Bioorganic & Medicinal Chemistry Letters 22 (2012) 5003–5007

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# Synthesis of vanadyl–hypocrellin A complex and its photodynamic properties research

Chao Yang [a,b], Fei Ma [d], Jing Tang [a,b], Lina Han [a,b], Shaohua Wei [b,c], Lin Zhou [b,c,*], Jiahong Zhou [a,c,*], Jian Shen [b,c], Fang Wang [a]

[a] Analysis and Testing Center, Nanjing Normal University, Nanjing 210046, PR China
[b] College of Chemistry and Materials Science, Nanjing Normal University, Nanjing 210046, PR China
[c] Jiangsu Key Laboratory of Biofunctional Materials, Nanjing Normal University, Nanjing 210046, PR China
[d] School of Geograph Science, Nanjing Normal University, Nanjing 210046, PR China

ARTICLE INFO

*Article history:*
Received 1 May 2012
Revised 1 June 2012
Accepted 9 June 2012
Available online 16 June 2012

*Keywords:*
$VO^{2+}$–HA complex
Hypocrellin A
Water solubility
Photodamage
Cytotoxicity

ABSTRACT

Hypocrellin A is an efficient photodynamic agent against many tumor cells and viruses. However, it was found that the preparation of injectable formula for HA was highly hampered by the poor water solubility of these compounds. So, here, a new water-soluble vanadyl–hypocrellin A complex was first synthesized and the complex forming process was studied using spectral and thermal dynamics methods. The results indicated that $VO^{2+}$–HA can stable in aqueous solutions and exhibit increased photostability, affinity and photocleavage ability toward ctDNA under anaerobic condition. Moreover, in vitro studies illustrated that $VO^{2+}$–HA also had strong anti-cancer activity.



Recently, photodynamic therapy (PDT) has been intensively studied as a modality for the treatment of various tumors.[1,2] PDT utilizes reactive oxygen species (ROSs), generated by irradiation the photosensitizers (PSs), to attack cancer cells and result in their death,[3–6] Hypocrellin A (HA, the CCDC deposition number is 663042) has been receiving intensive interest in the field of PDT due to its easy preparation and purification, high photo-toxicity but low dark-toxicity, and rapid clearance from normal tissues. However, it was found that the preparation of injectable formula for HA was highly hampered by the poor water solubility of these compounds. To solve this problem, many HA water-soluble derivatives and metal complexes were synthesized. Compared with the synthesis process of water-soluble derivatives, the preparation of metal complexes of HA is much easier.

Vanadium, exists in a variety of oxidation states.[7] In animals, they play an essential role even their biochemical functions have not been fully explained and still remain unclear.[8] In physiological conditions, $V^{IV}$ complexes are commonly observed in the form of vanadyl ion ($VO^{2+}$). Based on this conception, we synthesized a new complex of HA with $VO^{2+}$ ($VO^{2+}$–HA) to improve the water-solubility of HA.

The $VO^{2+}$–HA was synthesized as follows: in 3 mL water, various doses $VOSO_4$ and HA (20 μL, $1 \times 10^{-2}$ mol/L in DMSO) were mixed and the mixture was stirred for 2 h in dark. The initial mol ratio of HA and $VO^{2+}$ ratio were from 1:0 to 1:5. After the formation of $VO^{2+}$–HA, redundant regents were completely removed by dialyzing the solution against water in a 12–14 kDa cutoff cellulose membrane for 12 h. HA aqueous solution using DMSO as cosolvent was used as a control in all experiments. The samples were freeze drying for FTIR, EDS, TG and DSC analysis.

In the UV–Vis spectra,[9] as shown in Figure 1A, after connection with $VO^{2+}$, the two absorption peaks of HA at 472 nm and 590 nm shifted to 485 nm and 593 nm, respectively, which indicated that there was strong coordination interaction between $VO^{2+}$ and HA. Moreover, the intensity of 472 nm peak exhibited obvious decreasing but the 590 nm peak showed increasing and one set of isobestic points was observed, indicative of the complex formation. By molar ratio method,[10] we could calculate that $VO^{2+}$ can form 1:1 complex with HA (Fig. 1B).

To further verify the complex formation, FTIR spectra of HA or $VO^{2+}$–HA was analyzed (Fig. 2). In $VO^{2+}$–HA, the band of the stretching vibration of the carbonyl group in HA (1610 $cm^{-1}$) shifted to a lower frequency (1567 $cm^{-1}$). Besides, the band of the stretching vibration of V=O in $VOSO_4$ (1004 $cm^{-1}$) shifted to a higher frequency (1028 $cm^{-1}$). In addition, $VO^{2+}$–HA had two bands at 557 $cm^{-1}$ and 608 $cm^{-1}$ due to the V–O vibrating.[11–14]

* Corresponding authors. Tel./fax: +86 025 85898170.
*E-mail addresses:* zhoulin@njnu.edu.cn (L. Zhou), zhoujiahong@njnu.edu.cn (J. Zhou).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.06.025

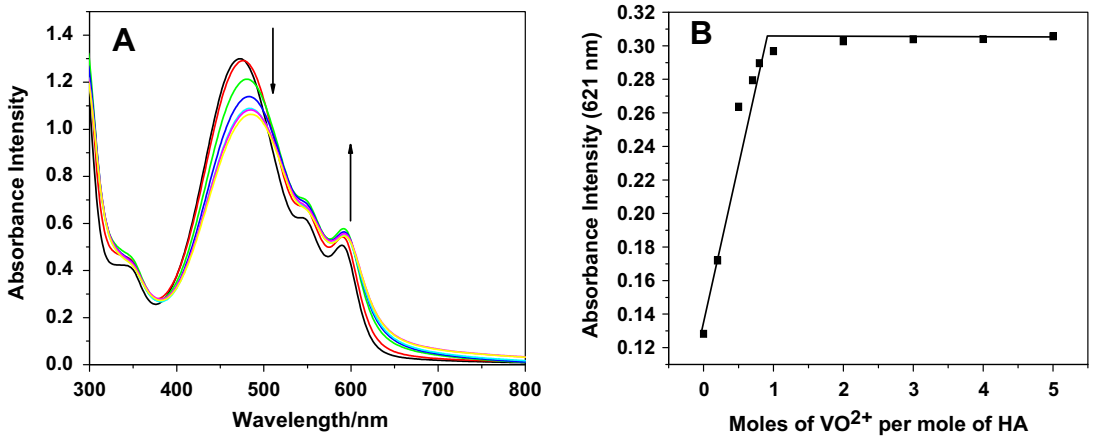


**Figure 1.** (A) Absorption spectrum changes of HA aqueous solution using DMSO as cosolvent upon the addition of $VO^{2+}$-[HA] = 66.7 μM, [$VO^{2+}$] = 0–266 μM and (B) Molar ratio plots for $VO^{2+}$–HA in water solution by the absorbance at 621 nm as a function of the molar ratio of $VO^{2+}$ to HA ([HA] = 66.7 μM).

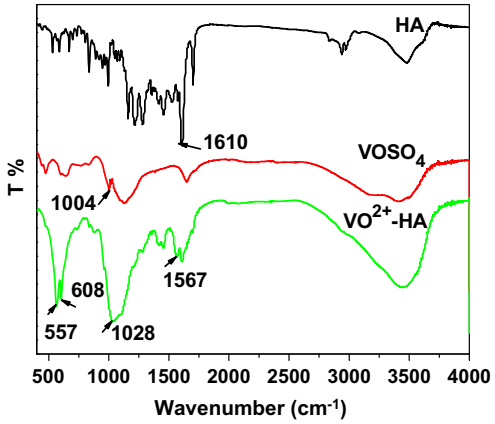


**Figure 2.** FTIR spectra of (a) HA; (b) $VOSO_4$; (c) $VO^{2+}$–HA.

Therefore, above results demonstrated that the complex of $VO^{2+}$ with HA was formed.[9]

The energy dispersion spectrum (EDS) was also used to verify the new complex had been formed. Comparing the energy disperse spectrum of HA and $VO^{2+}$–HA, the vanadium element can be detected in the complex, but in HA, vanadium peak cannot be detected.[9]

The thermogravimetry (TG) curve of HA exhibited its decomposition reaction at 299.82 °C (Fig. 3A). $VOSO_4$ possessed two lost weight process between 120–180 °C and 350–570 °C and its maximum decomposition temperature was 489.5 °C. The total lost weight ratio of $VOSO_4$ was 26.28% between the two steps. The curve for $VO^{2+}$–HA also exhibited two steps decomposition between 107.54–199.20 °C and 210–285 °C and its maximum decomposition temperature was 247.95 °C and the total lost weight ratio between the two steps was 13.06%, which was quite different from HA or $VO^{2+}$–HA. Moreover, in Figure 3B, the differential scanning calorimetry (DSC) curve of $VOSO_4$, one endothermic peak appeared at 241.33 °C, HA would melt at 220 °C. Conversely, $VO^{2+}$–HA had a new endothermic peak occurred at 478.50 °C. So, $VO^{2+}$–HA was not the simple mixture of HA and $VOSO_4$. Combining the above results of TG and DSC, we could conclude a new complex had been prepared.[9]

The absorption spectra of HA and $VO^{2+}$–HA solution after lifting to stand for 5 days were detected to evaluate the samples stability. The absorption intensity of the characteristic absorbance bands of $VO^{2+}$–HA had no obvious change but that of the HA aqueous solution sharply decreased in intensity (Fig. 4). Thus, comparing the changes in the absorption spectrum, it can be determined that $VO^{2+}$–HA had superior stability in the aqueous solution to free HA.

Most photosensitizers will degrade during the process of PDT by photobleaching. In this process, the intensity of the absorption will decrease.[15] Thus, the medicine used in PDT must not only have high photodynamic activity but also a slow photobleaching speed.[16] In photobleaching experiments,[17] photobleaching percent of $VO^{2+}$–HA and free HA were 3.42% and 15.46% during 30 min

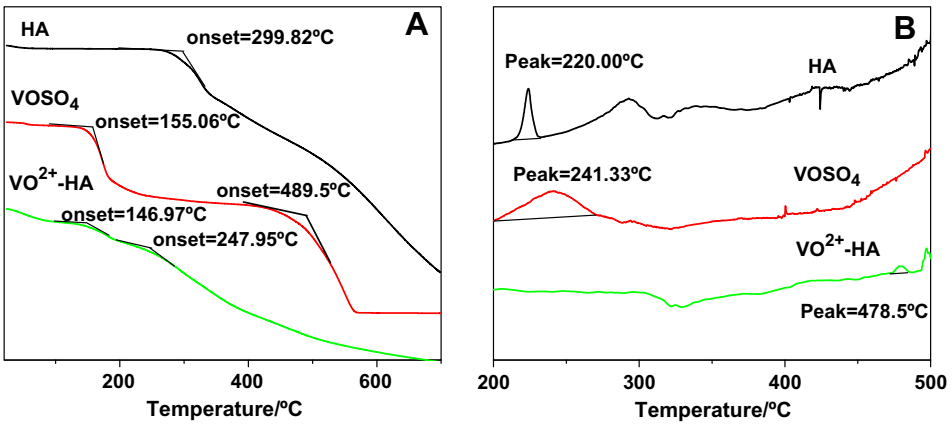


**Figure 3.** (A)TG and (B) DSC curves of (a) HA; (b) $VOSO_4$; (c) $VO^{2+}$–HA.