European Science Review 2024,
No 11–12.
ISSN 2310-5577

PREMIER Publishing
ppublishing.org

Section 3. Medicine

DOI:10.29013/ESR-24-11.12-18-21

DETERMINATION OF ELECTROMORPHES OF HYALURONIC ACID

Olga Venger [1]***, Denys Oslavskyi*** [2]

[1] Plant Breeding and Genetics Institute – National Center of Seed and Cultivars Investigations, Odesa, Ukraine

[2] Dnipro State Medical University, Dnipro, Ukraine

Cite: *Venger O., Oslavskyi D. (2024). Determination of Electromorphes of Hyaluronic Acid. European Science Review 2024, No 11–12*. https://doi.org/10.29013/ESR-24-11.12-18-21

Abstract

Hyaluronic acid is polymer widely used in the cosmetology and medicine. In the usage of HA the size of molecules has a very important significance. The use of hyaluronic acid is due, in particular, to its hydrophilic properties, which is why the size of the hyaluronic acid molecule is of great importance. Currently available methods for determining the length of hyaluronic acid molecules require significant reagent and time consumption. This work demonstrates the possibility of determining the lengths of hyaluronic acid electromorphes using agarose gel electrophoresis.

Keywords: *hyaluronic acid, molecular size, electrophoresis, electromorphes, molecular markers*

Introduction

Hyaluronic acid (HA) is a mucopolysaccharide consisting of glucuronic acid and N-acetylglucosamine residues linked by β-1,3' bonds (Wende, 2016). HA is a non-sulphated glycosaminoglycan that is part of connective, epithelial and nervous tissues and is one of the main components of the intercellular matrix of vertebrate connective tissue; it contributes significantly to cell proliferation and migration, and may also play a role in tumor formation. HA is used in the treatment of joints, conjunctivitis, infertility, cosmetic procedures, etc. (Collinsa & Birkinshaw, 2013; Lia & Hec, 2014; Kogan, 2007; Kong, 2013; Ming & Vivek, 2014).

When using HA, the size of the molecule is important (Lee & Banquy, 2014; Parka et. al., 2014; Kazuaki et. al., 2003). For example, in the preparation of ophthalmic preparations, larger HA fractions are preferred (Xu et. al., 2007; Hardingham & Muir, 1972; Owen & Fisher, 2013). The size of HA is determined either by paper chromatography or by filtration through micropores with molecular size markers (Oueslatia et. al., 2015; Lan et. al., 2013; Ünlüera & Ersöz, 2013; Zhoua et. al., 2006). These techniques are time-consum-

ing and costly, so the issue of determining the size of hyaluronic acid by a low-cost, fast, and high-quality method is relevant (Lee & Banquy, 2014; Ming, 2013; Price et. al., 1997; Volpi, 2000). One such method is electrophoretic separation in a polyacrylamide gel (PAGE), which is commonly used for proteins and nucleic acids. Electrophoretic mobility depends on the size of the molecule, thus, after electrophoretic separation, it is possible to determine the size of molecules relative to molecules of known size (Duan, 2008; Volpi, 2000).

The aim of the study was to determine the possibility of electrophoretic separation of HA in PAAG, visualization and determination of electrophoretic mobility relative to the molecular weight marker.

Methods

There have been used HA solutions in distilled water and HA-containing preparations, namely Oxyal (contains 15 % HA), Synocrom (contains 1 % water-soluble sodium hyaluronate), Suplasyin (contains 1 % sodium hyaluronate), 'Hyalgan (contains 2 % sodium hyaluronate), Syngial (contains 1 % HA) in concentrations of 100 % (for HA-containing preparations only), 10 %, 1 %, 0.1 %, 0.01 %, 0.001 %, 0.0001 %.

Solutions of appropriate concentrations were obtained by multistep dilutions in distilled water.

The separation of HA solutions and HA-containing preparations was carried out in a 10% polyacrylamide gel in 1x Tris-buffer at a constant voltage of 500 V and a temperature of 60 ˚C for 2-4 hours, depending on the size of the amplification fragments, in a vertical gel electrophoresis apparatus (Helicon). HA solutions, molecular weight marker LADDER 50 (to determine the electrophoretic mobility of HA solutions) and an aqueous solution of electrophoretic-neutral dye as a negative control were applied to the gel. Before applying the substances to the gel, prephoresis was performed for 30 min at a constant voltage of 300 V and a temperature of 60 ^{0}C. Visualization of the HA solutions separated in the PAGE was performed with silver nitrate (specific for organic compounds). The PAGE plate was washed with deionised water for 1 min and DNA was fixed with 10.0% ethanol for 10 min. The plate was transferred to 1.0 % nitric acid for 6 min and washed 3 times with deionised water with continuous shaking. The preparations do not contain organic compounds, except for HA, which makes it impossible to obtain false-positive results during staining.

The plate was placed in 0.012 M $AgNO_3$ for 30 min in the dark. After that the plate was washed twice with deionised water with vigorous shaking. The plate was incubated in a reducing solution (0.28 M Na_2CO_3, 0.019 % formalin), with the solutions being replaced after each darkening, until the amplification fragments were visually stained. The plate was fixed in 10.0% acetic acid for 5 min. The gel was washed for 2 min with deionised water and stored between two sheets of transparent plastic film.

The electrophoretic mobility of HA was determined by the molecular weight marker LADDER 50 using the TotalLab software.

Results

The results of the study are presented in Table 1.

Table 1. *Electrophoretic mobility of HA solutions, equivalent/bp*

Solution	Solution concentration, %						
	100	10	1	0.1	0.01	0.001	0.0001
	Electrophoretic mobility						
HA	1752, 1850	1752, 1850	1752, 1850	1752, 1850	1752, 1850	1752, 1850	1752, 1850
Oxyal	1745, 1841	1745, 1841	1745, 1841	1745, 1841	1745, 1841	1745, 1841	1745, 1841
Synocrom	1650	1650	1650	1650	1650	1650	1650
Suplasyn	1456, 1643	1456, 1643	1456, 1643	1456, 1643	1456, 1643	1456, 1643	1456, 1643

Solution	Solution concentration, %						
Hyalgan	1656, 1703	1656, 1703	1656, 1703	1656, 1703	1656, 1703	1656, 1703	1656, 1703
Syngial	1767	1767	1767	1767	1767	1767	1767

Discussion

The results of the study demonstrated the possibility of electrophoretic separation of HA in PAGE with subsequent visualization. The fractions of different lengths in HA solutions and HA-containing preparations were determined, their size relative to each other and the molecular weight marker was determined. It was shown that the preparations Oxyal, Suplasyn, and Hyalgan contain HA fractions of different sizes. The absence of concentration effect on the electrophoretic mobility of HA was found.

The use of electrophoretic distribution allows us to determine the electrophoretic mobility of HA and to compare the length of different HA fractions relative to each other and the molecular weight marker.

References

Collinsa, M. N., Birkinshaw, C. (2013). Hyaluronic acid based scaffolds for tissue engineering. Carbohydrate Polymers. – 92. – P. 1292–1279.

Duan, S. (2008). PAMAM dendrimer composite membrane for CO_2 separation: addition of hyaluronic acid in gutter layer and application of novel hydroxyl PAMAM dendrimer. Presented at the Fourth Conference of Aseanian Membrane Society. – 234. – P. 78 – 285.

Hardingham, T.E., Muir, H. (1972). The specific interaction of hyaluronic acid with cartilage proteoglycans. Biochimica et Biophysica Acta. – 279. – P. 401–405.

Kazuaki, K., Kinoshitaa, M., Yasuedab, S. (2003). Hyaluronic acid: separation and biological implications. Journal of Chromatography B. – 797. – P. 347–355.

Kogan, G. (2007). Hyaluronic acid: a natural biopolymer with a broad range of biomedical and industrial applications. Biotechnology Letters. – 29. – P. 17–25.

Kong, K. (2013). Construction of hyaluronic acid noisome as functional transdermal nanocarrier for tumor therapy. Carbohydrate Polymers, – P. 634–641.

Lan, S., Wu, X., Li, L. (2013). Synthesis and characterization of hyaluronic acid-supported magnetic microspheres for copper ions removal. Colloids and Surfaces A: Physicochemical and Engineering Aspects. – 425. – P. 42–50.

Lee, D.W., Banquy, X. (2014). Effects of molecular weight of grafted hyaluronic acid on wear initiation. Acta Biomaterialia. – 10. – P. 1817–1823.

Lia, J., Hec, Y. (2014). Hyaluronic acid-modified hydrothermally synthesized iron oxide nanoparticles for targeted tumor MR imaging. Biomaterials. – 35. – P. 3666–3667.

Ming, Li. (2013). Electrophoretic deposition and electrochemical behavior of novel graphene oxide-hyaluronic acid-hydroxyapatite nanocomposite coatings. Applied Surface Science. – 284. – P. 804–810.

Ming, C., Vivek, G. (2014). Topical delivery of hyaluronic acid into skin using SPACE-peptide carriers. Journal of Controlled Release. 2014. – V. 173. – P. 67–74.

Oueslatia, N., Leblanca, P., Bodina, A. (2015). A simple methodology for predicting the performances of hyaluronic acid purification by diafiltration. Journal of Membrane Science. – 490. – P. 152–159.

Owen, S. C., Fisher, S.A. (2013). Hyaluronic Acid Click Hydrogels Emulate the Extracellular Matrix. American Chemical Society. – 29. – P. 7393–7400.

Parka, J.H., Chob, J.H., Yoon, H.Y. (2014). Hyaluronic acid derivative-coated nanohybrid liposomes for cancer imaging and drug delivery. Journal of Controlled Release. – 174. – P. 98–108.

Price, N., Tuinman, A., Baker, C., Chisena, C., Cysyk, L. (1997). Isolation and characterization by electrospray-ionization mass spectrometry and high-performance anion-exchange

chromatography of oligosaccharides derived from hyaluronic acid by hyaluronate lyase digestion: Observation of some heretofore unobserved oligosaccharides that contain an odd number of units. Carbohydrate Research. – 303. – P. 303–311.
Xu, H., Ma, L., Shi, H., Gao, C., Han. C. (2007). Chitosan–hyaluronic acid hybrid film as a novel wound dressing: in vitro and in vivo studies. Polymers for Advanced Technologies. – 18. – P. 869–875.
Ünlüera, O., Ersöz, A. (2013). Separation and purification of hyaluronic acid by embedded glucuronic acid imprinted polymers into cryogel. Journal of Chromatography B. – 934. – P. 46–52.
Volpi, N. (2000). Hyaluronic Acid and Chondroitin Sulfate Unsaturated Disaccharides Analysis by High-Performance Liquid Chromatography and Fluorimetric Detection with Dansylhydrazine. - Analytical Biochemistry. – 277. – P. 19–24.
Wende, J. (2016). Determination of substitution positions in hyaluronic acid hydrogels using NMR and MS based methods. Carbohydrate Polymers. – 136. – P. 1348–1357.
Zhoua, H., Nia, J., Huanga, W., Zhanga, J. (2006). Separation of hyaluronic acid from fermentation broth by tangential flow microfiltration and ultrafiltration. - Separation and Purification Technology. – 52. – P. 29–38.

submitted 25.11.2024;
accepted for publication 9.12.2024;
published 31.01.2025

Contact: emerald-olga@ukr.net