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Short communication

Degradation of hyaluronan by an electrochemical process

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ABSTRACT

Degradation of hyaluronan (hyaluronic acid, HA) by electrochemical process was investigated and the effect on the degradation of HA was demonstrated by means of viscometry and size exclusion chromatography. The structures of the original and degraded HA were characterized by Fourier transform infrared spectra, nuclear magnetic resonance spectra and ultraviolet spectra analysis. The experimental results showed that when HA was treated by electrolysis using Ti/TiO_2–RuO_2 electrode, the molar mass of HA dramatically decreased with the increase of electrolysis time and current density, and the chemical structure of degraded HA was not obviously modified. It was concluded that the electrochemical method can be a useful alternative for degradation of HA.

1. Introduction

Hyaluronan (hyaluronic acid, HA) is a linear anionic polysaccharide, composed of repeating disaccharide units of *N*-acetyl-D-glucosamine and D-glucuronic acid. The high molecular mass HA plays an important role in many biological processes and has found many applications in medicine, pharmacy and cosmetics (Arshinoff, 1995; Lapcik, Lapcik, Smedt, Demeester, & Chabrecek, 1998). In recent years, the low molecular mass HA has also been reported to aid wound healing by promoting angiogenesis (West, Hampson, Arnold, & Kumar, 1985). HA fragments can also, under specific circumstances, promote early inflammation, which is critical to initiate wound healing, which can then modulate later stages of the process, allowing for matrix stabilization and reduction of long-term inflammation (Morra, 2005). Functional maturation of dendritic cells during inflammation, inhibition of anchorage-independent growth of tumour cells and protection of granulation tissue from oxygen free radical damage have also been ascribed to a mixture of HA fragments (Ghatak, Misra, & Toole, 2002).

Many methods have been applied to degrade HA into lower molecular mass fragments such as enzymatic hydrolysis (Li & Jedrzejas, 2001), acid hydrolysis (Tokita & Okamoto, 1995), oxidative degradation (Hokptusa, Jumel, Alexander, & Harding, 2003), high temperature in an autoclave (Bothner, Waaler, & Wik, 1988), ultrasonic radiation (Miyazaki, Yomota, & Okada, 2001), and microwave irradiation (Bezáková et al., 2008). Recently we reported an electrochemical method for the chitosan degradation (Cai et al., 2010). Chitosan was degraded efficiently by electrolysis using Ti/TiO_2–RuO_2 electrode. The objective of this work was to determine the feasibility of degradation of HA by electrochemical process. HA was treated by electrolysis using Ti/TiO_2–RuO_2 electrode. The degraded HA products were characterized and analyzed by viscometry, size exclusion chromatography and FT-IR, NMR and UV-spectroscopic techniques.

2. Experimental

2.1. Materials

HA (viscosity average molecular weight, M_v, 1463 kDa) was purchased from Freda Biochem. Co. (Shandong, China). All other chemicals were of analytical grade. Distilled water was used. Ti/TiO_2–RuO_2 electrode (5 cm × 7 cm) was prepared by thermal deposition of TiO_2 and RuO_2 (Ti/Ru molar ratio = 7:3) on Ti plate (Beer, 1972).

2.2. Analytical methods

The M_v of HA was determined by a viscometric method reported in literature (Laurent, Ryan, & Pietruszkiewicz, 1960). The samples were dissolved in 0.2 mol/L NaCl solutions. Viscometric measurements were carried out using an Ubbelohde capillary viscometer at 25 °C. The sample was introduced to the viscometer and four dilutions were made in situ. The reduced viscosity was plotted as a function of the concentration and, by extrapolating to zero concentration, the intrinsic viscosity, $[\eta]$, was obtained. Then, the M_v of

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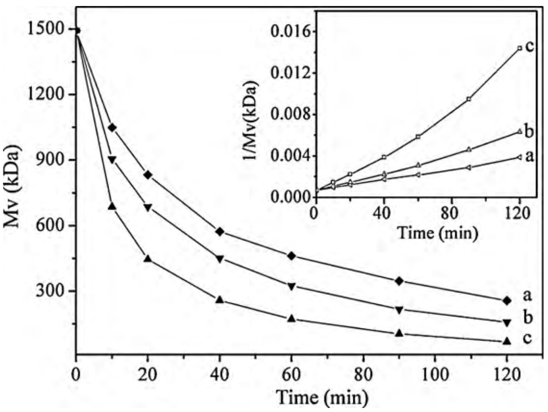

Fig. 1. Variation of M_v of HA with treatment time at different current densities of 50 mA/cm^2 (a), 100 mA/cm^2 (b), and 150 mA/cm^2 (c). Inset: $1/M_v$ versus treatment time.

HA was calculated based on the Mark–Houwink equation (Laurent et al., 1960): $[\eta] = k \cdot M_v^a$ with $k = 3.6 \times 10^{-2}$, $a = 0.78$.

The molecular properties or relative molar mass values were measured using size exclusion chromatography (SEC, Agilent Co. Ltd.). SEC was performed on a column, TSK-GMPW$_{XL}$ (7.8 × 300 mm) with 0.2 M NaCl solution at a flow rate of 0.6 mL/min. A 500 mL portion of the diluted sample containing about 0.05% HA was injected and the peak elution was monitored with DAWN EOS MALLS photometer (Wyatt Co. Ltd.) and G1362A differential refractometer.

FT-IR spectra of original and degraded HA were recorded in powder form in KBr discs at the range of 4000–400 cm^{-1} on a Bruker Vector 22 FT-IR spectrophotometer.

^{1}H and ^{13}C NMR spectra (in D_2O) of original and degraded HA were obtained in the inverse gated decoupling mode at 25 °C on a Bruker AVANCE-400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C.

UV spectra of original and degraded HA were obtained using Shimadzu UV-1201 spectrophotometer at the range of 200–500 nm.

2.3. Electrolysis of HA

0.1% (w/v) HA in 0.2 mol/L acetic acid–0.3 mol/L sodium acetate solution was prepared firstly. The electrolysis of 250 mL HA solution was performed in a reactor (Ø 7 cm × *H* 12 cm) with Ti/TiO_2–RuO_2 as anode, stainless steel as cathode (5 cm × 7 cm) and the spacing of 20 mm between two electrodes. A DC potentiostat was used as the power supply for the electrolysis. During the electrochemical treatment the solution was stirred constantly and the temperature was controlled at 50 °C. After degradation, three volumes of ethanol were added to the solution and after depositing overnight the formed precipitate was separated by filtration, then washed thoroughly with ethanol and dried overnight in a vacuum at 60 °C yielding the degraded HA products.

3. Results and discussions

3.1. Effect of electrolysis on the molar mass of HA

In order to examine whether an effect of electrochemical method is operative in the degradation of HA, a series of experiments were designed. The M_v of HA was plotted as a function of the reaction time. The results are illustrated in Fig. 1. As seen, the degradation proceeded in the whole stage of the reaction from 0 min to 120 min. Fig. 1c shows that the M_v of HA dramatically decreased from 1493 to 69 kDa at 120 min. It was obviously presented that electrolysis was a quite effective method for degradation of HA. The curves also show that when the current densities were varied from 50 to 150 mA/cm^2, the M_v of HA decreased to 258, 158, and 69 kDa at 120 min, respectively. It indicated that the increase of current density would result in the decrease of the M_v of HA, and therefore led to a better degradation of HA. Moreover, it can be seen that a linear relationship existed between the $1/M_v$ and reaction time (inset in Fig. 1). This indicated that a random scission of HA chains occurred.

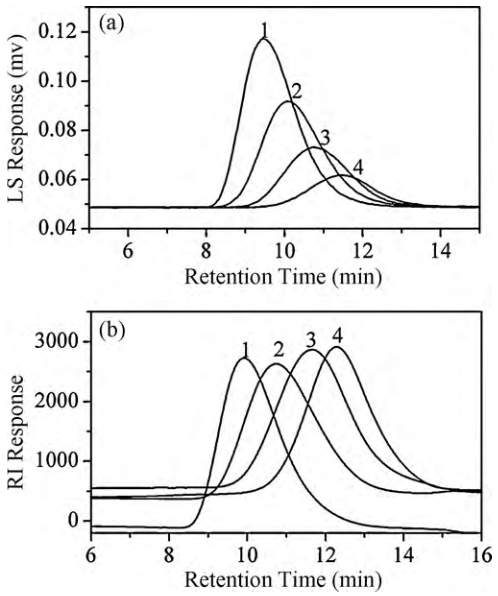

Fig. 2. MALLS (a) and RI (b) chromatograms of HA at the different electrolysis times: original HA (1), 20 min (2), 60 min (3), 120 min (4).

For further demonstrating the effect of electrochemical degradation of HA, the SEC method was used for qualitative evaluation of the reduction in molar mass of HA and its distribution. Fig. 2 shows SEC chromatograms of original HA and its degraded products resulting from electrolysis at the current density of 150 mA/cm^2 at 20, 60 and 120 min. The elution profiles of both series of chromatograms appeared as single peaks. As the electrolysis time increased, peak elution volumes by the MALLS detector became smaller and shifted towards lower molecular masses, what in accord with shiftening of peak elution volumes by the RI-detector to higher values indicate decrease in hydrodynamic volume and increasing extent of depolymerisation. Therefore, the results in Fig. 1 confirmed the decrease of the molar mass of HA with increasing electrolysis time.

3.2. FT-IR spectral analysis

The FT-IR spectra of original and degraded HA (M_v69 kDa) are shown in Fig. 3. There are several main bands of original HA in Fig. 3(a): COO$^-$ group at 1617 and 1411 cm^{-1}, Am I at 1653 cm^{-1}, Am II at 1563 cm^{-1}, C–O–C group at 1150 cm^{-1} (O-bridge), the C–O (exocyclic) and C–C groups at 1079 cm^{-1}, and the C–OH group at 1042 cm^{-1} (Kim et al., 2008). As can be seen, the overall spectral pattern did not change by decreasing the molecular size of HA and additional bands did not appear even in the spectrum of the degraded HA. Therefore, the result of FT-IR spectra suggested that there was no obvious modification of chemical structure of degraded HA.

3.3. ^{1}H and ^{13}C NMR spectral analysis

The ^{1}H and ^{13}C NMR spectra of the original and degraded HA (M_v69 kDa) are shown in Figs. 4 and 5, respectively. Their spec-

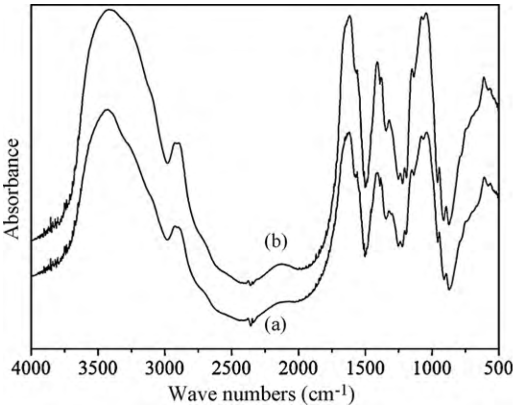

Fig. 3. FT-IR spectra of original HA M_v 1463 kDa (a) and degraded HA M_v69 kDa (b).

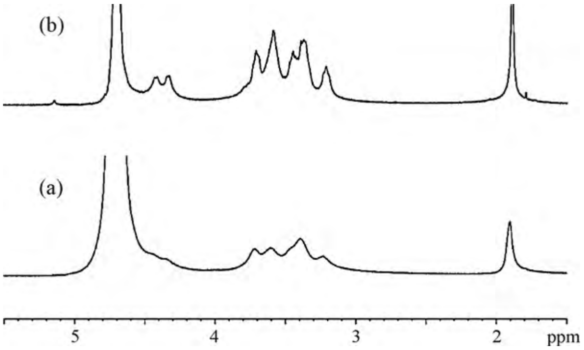

Fig. 4. ^{1}H NMR spectra of original HA M_v1463 kDa (a) and degraded HA M_v69 kDa (b).

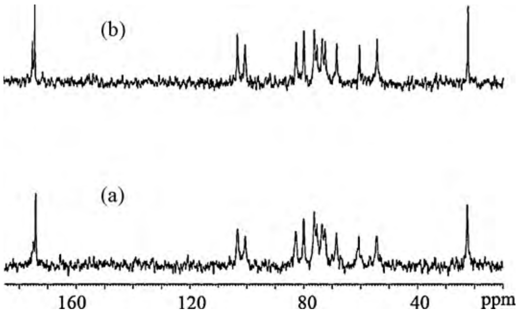

Fig. 5. ^{13}C NMR spectra of original HA M_v1463 kDa (a) and degraded HA M_v69 kDa (b).

tral pattern resembled that of the hitherto published spectra of HA (Bezáková et al., 2008). The very weak signal at 5.14 in the ^{1}H NMR spectra of the degraded HA was assigned to C1 of α-anomers of GlcpNAc reducing end residues. No differences were recorded between the chemical shifts of the carbon atoms of the original HA and its degraded products. Therefore, the result of ^{1}H and ^{13}C NMR spectra indicated that there was no obvious modification of chemical structure of degraded HA.

3.4. UV spectral analysis

The UV spectra of original and degraded HA (M_v69 kDa) are shown in Fig. 6. It revealed a new expanding absorption band at ~240 nm. Based on the data reported in the published literature (Bezáková et al., 2008; Kim et al., 2008), these absorption bands could be ascribed to carbonyl or carboxyl groups formed during the degradation process.

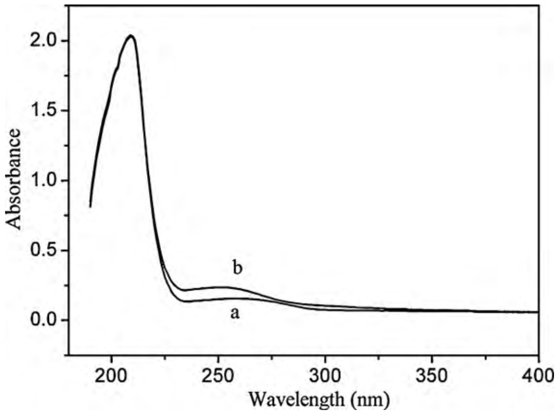

Fig. 6. UV spectra of original HA M_v1463 kDa (a) and degraded HA M_v69 kDa (b).

4. Conclusions

HA can be effectively degraded by electrochemical process which was verified by viscometry and SEC. The molar mass of HA decreased with the increase of electrolysis time and current density. The result of FT-IR, ^{1}H and ^{13}C NMR analysis suggested that there was no obvious modification of chemical structure of degraded HA. The result of UV spectra analysis indicated that the carbonyl or carboxyl groups were formed during the degradation process. Therefore the degradation method of HA by electrochemical process is feasible, convenient and potentially applicable.

References

Arshinoff, S. A. (1995). Dispersive and cohesive viscoelastic materials in phacoemulsification. *Ophthalmic Practice*, *13*, 98–104.

Beer, H. B. (1972). Electrodes and Coating therefor. *US Patent Office*, Patent No. 3 632 498.

Bezáková, Z., Hermannová, M., Dřímalová, E., Malovíková, A., Ebringerová, A., & Velebný, V. (2008). Effect of microwave irradiation on the molecular and structural properties of hyaluronan. *Carbohydrate Polymers*, *73*, 640–646.

Bothner, H., Waaler, T., & Wik, O. (1988). Limiting viscosity number and weight average molecular weight of hyaluronate samples produced by heat degradation. *International Journal of Biological Macromolecules*, *10*, 287–297.

Cai, Q., Gu, Z., Chen, Y., Han, W., Fu, T., Song, H., et al. (2010). Degradation of chitosan by an electrochemical process. *Carbohydrate Polymers*, *79*, 783–785.

Ghatak, S., Misra, S., & Toole, B. P. (2002). Hyaluronan oligosaccharides inhibit anchorage-independent growth of tumor cells by suppressing the phosphoinositide 3-kinase/Akt cell survival pathway. *The Journal of Biological Chemistry*, *277*, 38013–38020.

Hokptusa, S., Jumel, K., Alexander, C., & Harding, S. E. (2003). Hydrodynamic characterisation of chemically degraded hyaluronic acid. *Carbohydrate Polymers*, *52*, 111–117.

Kim, J. K., Srinivasan, P., Kim, J. H., Choi, J. I., Park, H. J., Byun, M. W., et al. (2008). Structural and antioxidant properties of gamma irradiated hyaluronic acid. *Food Chemistry*, *109*, 763–770.

Lapcik, L., Jr., Lapcik, L., Smedt, S. D., Demeester, J., & Chabrecek, P. (1998). Hyaluronan: Preparation, structure, properties, and applications. *Chemical Reviews*, *98*, 2663–2684.

Laurent, T. C., Ryan, M., & Pietruszkiewicz, A. (1960). Fractionation of hyaluronic acid, the polydispersity of hyaluronic acid from the bovine vitreous body. *Biochimica et Biophysica Acta*, *42*, 476–485.

Li, S., & Jedrzejas, M. J. (2001). Hyaluronan binding and degradation by *Streptococcus agalactiae* hyaluronate lyase. *The Journal of Biological Chemistry*, *276*, 41407–41416.

Miyazaki, T., Yomota, C., & Okada, S. (2001). Ultrasonic depolymerization of hyaluronic acid. *Polymer Degradation and Stability*, *74*, 77–85.

Morra, M. (2005). Engineering of biomaterials surfaces by hyaluronan. *Biomacromolecules*, *6*, 1205–1223.

Tokita, Y., & Okamoto, A. (1995). Hydrolytic degradation of hyaluronic acid. *Polymer Degradation and Stability*, *48*, 269–273.

West, D. C., Hampson, I. N., Arnold, F., & Kumar, S. (1985). Angiogenesis induced by degradation products of hyaluronic acid. *Science*, *228*, 1324–1326.