyme are able to partition from the aqueous phase to the membrane and vice versa (eventual hysteresis between the adsorption and desorption processes, is not considered).
b) The reaction mechanism is different in each phase, resulting in differences in the rate constants. This can be due to the possible occurrence of a nucleophilic-like effect (see Scheme 2) [4] induced by the polar head group of the membrane phospholipids or by the water structured at the membrane surface proposed in previous works [1,2].



Scheme 1. *E*, ES, *S* and *P* represent the enzyme, enzyme–substrate complex, substrate and the product respectively. *k* = rate constants. Subindexes w and m refer to the water and the membrane phases, respectively.



Scheme 2. *P*1, ES′, *P*2, *P*3 and N, represent the aglycone, glycosylated enzyme, galactose, galactose derivative and the nucleophile, respectively. Other symbols were described in the text.

2.1.1.1. Mass balance. The total amount of each chemical species in the system is the sum of the amounts in each phase

$$C_T \cdot V_T = C_m \cdot V_m + C_w \cdot V_w \quad (1)$$

where C, V and the subindexes T, m and w refer to concentration, volume, total system, membranous phase and water phase, respectively.

The partition coefficient between the membrane and the aqueous phase is:

$$P = C_m / C_w \quad (2)$$

The sum of the fractions of the total volume corresponding to membrane ($f_m = V_m / V_T$) and water ($f_w = V_w / V_T$) phases correspond to

$$f_w + f_m = 1 \quad (3)$$

From the Eqs. (1), (2) and (3):

$$C_w = \frac{C_T}{f_w + P \cdot f_m} \quad \text{and} \quad C_m = \frac{C_T \cdot P}{P \cdot f_m + f_w} \quad (4)$$

From the mass of solute partitioned in each phase

$$m_w = C_w \cdot V_w \quad \text{and} \quad m_m = C_m \cdot V_m \quad (5)$$

the contribution of the amount of mass in the membranous and aqueous phases to the global concentration in the whole system can be calculated:

$$C'_w = \frac{m_w}{V_T} \quad \text{and} \quad C'_m = \frac{m_m}{V_T} \quad (6)$$

Then, combining Eqs. (3), (4) and (5), solving for C_T and C_m and replacing into Eq. (6) we got:

$$C'_w = \frac{C_T \cdot f_w}{P \cdot f_m + f_w} \quad \text{and} \quad C'_m = \frac{C_T \cdot P \cdot f_m}{P \cdot f_m + f_w}. \quad (7)$$

From the kinetics of releasing of the aglycone (ONP in this case) represented by *P*1 in the Scheme 2, k_{cat} in each phase results [4]:

$$k_{cat} = \frac{k_2 \cdot (k_3 + k_4 \cdot N)}{k_2 + k_3 + k_4 \cdot N}$$

where N represents the concentration of a nucleophile.