Section 1. Biology

Venger Andrii,
Assistant, of department of microbiology,
virology and immunology, Ph D.,
Odessa national medical university,
E-mail: venger87@ukr.net;

Kolesnyk Olga,
junior scientific researcher, Ph D.,
The Plant breeding and genetics Institute
National center of seed and cultivar investigation,
E-mail: emerald-olga@ukr.net

Malynovsky Volodymyr,
professor assistant, of department of medical
chemistry and biology, Ph D.,
International Humanitarian University,
E-mail: vmalinovskii@gmail.com

Zaitsev Andrii,
Student, of department of microbiology,
virology and immunology
Odessa national medical university,
E-mail: zayceva1957@ukr.net

Masloboeva Taisia,
Student, of department of microbiology,
virology and immunology
Odessa national medical university,
E-mail: sovushka21tasa@gmail.com

Hruzevskyi Oleksandr,
professor assistant, of department of microbiology,
virology and immunology, Ph D.,
Odessa national medical university,
E-mail: hruzevskiy@ua.fz

MOLECULAR SIZE DETERMINATION OF HYALURONIC ACID

Abstract: Hyaluronic acid (HA) is polysaccharide widely used in the medicine. In the usage of HA the size of molecules has a very important significance. Identification of HA size is usually carried out by paper chromatography method or by filtration through micropores with markers of molecular

size. However, the mentioned methods are very expensive and need extra time to be completed. The aim of scientific research was to detect the possibility of separation and identification of molecular size of HA by electrophoresis in the polyacrylamide gel. Possibility to separate and identificate the specific size of HA molecules was shown in polyacrylamide gel by electrophoretic method. This method requires less time and resources than ones which are usually used and can be applied both in industry and medicine.

Keywords: hyaluronic acid, molecular size, electrophoresis, molecular markers.

Introduction

Hyaluronic acid (HA) is polysaccharide, which is consisting of remainders of glucuronic acid and n-acetylglucoseamine, connected by β 1.3' – bonds. HA is used for treatment of bones, conjunctivitis, infertility, and also it is widely applied in cosmetology etc [1, P. 612–618]. HA is a glicosamineglycane present in the extracellular matrix of cumulus oophorus around the oocyte that proves to play the important role in natural human fertilization has anti-adhesive, anti-inflammatory, and lubricating properties, so could potentially be useful for spinal pain [2, P. 573–581]. HA administered epidurally had a therapeutic effect on the allodynia and hyperalgesia induced by chronic compression of the dorsal root ganglion. Degradation of HA is a step-wise process that can occur via enzymatic or non-enzymatic reactions [3, 12]. A reduction in HA mass or molecular weight via degradation or slowing of synthesis affects physical and chemical properties such as tissue volume [1, P. 612–618]. It was shown, that extracellular matrix contains high molecular weight hyaluronic acid (HMWHA; ~ 2×106 Da). During injury, HMWHA breaks down to low molecular weight hyaluronic acid (LMWHA; ~ $0.8 – 8 \times 105$ Da) [4, 10].

In the usage of HA the size of molecules has a very important significance. Thus, in the producing of ophthalmology drugs it is preferred to use fractions of HA with larger size. Identification of HA size is usually provided by paper chromatography method or by filtration from micropores with markers of molecular size. However, the mentioned methods are very expensive and need extra time to be completed [5, 2359–2367].

The aim of scientific research was the finding of possibility to separate and identificate the molecular size of HA by electrophoresis in the polyacrylamide gel.

Materials and methods. The electrophoresis of 0.1%, 0.2%, 10% solutions of HA, "Oxyal" and "Biotrue"medicaments, which contain HA conducted in polyacrylamide gel. The size of HA molecule was calculated by markers of molecular weight Ladder 50 and pUC19 Msp I with the help of TotalLab program. Visualization of HA and markers in polyacrylamide gel was provided by $AgNO_3$.

Results. The identified molecular sizes of HA are present in table.

Table – 1. Molecular sizes of HA

Substance	Concentration of HA,%	Size of HA according to base pairs of DNA
HA solution	0.10	1752; 1850
	0.20	1752; 1850
	10.00	1752; 1850
Oxyal	0.20	1745; 1841
Biotrue	0.24	1747

By results, HA in 0.1%, 0.2%, 10% solutions has the size which is equivalent to 1752–1850 base pairs of DNA. HA in "Oxyal" medicament was consisted of two fractions equivalent to 1745 and 1841 base pairs of DNA. HA in "Biotrue" medicament was consisted of two fractions equivalent to 1747 base pairs of DNA.

Discussion. As follows, possibility to separate and identificate the specific size of HA molecules in polyacrylamide gel by electrophoretic method was shown. This method requires less time and resources and can be used in the industry and medicine.

References:

1. Fakhari A., Phan Q., Berkland C. Hyaluronic acid colloidal gels as self-assembling elastic biomaterials. J Biomed Mater Res B Appl Biomater. 102 (3),– 2014.– P. 612–618.
2. Sati L., Cayli S., Delpiano E., et al. The pattern of tyrosine phosphorylation in human sperm in response to binding to zona pellucida or hyaluronic acid. Reprod Sci. 21 (5),– 2014.– P. 573–581.
3. Borzacchiello A., Russo L., Malle B., et al. Hyaluronic acid based hydrogels for regenerative medicine applications. Biomed Res Int.– 2015.– 12 p.
4. Maharjan A., Pilling D., Gomer H. High and low molecular weight hyaluronic acid differentially regulate human fibrocyte differentiation. PLoS One. 6 (10),– 2011.– 10 p.
5. Hansen I., Ebbesen M., Kaspersen L., et al. Hyaluronic acid molecular weight-dependent modulation of mucin nanostructure for potential mucosal therapeutic applications. Mol Pharm. 14 (7),– 2017.– P. 2359–2367.