Biophysical Chemistry 145 (2009) 126–134

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

How hyaluronan–protein complexes modulate the hyaluronidase activity: The model

Jean-Claude Vincent *, Hélène Lenormand

Laboratoire "Polymères, Biopolymères, Surfaces", FRE 3101 CNRS – Université de Rouen, 76821 Mont-Saint-Aignan cedex, France

ARTICLE INFO

Article history:
Received 4 August 2009
Received in revised form 22 September 2009
Accepted 22 September 2009
Available online 1 October 2009

Keywords:
Hyaluronan
Hyaluronidase
Polysaccharide–protein complex
Enzyme activity
Modeling

ABSTRACT

Hyaluronan (HA) is the substrate of hyaluronidase (HAase). In addition, HA is able to form electrostatic complexes with many proteins, including HAase. Experiments have shown the strong inhibition of the HA hydrolysis catalyzed by HAase when performed at low HAase over HA concentration ratio and under low ionic strength conditions. Non-catalytic P proteins are able to compete with HAase to form electrostatic complexes with HA and thus to modulate HAase activity. We have modeled the HA–HAase–P system by considering the competition between the two complex equilibria HA–P and HA–HAase, the Michaelis–Menten type behavior of HAase, and the non-activity of the electrostatically complexed HAase. Simulations performed by introducing experimental data produce a theoretical behavior similar to the experimental one, including all the atypical phenomena observed: substrate-dependence, enzyme-dependence and protein-dependence of HAase. This shows that our assumptions are sufficient to explain the behavior of the system and allow us to estimate unknown parameters and suggest new developments.

1. Introduction

Hyaluronidase (HAase) is an enzyme involved in several fundamental biological phenomena, such as fertilization and cancer [1–4]. Its natural substrate is hyaluronan (HA) which is hydrolyzed into HA oligosaccharides. HA is a linear high molar mass polysaccharide composed of D-glucuronic acid-β(1,3)-N-acetyl-D-glucosamine disaccharide units linked together through β(1,4) glycosidic bonds. It is a major constituent of the extracellular matrix (ECM) of vertebrates and is involved in many biological processes, such as cellular adhesion, mobility and differentiation processes [5–9]. Its properties are a function of its chain length: HA oligosaccharides (4 to 25 disaccharides) have an angiogenic action [9–11] contrary to native HA [12]. In the ECM, the action of HAase produces HA oligosaccharides which favor the production of new blood-vessels facilitating the development of cancer tumors. In addition to many polysaccharides, a lot of proteins are components of the ECM [13,14].

Several authors have shown that non-catalytic proteins, like bovine serum albumin (BSA), are able to increase the activity of HAase in order to enhance the sensitivity of its assay or detection [15]. They have shown that an optimal BSA concentration exists for enhancing the HAase activity: low BSA concentrations activate HAase, whereas high concentrations inhibit HAase. Some other works also report that BSA can activate the HAase activity. Gacesa et al. [16] report that serum proteins are able to enhance the HAase catalytic activity, and show that among the serum proteins, albumin has the greatest effect. Gold [17] observes that BSA is able to activate both human liver HAase and bovine testicular HAase at pH 4. Maingonnat et al. [15] show that other proteins such as hyaluronectin, hemoglobin and immunoglobulins are also able to enhance HAase activity and so, to increase the sensitivity of the HAase detection. All these results have been reported without any definite explanation. We have also shown that, in vitro, proteins like BSA or lysozyme (LYS) modulate the activity of HAase. All these observations have recently received an explanation when we have shown that the positive charges of HAase interact with the negative charges of HA to form non-specific electrostatic HA–HAase complexes in which HAase is no more catalytically active, and that the non-catalytic proteins compete with HAase to form electrostatic complexes with HA, resulting in the modulation of the HAase activity [18–22]. We have also shown that the phenomena of complex formation between HAase and HA is maximum at low ionic strength and pH 4 which is the optimal pH for HAase [23], but also exists at higher ionic strength including 150 mM [21] and higher pH values including pH 7 [23].

The mechanism we proposed can be summarized as follows [22]: At low HAase over HA stoichiometric ratio, the binding of HAase to HA is total and the HAase activity is nil. When the concentration of the non-catalytic protein is increased and if its net charge is positive, the protein is able to form complexes with HA by occupying the free HA complexation sites, non-occupied by HAase. When there are no more free sites and if the protein has a higher affinity for HA than that of HAase, the non-catalytic protein competes with HAase and forms complexes with HA by releasing HAase. HAase being free, recovers its catalytic activity. This phenomenon exists as long as HAase molecules form complexes with HA. The complex formation system is thus a ternary system involving HA, HAase and the non-catalytic protein [22]. When all the HAase molecules are released, they are all active and the HA hydrolysis rate is maximum. However, the non-catalytic protein continues to form complexes with HA. The complex formation system is

* Corresponding author. Tel.: +33 2 35 14 67 42; fax: +33 2 35 14 67 04.
E-mail address: jean-claude.vincent@univ-rouen.fr (J.-C. Vincent).

0301-4622/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2009.09.010

now a binary system involving HA and the non-catalytic protein. At each time that one protein molecule forms a complex with HA, it occupies a given number of negative carboxyl groups on the HA molecule and hides a given number of potential cleavable β(1,4) sites for hydrolysis by HAase. These potential cleavable β(1,4) sites being the actual substrate of HAase [24], the concentration of the HAase substrate decreases. The HAase activity thus decreases at high concentrations of the non-catalytic protein [21,22].

To our knowledge, this is the only explanation for the dual role of inhibition/activation of the HAase activity by proteins. The facts were observed by several authors but no explanation was reported in the literature. As the reason lies in the competition between HAase and other proteins for their binding to HA, we propose here a simple and original modeling for this mechanism by using the physicochemical equations at equilibrium.

2. The experimental work

The experimental work concerning the dependence of the HAase activity with respect to the BSA concentration has been reported in previous papers [19,22,23]. The generalized shape of the BSA-dependence is drawn in Fig. 1a and shows four intervals separated by three critical points: i) Point A, at the interface between intervals ① and ②, corresponded to the concentration of the BSA molecules able to form additive complexes with the HA molecules already complexed with the HAase present in the system. This BSA concentration was noted $[BSA]_{HAase\ zero}$ because all the HAase molecules were complexed with HA and no free HAase molecules remained in solution leading to a quasi zero hydrolysis rate. ii) Point B, at the interface between intervals ② and ③, corresponded to the minimum BSA concentration needed so that the HA molecules were complexed with BSA alone. In B, all the HAase molecules were free in solution and able to catalyze the HA hydrolysis. The HA hydrolysis rate was maximum. This BSA concentration was noted $[BSA]_{min}$. In B, all the HA molecules are complexed with BSA with a characteristic BSA over HA ratio noted ψ_{min} [22]. iii) Point C, at the interface between intervals ③ and ④, corresponded to the highest BSA concentration able to produce a hydrolysable complexed HA. This BSA concentration was noted $[BSA]_{max}$. At this point, the HA molecules were too tightly complexed with BSA to be accessible to HAase and the HA hydrolysis rate was nil. All the potentially cleavable HA sites were hidden by the BSA molecules.

Our experimental studies [22] have also shown that we have to consider two types of system: i) a ternary HA/HAase/BSA system when the BSA concentration is lower than that corresponding to the B point, and ii) a binary HA/BSA system with all the HAase molecules free in solution when the BSA concentration is higher than that corresponding to the B point. When the ternary system is considered, we have shown that one HA molecule of 1 MDa can form complexes with approximately either 10 HAase molecules or 10 BSA molecules [22]. It means that statistically one HAase molecule, or one BSA molecule, forms an electrostatic complex with an HA fragment of about 265 disaccharides. When the binary system is considered, all the HAase molecules are free in solution and do not interact with HA for complex formation. In that case, we have shown that one HA molecule of 1 MDa can interact with a maximum of 64 BSA molecules [22]. It means that statistically one BSA molecule forms a non-substrate electrostatic complex with an HA fragment of about 38 disaccharides. The complexed HA fragment can no more be hydrolyzed by HAase because there is no place for HAase to catalytically interact with the corresponding cleavable β(1,4) sites of the HA fragment.

Other experiments, performed in the presence of 0.15 $mol\,L^{-1}$ ionic strength have shown that when the concentration of small ions in the medium is high enough to screen the charges on the two biopolymers, the HA/HAase system behaves as a Michaelis–Menten type enzyme [20]. This Michaelis–Menten type behavior is also observed at low ionic strength in the presence of BSA when the HA molecule complexed with BSA in a constant BSA over HA ratio is considered as the substrate entity.

The formation of electrostatic complexes between HA and proteins and the modulation of the HAase activity by proteins are not specific to BSA and have been observed with other proteins such as hyaluronectin [15], immunoglobulins [15] and lysozyme [23]. The modulation of the HAase activity in the presence of non-catalytic proteins requires two conditions: i) the protein has to be able to form a complex with HA and ii) this complex has to be more stable than the electrostatic complex formed between HA and HAase [22]. In order to reflect the non-specificity of the protein forming electrostatic complexes with HA, we shall use in our model the generic symbol of P for protein. For the ternary system, we assume that an HA fragment of n carboxyl groups, written HA_n, is able to form an electrostatic complex with either one HAase molecule or one P molecule. For the binary system, we assume that an HA fragment of m carboxyl groups, written HA_m, is able to form an electrostatic complex with one P molecule.

3. Theory

We assume that the enzymatic system is governed by the classical Michaelis–Menten equation giving the initial hydrolysis rate, V_i, as a function of the substrate S concentration:

$$V_i = k_2 \times [HAase] \times [S] / (Km + [S]) \tag{1}$$

Where [HAase] is the concentration of the free active enzyme and [S] is the substrate concentration which is equal to the concentration of the potentially cleavable β(1,4) bonds of HA [24]. Modeling of the system takes into account the two intervals successively, before the B point where the system is a ternary system and after the B point where the system is a binary system, HAase being no more involved in the complexes with HA.

3.1. Modeling of the ternary HA/HAase/P system: an expanded complex

Two complex formation equilibria exist in the system:

$$HA_n + HAase \rightleftarrows HA_n - HAase\ electrostatic\ complex \tag{2}$$

this complex is a potential substrate for HAase, but the complexed HAase is not active.

$$HA_n + P \rightleftarrows HA_n - P\ electrostatic\ complex \tag{3}$$

this complex is a potential substrate for HAase. The two equilibria are characterized by their dissociation constants K_{HAase} and K_P:

$$K_{HAase} = [HA_n] \cdot [HAase] / [HA_n - HAase] \tag{4}$$

$$K_P = [HA_n] \cdot [P] / [HA_n - P] \tag{5}$$

In addition to these equations, the mass conservation laws give:

$$[HAase] + [HA_n - HAase] = [HAase]_0 \tag{6}$$

$$[P] + [HA_n - P] = [P]_0 \tag{7}$$

$$[HA_n] + [HA_n - HAase] + [HA_n - P] = [HA_n]_0 \tag{8}$$

By expressing $[HA_n]$, $[HA_n - HAase]$ and $[HA_n - P]$ as a function of [HAase] by using Eqs. (4)–(8), we obtain the following third degree equation in [HAase]:

$$\begin{aligned}[HAase]^3 \times [K_{HAase} - K_P] + [HAase]^2 \times [K_{HAase}^2 - K_P \cdot K_{HAase} \\ + K_P \cdot [HAase]_0 - K_{HAase} \cdot [P]_0 - 2K_{HAase} \cdot [HAase]_0 - K_P \cdot [HA_n]_0 \\ + K_{HAase} \cdot [HA_n]_0] + [HAase] \times [HAase]_0 \times [K_P \cdot K_{HAase} - 2K_{HAase}^2 \\ + K_{HAase} \cdot [HAase]_0 + K_{HAase} \cdot [P]_0 - K_{HAase} \cdot [HA_n]_0] \\ + K_{HAase}^2 \cdot [HAase]_0^2 = 0\end{aligned} \tag{9}$$