Reveal the Relationship Between Hyaluronic Acid and Water Using Aquaphotomics

Weilu Tian[1], Qin Dong[1], Boran Lin[1], Xiangchun Yang[1], Hui Zhang[1], Lian Li[1], Lei Nie[1], Xueping Guo[2], Siling Huang[2] and Hengchang Zang[1]*

[1]School of Pharmaceutical Sciences, Shandong University, Jinan, Shandong, PR China;
[2]Bloomage Biotechnology Corporation Limited, Jinan, Shandong, PR China

Asian Journal of Complementary and Alternative Medicine. Volume 09 Issue 1

Published on: 06/03/2021

***Author for Correspondence:** Hengchang Zang, School of Pharmaceutical Sciences, Shandong University, Wenhuaxi Road 44, Jinan 250012, PR China. E-mail address: zanghcw@126.com.

Cite this article as: Tian W, Dong Q, Lin B, Yang X, Zhang H, et al. *Reveal the Relationship Between Hyaluronic Acid and Water Using Aquaphotomics.* Asian Journal of Complementary and Alternative Medicine, Vol 9(1), 28-31:2021.

ABSTRACT

Hyaluronic acid (HA) is a kind of biological macromolecule with strong water binding ability. It has rich biological functions and plays an important role in the living body. It has extremely high application value in the fields of medical beauty, medicine, medical treatment and food. In the past, the thinking of studying HA was rather rigid, which is reflected in the direct study of HA itself, which is quite difficult in a complex system because there are too many influencing factors in the real biological environment. The proposal of aquaphotomics allows researchers to focus on the water molecules in complex biological systems, which leads us to shift the angle of thinking about HA-related issues to the water molecules that are closely bound to it. In previous and ongoing work, we use spectroscopy technology and aquaphotomics to study water species, focus on the widely used HA and its derivatives on the market, and apply multivariate analysis methods to analyze the interaction between HA and water molecules to further clarify the material properties of HA form the basis for monitoring its process of binding water in the body. This paper briefly reviews important knowledge concerning the relationship between HA and water, and explains our past and ongoing related research in this field.

Key words: hyaluronic acid, water, aquaphotomics, spectroscopy

OVERVIEW

HA, also called hyaluronan, is a glycosaminoglycan composed of the basic structure of disaccharides (formed by repeated connection of D-glucuronic acid and N-acetylglucosamine through β-1,4 and β-1,3 glycosidic bonds) [1]. HA is widely distributed in human and animal tissues and extracellular matrix, and the content is higher in the vitreous body, skin, umbilical cord, joints and other parts of the eyeball. Besides, HA is also the main component in the capsules of some bacteria, such as *Streptococcus* and *Pseudomonas aeruginosa* [1]. The synthesis of HA in cells is a complex process that requires the participation of multiple enzymes, the most important of which is HA synthase (HAS) [2]. The early way to obtain HA was to extract it from human or animal tissues, but the yield of products obtained in this way is limited [3,4]. In recent years, with the advancement of genetic engineering and fermentation engineering, the use of microbial fermentation to obtain large quantities and high purity HA has become the mainstream. At present, bacteria *Streptococcus* and *Lactococcus* are mostly used as fermentation strains [5-8]. At the same time, some scientists studied the *in vitro* enzymatic synthesis of HA and made some progress [9,10]. However, the limitation of this method lies in the expensive raw materials and the difficulty in achieving large-scale industrial production.

HA is a physiologically active substance that has many functions such as moisturizing, lubricating, promoting tissue repair, cell protection, promoting the proliferation of immune cells and targeting tumor cells [11,12]. In addition, it also has the characteristics of non-toxicity, low immune response, high biocompatibility, biodegradability and human body absorption, so it has mature applications in the fields of cosmetics, skin care products and pharmaceuticals [13]. In December 2020, HA was approved as a new food raw material in China, which can be applied to ordinary food additives, which means that HA will have a broad space for development in the food field in the future.

Molecular weight is one of the most noteworthy indicators in the physical and chemical properties of HA, and it directly affects the biological activity of HA. According to the general division principle, HA can be divided into high molecular weight HA (>1000 kDa), medium molecular weight HA (100 kDa~1000 kDa) and low molecular weight HA (<100 kDa) including oligomeric HA (polymerization degree less than 25) [14]. The low molecular weight HA exhibits linearity in spatial morphology, while the high molecular weight HA is entangled and develops toward the formation of a network [15]. Molecular weight affects its rheological properties, which affects its hydration capacity and other biological activities, and ultimately determines the appropriate use [16,17]. The penetration of low molecular weight HA is stronger, and the viscoelasticity of high molecular weight HA is stronger. Essendoubi et al. [18] used Raman microscopy to monitor the skin penetration of HA of different molecular weights, and found that HA of different molecular weights can be absorbed by the skin. HA with a molecular weight of 1,000 kDa to 1,400 kDa mainly enters the stratum corneum, while HA of 20 kDa to 300 kDa HA penetrates deeper.

At present, the molecular weight range of most HA produced by fermentation is in the range of 10^5~10^7 Da [19]. If the goal is to obtain HA with a specific molecular weight distribution range, the traditional method is to degrade high molecular weight HA through enzymatic, chemical or physical methods. Studies have shown that the molecular weight of HA is affected by the primary structure of HAS, that is, the amino acid sequence [20,21]. Along this line of thinking, trying to change the amino acid composition of HAS through genetic engineering means may be able to produce HA with a specific molecular weight distribution range, which is undoubtedly a promising attempt to respond to the corresponding market demand.

Since HA contains a large number of hydroxyl and carboxyl groups in its chemical structure, intramolecular and molecular hydrogen bonds have been formed in the aqueous solution, so it has excellent hydrophilicity. The rigid helical columnar structure of HA can keep the water molecules it binds inside the structure, so that water is not easy to lose, which is also an important reason for its strong ability to lock water [22]. In the past, it was generally believed that a molecule of HA can hold up to 500 water molecules, and this is why HA is known as natural moisture factor (NMF) [4]. The moisture content in the skin is directly related to the HA content. Studies have shown that HA has a higher moisture absorption capacity at low relative humidity, but has a lower moisture absorption capacity at high relative humidity [23]. The moisturizing effect of high molecular weight HA is stronger than that of low molecular weight HA [23].

As people age, the HA content and water content in the body show a gradual downward trend almost simultaneously [24]. This law is mysterious and vague, because it is especially difficult to obtain the precise relationship between the two in different life forms, different organizations, and in different environments. However, when a certain specification of HA is specifically applied under specific requirements, the relationship between the two must be considered. The loss of HA and water molecules has a major impact on the beauty of the skin and the freedom of joint movement. Therefore, the use of injections or smear filling and other means to replenish HA in time to stay youth and maintain normal exercise ability are two areas with huge demand in the medical aesthetics market. However, there is currently no in-depth research on the interaction between HA and water molecules, which restricts the precise control of the usage and dosage of HA and its derivatives. The reason for the unclear understanding of this scientific problem is that no relevant research system has been found in the past to give enlightenment. It was not until the theory of aquaphotomics was put forward by Prof. Tsenkova in 2005 that we realized that this might be a feasible way to clarify the relationship between HA and water [25].

Aquaphotomics represents a unique tool to study and understand water and biological systems in real-time and non-destructively [26]. It takes the water in the life system as the research object, uses spectroscopy to detect the structural changes of water molecules in different environments, and reflects the interaction between water molecules and other molecules in the life system or the function of water in the life system at the molecular level [27]. Aquaphotomics uses the influence of disturbance factors on the spectrum of water to analyze the life system, and uses the multivariate analysis method to find the peak changes in the spectrum, which can reflect the changes of other molecules in the solution at the molecular level [27,28]. Prof. Tsenkova analyzed the information of water molecules in a large number of aqueous systems and established the concept of water matrix coordinate (WAMACS), which is of great benefit to the qualitative and quantitative research of water molecules and target substances in the system [26-29]. With the help of aquaphotomics, it has become easier to reveal the information change laws of target substances in complex systems in chemistry, biology, physics and other fields. At the same time, more and more researchers have attracted more and more researchers to use this theory to solve the problem in recent years.

It is a systematic and complex task to clarify the dynamic change law of HA in the skin and combined with water. Therefore, our strategy is to start with the preparation of HA solution for *in vitro* experiments and conduct in-depth exploration. In the previous research, our research group used near-infrared spectroscopy (NIR) to study HA (7,775 Da) aqueous solutions under different concentrations and temperature disturbances, and analyzed the hydration of HA in aqueous solutions based on aquaphotomics [30]. Studies showed that as the concentration of HA increased, the absorption value of the first overtone region of OH (reflecting hydration water) changed nonlinearly. Water species with two hydrogen bonds (S_2) and three hydrogen bonds (S_3) showed a decrease in the low concentration range of 0-40 mg/mL, but increased at higher concentrations, indicating the water species were different under different HA concentrations. At the same time, it was also found that HA has the ability to improve the thermal stability of the water structure, indicating a potential biological protection function.

In recent work, we used NIR, applied aquaphotomics, used temperature and pH value as disturbance factors, and used qualitative and quantitative chemometric methods to study the changes of water species and relative content in HA solution, revealing the correlation between the ability to bind water and the concentration, molecular weight and aqueous environment of HA. As a result, it was found that the interaction between different specifications of HA and water molecules is different and will be affected by environmental factors. In the study of HA hydrogels, we used Raman spectroscopy, hoping to use aquaphotomics and chemometrics processing methods to quickly identify the water content of the filler and the corresponding water species. These parts of the work provided a basis for the scientific application of HA products, and the relevant research results will be published in the near future.

SUMMARY

HA forms a large number of intramolecular and intermolecular hydrogen bonds in the water environment. When the external conditions change, the intermolecular interaction mode changes immediately. Exploring the specific conditions of HA bound to water molecules in a specific environment is of great significance for precise medication. Therefore, studying to understand the interaction between HA and water in a complex environment will deeply understand the essential reasons for HA to exert its biological activity, and it will also be of great benefit to the development and use of HA products. In short, regardless of the physical and chemical properties, functions, and uses of HA, the role played by water molecules cannot be ignored. For the analysis of complex situations, aquaphotomics is an inevitable tool.

AUTUOR CONTRIBUTION

W.T. collected, sorted out the reference materials, and wrote the draft. Q.D., B.L., X.Y., H.ZH., L.L, L.N, X.G. and S.H. revised different parts of the draft. H.Za. pointed out the content framework to be described in this review, and made the final correction to the draft.

COMPETING INTERESTS

The authors declare no competing interests.

REFERENCES

1. Fallacara A, Baldini E, Manfredini S, Vertuani S (2018) Hyaluronic acid in the third millennium. *Polymers*, 10(7): 701-736.
2. Yang J, Cheng F, Yu H, Wang J, Guo Z, et al. (2017) Key role of the carboxyl terminus of hyaluronan synthase in processive synthesis and size control of hyaluronic acid polymers. *Biomacromolecules*, 18(4): 1064-1073.
3. Meyer K, Palmer J W (1934) The polysaccharide of the vitreous humor. *Journal of Biological Chemistry*, 107(3): 629-634.
4. Zadorojnâi L, Zadorozhnâi A (2012) Hyaluronic acid: obtaining, properties and application. *Chemistry Journal of Moldova*, 7(2):57-66.
5. Dougherty B A, Van de Rijn I (1994) Molecular characterization of hasA from an operon required for hyaluronic acid synthesis in group A *Streptococci*. *Journal of Biological Chemistry*, 269(1): 169-175.
6. Chien L J, Lee C K (2007) Hyaluronic acid production by recombinant *Lactococcus lactis*. *Applied Microbiology and Biotechnology*, 77(2): 339-346.
7. Prasad S B, Jayaraman G, Ramachandran K B. (2010) Hyaluronic acid production is enhanced by the additional co-expression of UDP-glucose pyrophosphorylase in *Lactococcus lactis*. *Applied Microbiology and Biotechnology*, 86(1): 273-283.
8. Jeeva P, Doss S S, Sundaram V, Jayaraman G (2019) Production of controlled molecular weight hyaluronic acid by glucostat strategy using recombinant *Lactococcus lactis* cultures. *Applied Microbiology and Biotechnology*, 103(11): 4363-4375.
9. Li S, Wang S, Fu X, Liua X, Wang PG, et al. (2017) Sequential one-pot multienzyme synthesis of hyaluronan and its derivative. *Carbohydrate Polymers*, 178(12): 221-227.
10. Li J, Qiao M, Ji Y, Linc L, Zhang X, et al. (2020) Chemical, enzymatic and biological synthesis of hyaluronic acids. *International Journal of Biological Macromolecules*, 152(6): 199-206.
11. Garantziotis S, Savani R C. Hyaluronan biology: A complex balancing act of structure, function, location and context. *Matrix Biology*, 2019, 78(5): 1-10.
12. Tavianatou A G, Caon I, Franchi M, Piperigkou Z, Galesso D, et al. (2019) Hyaluronan: molecular size-dependent signaling and biological functions in inflammation and cancer. *The FEBS Journal*, 286(15): 2883-2908.
13. Gupta R C, Lall R, Srivastava A, Sinha A (2019) Hyaluronic acid: Molecular mechanisms and therapeutic trajectory. *Frontiers in Veterinary Science*, 6(6): 192-215.
14. Saranraj P, Naidu M A (2013) Hyaluronic acid production and its applications: a review. *International Journal of Pharmaceutical & Biological Archives*, 4(5): 853 - 859.
15. Taweechat P, Pandey R B, Sompornpisut P (2020) Conformation, flexibility and hydration of hyaluronic acid by molecular dynamics simulations. *Carbohydrate Research*, 493(5): 1-9.

16. Rayahin J E, Buhrman J S, Zhang Y, Koh TJ, Gemeinhart RA, et al. (2015) High and low molecular weight hyaluronic acid differentially influence macrophage activation. *ACS Biomaterials Science & Engineering*, 1(7): 481-493.

17. Chistyakov DV, Astakhova AA, Azbukina NV, Goriainov SV, Chistyakov VV, et al. (2019) High and low molecular weight hyaluronic acid differentially influences oxylipins synthesis in course of Neuroinflammation. *International Journal of Molecular Sciences*, 20(16): 3894-3907.

18. Essendoubi M, Gobinet C, Reynaud R, Angiboust JF, Manfait M, et al. (2016) Human skin penetration of hyaluronic acid of different molecular weights as probed by Raman spectroscopy. *Skin Research and Technology*, 22(1): 55-62.

19. Liao Y H, Jones SA, Forbes B, Martin GP, Brown MB (2005) Hyaluronan: pharmaceutical characterization and drug delivery. *Drug Delivery*, 12(6): 327-342.

20. Jin P, Kang Z, Yuan P, Du G, Chen J (2016) Production of specific-molecular-weight hyaluronan by metabolically engineered *Bacillus subtilis* 168. *Metabolic Engineering*, 35: 21-21.

21. Mandawe J, Infanzon B, Eisele A, Zaun H, Kuballa J, et al. Directed Evolution of Hyaluronic Acid Synthase from *Pasteurella multocida* towards High-Molecular-Weight Hyaluronic Acid. *Combining Chemistry and Biology*, 2018, 19(13): 1414-1423.

22. Sun X, Gong H, Xie M, Chun-LiuZhao (2020) All-fiber humidity sensor based on Michelson interferometer with hyaluronic acid and polyvinyl alcohol composite film. *Optical Fiber Technology*, 60(11):1-6.

23. Guo X, Guo A, Ling P. Application of hyaluronic acid in cosmetics, health foods and soft tissue fillers. *Food and Drug (in Chinese)*, 2005, 7(1):20-23.

24. Papakonstantinou E, Roth M, Karakiulakis G (2012) Hyaluronic acid: a key molecule in skin aging. *Dermato-Endocrinology*, 4(3): 253-258.

25. Tsenkova, R (2005) “Visible-near infrared perturbation spectroscopy: water in action seen as a source of information” in 12th International Conference on Near-infrared Spectroscopy (Auckland), 607–612.

26. Tsenkova R, Munćan J, Pollner B, Zoltan Kovacs (2018) Essentials of aquaphotomics and its chemometrics approaches. *Frontiers in Chemistry*, 6(8): 1-25.

27. Tsenkova R. Aquaphotomics: dynamic spectroscopy of aqueous and biological systems describes peculiarities of water. *Journal of Near Infrared Spectroscopy*, 2009, 17(6): 303-313.

28. Gowen A A, Amigo J M, Tsenkova R. Characterisation of hydrogen bond perturbations in aqueous systems using aquaphotomics and multivariate curve resolution-alternating least squares. *Analytica Chimica Acta*, 2013, 759(1): 8-20.

29. Tsenkova R (2010) Aquaphotomics: water in the biological and aqueous world scrutinised with invisible light. *Spectroscopy Europe*, 22(6): 6-10.

30. Dong Q, Guo X, Li L, Yu C, Nie L, et al. (2020) Understanding hyaluronic acid induced variation of water structure by near-infrared spectroscopy. *Scientific Reports*, 10(1): 1-8.