Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 816 (2005) 289–295
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Analysis of the interaction between hyaluronan and hyaluronan-binding proteins by capillary affinity electrophoresis: significance of hyaluronan molecular size on binding reaction

Mitsuhiro Kinoshita*, Kazuaki Kakehi

Faculty of Pharmaceutical Sciences, Kinki University, Kowakae 3-4-1, Higashi-Osaka 577-8502, Japan

Received 14 April 2004; accepted 23 November 2004
Available online 15 December 2004

Abstract

We developed a method for the analysis of the interaction between hyaluronan (HA) oligosaccharides and hyaluronan-binding proteins (HABPs) using capillary affinity electrophoresis (CAE). The method is based on high-resolution separation of fluorescent-labeled HA molecules in the presence of hyaluronan-binding proteins at different concentrations by capillary electrophoresis (CE) with laser-induced fluorescent detection. Hyaluronan-binding protein from bovine nasal cartilage interacts strongly with HA decasaccharide or larger oligosaccharides. Effect of the molecular size of HA oligomers clearly showed that longer carbohydrate chains than decasaccharide were required for recognition by HA binging protein. Interestingly, the interaction did not cause retardation of HA oligomers as observed in many binding reactions such as the interaction between pharmaceuticals and serum albumin, but showed disappearance of the oligomer peak. Although we cannot explain the accurate mechanism on the interaction, disappearance is probably due to low equilibrium rate between free and conjugate states. The present technique will be useful to compare the relative binding affinity, and to understand the mechanism on the interaction between hyaluronan and hyaluronan-binding proteins.

Keywords: Capillary affinity electrophoresis; Hyaluronan; Hyaluronan-binding protein

1. Introduction

Hyaluronan (HA) is a linear polysaccharide composed of simple disaccharide units of –(4GlcAβ1-3GlcNAcβ)–. HA has various biological functions such as tissue morphogenesis, wound repair, cell migration, tumor invasion, and immune recognition [1,2]. These functions are considered to be mediated through specific interactions with HA-binding proteins (HABPs). Therefore, characterization of the molecular basis of recognition and modulation of HA molecules by proteins are a key determinant in understanding the biology of HA. West et al. reported that a mixture of HA oligomers induced the angiogenic response of the chick chorioallantoic membrane and the activity was restricted to HA oligomers with 4–25 disaccharide units [3]. Xu et al. reported that tetrasaccharide of HA up-regulated heat shock protein 72 (Hsp72) expression, and suppressed cell death in K562 cell, but HA oligomers having larger sizes did not show such effect [4]. On the other hand, Termeer et al. investigated the effects of HA on dendritic cells and found that only small HA oligomers of tetra- and hexasaccharides-induced immunophenotypic maturation of human monocyte-derived dendritic cells, and increased production of cytokines such as IL-1β, TNF-α and IL-12 [5]. It has been proposed that HA exerts its function by interacting with HA-binding proteins in the extracellular matrix and HA receptors on the cell surface. At present it is

Abbreviations: CAE, capillary affinity electrophoresis; HA, hyaluronan; HABP, hyaluronan-binding protein; AMAC, 2-aminoacridone; TA, Tris–acetate; MALDI-TOF MS, matrix-assisted laser desorption ionization/time-of-flight mass spectrometry

* Corresponding author. Tel.: +81 6 6721 2332x3868; fax: +81 6 6721 2353.
E-mail address: m-kino@phar.kindai.ac.jp (M. Kinoshita).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.11.050

not well understood why these functions exhibit correlation with the size of HA.

Previously, we reported high-resolution separation of HA using capillary electrophoresis (CE) and a buffer containing neutral polymer as sieving matrix, and found that smaller oligosaccharides than octasaccharide migrated in the reverse order of their molecular sizes and larger oligosaccharides migrated in the order of molecular sizes, and the reversal of migration order of these oligomers exhibited good correlation with the expression of biological functions [6]. Furthermore, we showed that hyaluronidase hydrolyzed larger oligomers than hexamer and the activity was well correlated to migration profiles of oligomers [7]. These results suggested that formation of a three-dimensional structure of HA oligomer regulated the enzyme susceptibility.

To identify and characterize HABP, various methods have hitherto been established such as the transblot assay using [^{3}H] labeled HA [8–10], HA-Sepharose column chromatography [11], co-precipitation using HA-Sepharose or cetylpyridinium chloride [12], and enzyme-linked immunosorbent assay [13]. However, these methods often lack accuracy and need large amounts of proteins and ligands. Separation techniques such as affinity chromatography or conventional gel electrophoretic techniques require immobilization steps. It should be noticed that there has been no information on the relationship between the size of HA and their biological functions.

Among various techniques for binding studies, capillary electrophoresis is a unique one that can be employed to observe the interaction under conditions similar to physiological condition. The technique for studies of molecular interaction by CE is called “capillary affinity electrophoresis (CAE)” or “affinity capillary electrophoresis (ACE)”. We use the term “capillary affinity electrophoresis” for this approach throughout the present paper according to our previous reports [14,15]. Capillary affinity electrophoresis is an analytical approach in which the migration patterns of interacting molecules in an electrical field are recorded and used to quantify and identify specific binding and estimate binding constants [16,17]. Only minute amounts of both protein and ligand molecule are needed for observation of the binding reaction. Because binding occurs in free solution, we can easily control all experimental conditions. Recently, we proposed a method for simultaneous determination of the interactions between a mixture of oligosaccharides and a carbohydrate-recognizing protein using capillary affinity electrophoresis [14]. The method is based on the separation of a mixture of carbohydrate chains in a buffer containing a carbohydrate-recognizing protein (i.e. lectin). When a carbohydrate in the mixture interacts with a lectin, we can observe the different migration as compared to that in the absence of the lectin. The method allows determining subtle difference in affinities among complex mixtures of carbohydrate chains.

In this report, we present a method for the simultaneous analysis of the interaction between HA oligosaccharides labeled with fluorescent 2-aminoacridone (AMAC) and HABP derived from bovine nasal cartilage using capillary affinity electrophoresis. We also showed an inhibition assay method that provided a more quantitative assessment of HA binding with HABP than other methods previously reported. Furthermore, we discuss the relationship between the sizes of HA oligomers and the binding properties of HABP.

2. Materials and methods

2.1. Materials

HA samples from *Streptococcus zooepidemicus* and pig skin were purchased from Wako Pure Chemicals (Doshomachi, Osaka, Japan). Hyaluronidase from ovine testes was obtained from Roche diagnosis (Minato-ku, Tokyo, Japan). Hyaluronic acid-binding protein from bovine nasal cartilage was purchased from Seikagaku Kogyo (Nihon-bashi, Tokyo, Japan). 2-Aminoacridone (AMAC) was obtained from Molecular Probes (Eugene, OR, USA). Human IgG, human fibrinogen, and sodium cyanoborohydride ($NaBH_3CN$) were from Sigma–Aldrich Japan (Minato-ku, Tokyo, Japan). All aqueous solutions were prepared with water purified with a Milli-Q Purification System (Millipore, Bedford, MA, USA). A capillary coated with dimethylpolysiloxane (DB-1 capillary) was obtained from GL Science Co. (Shinjuku, Tokyo, Japan). Polyethyleneglycol 70000 was from Wako. All other reagents were of the highest grade commercially available, and used without further purification.

2.2. Preparation of HA oligosaccharides

Hyaluronan (100 mg) from *S. zooepidemicus* was dissolved in 0.15 M citrate/HCl (pH 5.3, 10 ml), digested at 37 °C overnight with ovine testicular hyaluronidase (2000 units), and lyophilized to dryness. A portion (1 mg) of the lyophilized material was dissolved in 100 mM NaH_2PO_4 (0.1 ml), and the solution was filtered through an ultrafiltration membrane (10 kD cut-off; Millipore) and a portion (10 μl) was injected to a YMC-PAC PA5 column (YMC, Tokyo, Japan) on a Shimadzu 10AD HPLC apparatus equipped with a UV detector. Separation was performed in linear gradient elution using (a) 100 mM NaH_2PO_4 to (b) 1000 mM NaH_2PO_4 at a flow rate of 1.0 mL/min. The gradient elution was from 0 to 60% of solvent (b) over 40 min. Fractions containing hyaluronan oligomers consisting of 2–11 disaccharide units were pooled, and lyophilized to dryness. The lyophilized fractions were purified on a column of Sephadex G-50 (1 cm × 100 cm, Pharmacia, Upsala, Sweden) using water as eluent. Fractions containing uronic acids examined by the carbazole-H_2SO_4 method [18] were collected, and lyophilized to dryness.

2.3. Derivatization of HA oligomers with AMAC

HA oligomers were labeled with AMAC according to the method reported by Lamari et al. [19] and Kitagawa et

al. [20]. Briefly, the dried sample (50 μg) of HA oligomer was dissolved in a mixture (20 μl) of dimethyl sulfoxide and acetic acid (17:3, v/v) containing 100 mM AMAC. A solution (20 μl) of 1 M sodium cyanoborohydride in the same mixture of dimethyl sulfoxide and acetic acid (17:3) was added. The mixture was kept at 90 °C for 30 min and diluted with water (160 μl). Because the AMAC reagent is soluble in ethanol, HA oligomers can be collected after precipitation by addition of excess alcohol. The reaction mixture was mixed with 5 mL of cold ethanol containing 1.3% potassium acetate, and kept for 1 h on an ice bath. The alcohol layer containing the excess reagent was removed after centrifugation. The precipitate of AMAC-labeled HA oligomers was dissolved in 200 μl of water, and a portion was used for the binding studies. Concentration of the labeled oligosaccharide was adjusted by comparing the fluorescence intensity using a solution of AMAC-labeled maltose at a fixed concentration as reference compound.

2.4. MALDI-TOF MS

MALDI-TOF MS was performed on a Voyager DE-PRO (PE Biosystems, Framingham, MA, USA). A nitrogen laser at 337 nm was used to irradiate samples, and an average of 100 shots was taken. The instrument was operated in linear operation using positive polarity. An accelerating voltage of 22 kV was used. A portion (0.5 μl) of a sample solution was applied to a polished stainless steel target, to which added a solution (0.5 μl) of 2,5-dihydroxybenzoic acid in a mixture of methanol and water (1:1, v/v). The mixture was dried in atmosphere by keeping it at room temperature for several hours.

2.5. Capillary affinity electrophoresis

Capillary affinity electrophoresis was carried out in the similar manner reported previously using a Beckman MDQ glycoprotein systems (Beckman Coulter, MA) equipped with an Ar-laser-induced fluorescence detection system [14,15]. Detection was done by installing a 520-nm emission filter with a 488-nm excitation filter. All experiments were carried out with a DB-1 capillary (60 cm total length, effective length 50 cm, 50 μm i.d.). The detection window was made at 10 cm from the outlet end of the capillary. Injection of a sample solution was performed by the pressure method at 1 psi for 10 s. Data were collected and analyzed with a 32 Karat Gold Software (Beckman). A 0.1 M Tris–acetate (TA) buffer with or without HA-binding protein was used as an electrolyte, and filled in the capillary prior to the analysis after washing with water and TA buffer without protein for 1 min. All other conditions are described in figure legends.

3. Results

CAE can utilize a few modes for determination of molecular interactions [16]. In the interaction in stationary phase, the ligand/protein molecule is immobilized on the capillary wall or imprinted in a column or gel. Migration of the molecule having affinity to the immobilized molecules is delayed compared with that in case of their absence [21,22]. On the contrary, the interaction in the mobile phase takes place when both the ligand and the receptor are in free solution. The migration time of a ligand molecule is dependent on the binding and the concentration of the protein [23,24]. Using this mode of interaction, we proposed an approach for screening of post-translational modification of carbohydrates in glycoproteins. Interaction between a mixture of fluorescent-labeled carbohydrates and carbohydrate-binding proteins was studied, and affinities to carbohydrate chains were determined simultaneously [14].

3.1. Interaction between a mixture of HA oligosaccharides and some HA-binding proteins (HABPs)

A number of proteins possessing ability to bind HA have been reported. Aggrecan, a macromolecular aggregating proteoglycan, is one of the major structural components of cartilage [25]. Versican and brevican have been also reported to bind HA [26–28]. These HABPs contain a common domain, termed “Link module”, which is involved in HA binding [26–28]. Some blood proteins such as fibrinogen, IgG and IgM have also been shown to possess HA-binding ability [29,30]. As an initial approach to investigate the interaction between HA and HABP, we examined the interaction between the mixture of AMAC-labeled HA oligomers (tetrasaccahride to the oligomer composed of 22 monosaccharide units; HA_4–HA_{22}) and HABPs using CAE. The results are shown in Fig. 1.

In the present study, we used a DB-1 capillary and a running buffer containing polyethyleneglycol to suppress protein adsorption on the capillary surface. Electroosmotic flow also became negligible when a small amount of polyethyleneglycol was added in the running buffer. When AMAC-labeled oligosaccarides were analyzed in the absence of the ligand, these oligomers were well resolved within 18 min (Fig. 1a). In this mode of separation, larger oligomers appeared earlier and smaller molecules later based on the charge/mass ratio. The present study was performed in the running buffer around isoelectric point of each protein (pI = 5.9–7.3). Therefore, the mass/charge ratio of protein is very large, and the electrophoretic velocity of HABP was quite small compared with that of AMAC-labeled HA molecules, and the binding reaction was observed as the change in migration of HA oligomers. HABPs used in the present study showed two types of interactions with HA oligomers. In the presence of HA-binding protein from bovine nasal cartilage, oligomer peaks except for HA_4 disappeared at the concentration of 1 μM (Fig. 1b). On the other hand, human fibrinogen showed retardation of migration times of AMAC-labeled HA oligomers (Fig. 1c). However, human IgG did not show affinity to HA oligomers at 1 μM level (Fig. 1d).

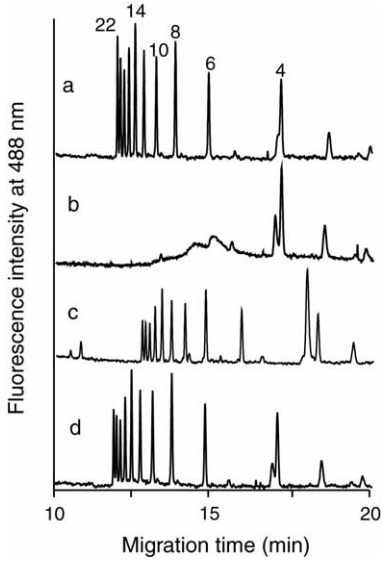

Fig. 1. Capillary affinity electrophoresis of the interaction between AMAC-labeled HA oligosaccharides and some HA-binding proteins. (a) AMAC-labeled HA oligosaccharides in the absence of HA-binding protein, in the presence of (b) HABP, (c) fibrinogen, and (d) IgG. Protein concentrations were set to 1 μM. Analytical conditions: capillary, fused silica capillary coated with dimethylpolysiloxane (60 cm, 50 μm i.d., effective length of 50 cm); buffer, 0.1 M Tris–acetate (pH 7.5) containing 0.5% polyethyleneglycol 70,000; applied voltage, 20 kV; temperature, 25 °C. Fluorescence detection was performed with a 520 nm light filter for emission by irradiating with an argon laser-induced 488 nm light. The sample solutions were introduced for 10 s by pressure method (1 psi).

3.2. Effect of molecular size of HA oligomers on the interaction with HABP from bovine nasal cartilage

HA having higher molecular masses than 10^6 Da strongly binds to HABP from bovine nasal cartilage, which is one of the well-defined HABPs. Hascall and Heinegard investigated the interaction between high molecular mass preparations of HA and HABP isolated from the extracts of bovine nasal cartilage by inhibition studies using HA oligosaccharides as inhibitor [31]. We applied capillary affinity electrophoresis to simultaneous determination of binding characteristics between HA oligosaccharides with HABP (Fig. 2A).

Peak intensities of large oligosaccharides (>HA_{10}) observed between 12 and 13 min were gradually decreased with increase of concentrations of HABP. These oligomer peaks completely disappeared at 250 nM of HABP. In general, interaction is usually observed as peak retardation in CAE. But we also observe peak disappearance for the interaction between a specific carbohydrate and a lectin such as high-mannonse oligosaccharides and Concanavalin A (ConA). In the present study, interaction between HA-oligomers and HABP caused disappearance of HA oligomer peaks as observed in case of the interaction between ConA and high-mannose type oligosaccharides. On the contrary, affinities of hexa (HA_6)- and octa (HA_8)-saccharides to HABP were not obvious and peak shapes were not changed even at 125 nM concentration (Fig. 2A(e)). Finally, these peaks were fused to a broad peak and decreased at 1000 nM. The affinity of tetrasaccharide to HABP was not obvious at 1000 nM. Fig. 2B shows the relationship between peak intensities of AMAC-labeled HA oligomers and concentrations of HABP. Hexa- and octasaccharides showed obviously lower affinities than those observed for larger oligosaccharides (>HA_{10}). Concentrations required for complete disappearance of peaks derived from hexa- and octasaccharides were 1000 and 500 nM, respectively. On the other hand, peaks of larger oligosaccharides than decasaccharide completely disappeared at lower concentrations of 250 nM.

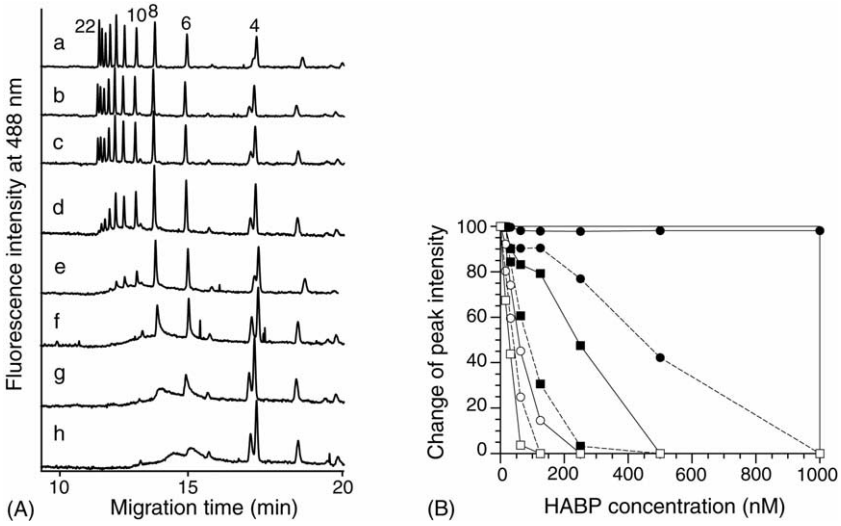

Fig. 2. Effect of concentrations of HABP on the binding reaction. (A) Capillary affinity electrophoreses were performed without HABP (a) and with HABP at a concentrations of 15.6 nM (b), 31.2 nM (c), 62.5 nM (d), 125 nM (e), 250 nM (f), 500 nM (h), and 1000 nM (i), respectively. Analytical conditions were the same as in Fig. 1. (B) Changes of peak intensity of AMAC-HA oligomers were plotted against HABP concentrations using the data in (A). The symbols were: HA_4 (filled circle, solid line), HA_6 (filled circle, dashed line), HA_8 (filled square, solid line), HA_{10} (filled square, dashed line), HA_{12} (open circle, solid line), HA_{16} (open circle, dashed line), HA_{20} (open square, solid line), respectively.

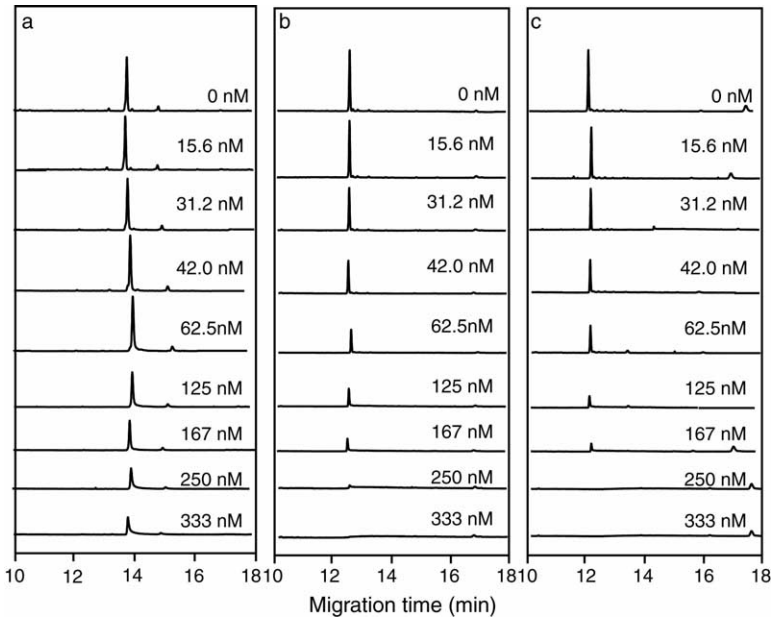

Fig. 3. Interaction between purified HA oligosaccharides and HABP. (a) AMAC-labeled HA_8, (b) AMAC-labeled HA_{10}, and (c) AMAC-labeled HA_{20}, respectively. Analytical conditions were the same as in Fig. 1.

We also examined the binding characteristics of HABP using purified HA oligosaccharides. The results are shown in Fig. 3.

Decrease in peak intensity of HA octasaccharides began at 125 nM of HABP, but not completed even at 333 nM (Fig. 3a). In contrast, binding studies for HA_{10} and HA_{20} with HABP showed obvious decrease of peak intensities at 31.2 and 15.6 nM, respectively (Fig. 3b and c). These oligomer peaks completely disappeared at 250 nM. These results were in good agreement with those observed for the determination of binding using a mixture of HA oligomers (Fig. 2Aa)

3.3. Inhibition studies

Competitive binding is one of the common methods to analyze the molecular interaction, and generally available for comparison of relative intensities of binding among ligands. The method is especially useful for the binding studies using unlabeled oligosaccharides, because competitive binding curve yields IC_{50} values, which are the concentrations of unlabeled ligands necessary to displace 50% of the labeled ligand from receptor.

We applied CAE to competitive studies on the binding between HA-oligomers and HABP. In the initial step, AMAC-HA_{20} was analyzed in the presence of HABP at 1 μM, the peak of AMAC-HA_{20} was not observed (Fig. 4b).

When unlabeled HA_{20} (0–100 μM) as a competitor was added to the buffer, the peak intensity of AMAC-HA_{20} was gradually recovered with increasing of the concentrations of unlabeled HA_{20}, and completely recovered at a concentration of 10 μM (Fig. 4c–f). Inhibition studies on unlabeled HA oligomers (HA_4–HA_{20}) and high MW HA preparations (HA_{PS} from pig skin (MW: 100,000) and HA_{SZ} from *S. zooepidemicus* (MW: 1,500,000)) are shown in Fig. 5.

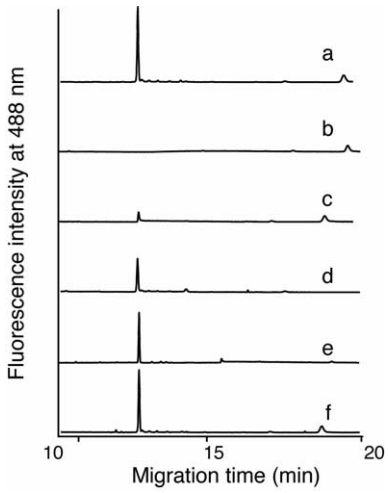

Fig. 4. Inhibition of the binding between AMAC-labeled HA_{20} and HABP by unlabeled HA_{20}. Capillary affinity electrophoresis was performed without unlabeled HA oligosaccharide as competitor (a) and with HA oligosaccharide as competitor at a concentrations of 0.5 μM (b), 1 μM (c), 2 μM (d), 5 μM (e), and 10 μM (f), respectively. Analytical conditions were the same as in Fig. 1.

High MW HA preparations (HA_{PS} and HA_{SZ}) were good inhibitors and inhibited binding between HA_{20} and HABP at a concentration of 1 μM. In contrast, IC_{50} values for HA_{10} and HA_{20} were 6 and 3 μM, respectively. HA_8 was a poor inhibitor and a concentration of higher than 200 μM was required to give 50% inhibition. The jump of binding with increasing oligomer sizes was clearly observed

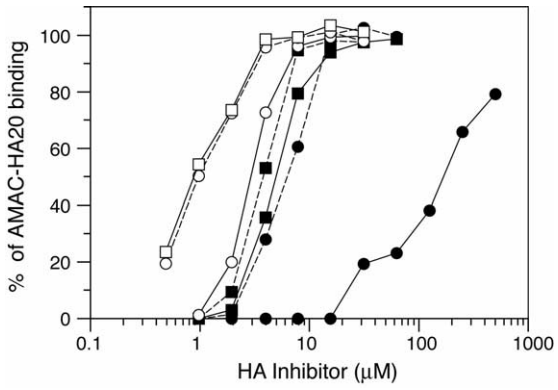

Fig. 5. Inhibition curves of the binding between AMAC-labeled HA_{20} and HABP by unlabeled HA oligosaccharides. AMAC-labeled HA_{20} was analyzed in the presence of HABP (1 μM) and unlabeled HA oligosaccharides at various concentrations. HA_8 (filled circle, solid line), HA_{10} (filled circle, dashed line), HA_{12} (filled square, solid line), HA_{16} (filled square, dashed line), HA_{20} (open circle, solid line), HA_{PS} from pig skin (open circle, dashed line), and HA_{SZ} from *S. zooepidemicus* (open square, solid line).

Table 1
Inhibition of AMAC-HA_{20} binding by unlabeled HA oligomers

Oligomer size	50% inhibition (μM) AMAC-HA_{20}
8	255
10	6.05
12	4.82
14	4.51
16	3.80
18	3.54
20	3.03
HA_{PS}	1.18
HA_{SZ}	1.01

for HA oligomers between HA_8 and HA_{10}. These observations indicate that HA_{10} is a minimum size for binding to HABP. Table 1 summarizes the 50% inhibition concentrations (IC_{50}) of AMAC–HA_{20} binding to HABP using various HA oligomers and polymers.

The IC_{50} values were calculated based on the data obtained from Fig. 5. Oligomers between HA_{10} and HA_{20} showed little difference in IC_{50} values. However, there are ca. three folds increase in the 50% inhibition concentration with HA polymers, compared to those of HA oligomers. These data were well comparable to the data reported using analytical ultracentrifugation [32].

4. Discussion

The present study demonstrates that a technique based on capillary affinity electrophoresis is a powerful approach for evaluation of interaction between HABP and AMAC-labeled HA oligomers having different sizes as a mixture. We found that the binding between AMAC-labeled HA and HABP was observed in two different manners: i.e. peak retardation for observed in the binding with fibrinogen and peak disappearance observed in the binding with hyaluronan-binding protein from bovine nasal cartilage.

Peak intensity of higher oligosaccharides (>HA_{10}) was gradually decreased at higher concentrations than 15.6 nM of HABP, and was disappeared above a concentration of 250 nM. Peak disappearance suggested that ligand exchange rate were quite slow compared with electrophoretic migration of HA molecules as suggested in our previous paper [14,15]. Studies on size-requirement of HA molecules for the binding to HABP indicated that octasaccharide or decasaccharide were minimum size for recognition by HABP. These observations indicate that binding ability of HA oligomers to HABP is dependent on their molecular sizes and decasaccharide is the minimum size for complete recognition by HABP. Furthermore, these results are well correlated with our previous report concerning the unusual migrations of HA oligomers [6] by capillary electrophoresis.

To investigate the mechanism underlying the recognition of HA oligosaccharides of different sizes by HABP, inhibition studies were performed using fluorescent-labeled HA_{20} sample and HABP. The binding reaction was weakly inhibited by unlabeled HA_8. On the contrary, binding of HA_{20} was completely inhibited in the presence of oligosaccharide of HA_{10}-HA_{20} in the similar concentrations as observed for the binding of native hyaluronan. Christer et al. reported the binding reaction between HA oligomers and HABP from bovine nasal cartilage using analytical ultracentrifugation method [32]. They also reported that the jump of binding affinity was clearly observed for HA oligomers between HA_8 and HA_{10}. We would like to emphasize that the present technique can determine the minimum size for recognition in the strict manner, because a mixture of oligomers can be used, and relative affinities can be accurately compared.

In a previous work, we found that smaller oligosaccharides than octamer is migrated in the reverse order of their molecular masses in a buffer containing polymer matrix, when analyzed by capillary electrophoresis. But larger oligomers than decasaccharide are migrated in the order of molecular sizes [6]. We found that this anomalous migration was well correlated with reported biological activity of HA oligosaccharides, and speculated that this anomalous migration was based on the formation of a fixed conformation such as helix structure supported by intramolecular hydrogen bonding between carboxylic acid and amide groups in HA molecules [33]. The previous studies clearly support the present results obtained by capillary affinity electrophoresis.

In conclusion, CAE used in this paper provides more precise assessment of HA binding by HA-binding protein from bovine nasal cartilage than the methods previously reported, such as a conventional affinity chromatography and ELISA method. From the view of increasing evidence that the molecular size of HA is critical to control their cellular functions, it is important to investigate how HA preparations of different sizes interact with their binding protein family such as CD44, vercian, neurocan and brevican, that contain a common structural domain of ca. 100 amino acids, termed as a link module

that is involved in HA binding [34]. The technique used in the present study will provide a useful approach for comparing the relative binding affinity of HA oligosaccharides with HA-binding protein.

References

[1] C. Knudson, W. Knudson, FASEB. J. 7 (1993) 1233.
[2] L. Sherman, J. Sleeman, P. Herrlich, H. Ponta, Curr. Opin. Cell Biol. 6 (1994) 726.
[3] D.C. West, I.N. Hampson, F. Arnold, S. Kumar, Science 228 (1985) 1324.
[4] H. Xu, T. Ito, A. Towada, H. Maeda, H. Yamanokuchi, K. Isahara, K. Yoshida, Y. Uchiyama, A. Asari, J. Biol. Chem. 277 (2002) 17308.
[5] C.C. Termeer, J. Hennies, U. Voith, J.M. Weiss, P. Prehm, J.C. Simon, J. Immunol. 165 (2000) 1863.
[6] K. Kakehi, M. Kinoshita, S. Hayase, Y. Oda, Anal. Chem. 71 (1999) 1592.
[7] M. Kinoshita, A. Okino, Y. Oda, K. Kakehi, Electrophoresis 22 (2001) 3458.
[8] Q. Yu, B.R. Toole, Biotechniques 19 (1995) 122.
[9] B. Yang, B.L. Yang, P.F. Goetinck, Anal. Biochem. 228 (1995) 299.
[10] K. Hoare, R.C. Savani, C. Wang, E.A. Turley, Connect. Tissue Res. 30 (1993) 117.
[11] A. Tengblad, Biochim. Biophys. Acta 578 (1979) 281.
[12] T.H. Lee, H.G. Wisniewski, J. Vilcek, J. Cell. Biol. 116 (1992) 545.
[13] R.J. Peach, D. Hollenbaugh, I. Stamenkovic, A. Aruffo, J. Cell. Biol. 122 (1993) 254.
[14] K. Nakajima, Y. Oda, M. Kinoshita, K. Kakehi, J. Proteome Res. 2 (2003) 81.
[15] K. Nakajima, M. Kinoshita, Y. Oda, T. Masuko, H. Kaku, N. Shibuya, K. Kakehi, Glycobiology 14 (2004) 793.
[16] R.M. Guijt-van Dujin, J. Frank, G.W. van Dedem, E. Baltussen, Electrophoresis 21 (2000) 3905.
[17] K. Shimura, K. Kasai, Anal. Biochem. 251 (1997) 1.
[18] T. Bitter, H. Muir, Anal. Biochem. 4 (1962) 330.
[19] F. Lamari, A. Theocharis, A. Hjerpe, N.K. Karamanos, J. Chromatogr. B, Biomed. Sci. Appl. 730 (1999) 129.
[20] H. Kitagawa, A. Kinoshita, K. Sugahara, Anal. Biochem. 232 (1995) 114.
[21] X.J. Wu, R.J. Linhardt, Electrophoresis 19 (1998) 2650.
[22] X.X. Zhang, J. Li, J. Gao, J. Sun, W.B. Chang, Electrophoresis 20 (1999) 1998.
[23] M. Hong, A. Cassely, Y. Mechref, M.V. Novotony, J. Chromatogr. B 752 (2001) 207.
[24] K. Shimura, K. Kasai, J. Biochem. 120 (1996) 1146.
[25] H. Watanabe, Y. Yamada, K. Kimata, J. Biochem. 124 (1998) 687.
[26] D.R. Zimmermann, E. Ruoslahti, EMBO J. 8 (1989) 2975.
[27] T. Shinomura, Y. Nishida, K. Ito, K. Kimata, J. Biol. Chem. 268 (1993) 14461.
[28] U. Rauch, L. Karthikeyan, P. Maurel, R.U. Margolis, R.K. Margolis, J. Biol. Chem. 268 (1992) 19536.
[29] R.D. LeBoeuf, H.R. Raja, G.M. Fuller, P.H. Weigel, J. Biol. Chem. 261 (1986) 12586.
[30] C.B. Underhill, Biochem. Biophys. Res. Commun. 108 (1982) 1488.
[31] V.C. Hascall, D. Heinegard, J. Biol. Chem. 249 (1974) 4242.
[32] J.E. Christner, M.L. Brown, D.D. Dziewiatkowski, J. Biol. Chem. 254 (1979) 4624.
[33] J.E. Scott, F. Heatley, W.M. Hull, Biochem. J. 220 (1984) 197.
[34] A.J. Day, G.D. Prestwich, J. Biol. Chem. 277 (2002) 4585.