

Photochemical properties of a new kind of anti-cancer drug: *N*-glycoside compound

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Due to the nontoxicity and efficient anti-cancer activity, more and more attention has been paid to *N*-glycoside compounds. Laser photolysis of *N*-(α -*D*-glucopyranoside) salicyloyl hydrazine (NGSH) has been performed for the first time. The research results show that NGSH has high photosensitivity and is vulnerable to be photo-ionized via a monophotonic process with a quantum yield of 0.02, generating NGSH⁺ and hydrated electrons. Under the aerobic condition of cells, the hydrated electrons are very easy to combine with oxygen to generate ¹O₂ and O₂⁻, both of which are powerful oxidants that can kill the cancer cells. In addition, NGSH⁺ can be changed into neutral radicals by deprotonation with a p*K*_a value of 4.02 and its decay constant was determined to be 2.55×10⁹ dm³·mol⁻¹·s⁻¹. NGSH also can be oxidized by SO₄⁻ with a rate constant of 1.76×10⁹ dm³·mol⁻¹·s⁻¹, which further confirms the results of photoionization. All of these results suggest that this new *N*-glycoside compound might be useful for cancer treatment.

N-glycoside compound, laser photolysis, photoionization, quantum yield, anti-cancer mechanism

Cancer, which is only inferior to heart disease, has become the second leading cause of death of human beings. A survey conducted by the World Health Organization showed that the number of cancer patients is presenting a rising trend year by year. The cancer incidence in China is about 1.2 million per year, and the number of people who die each year from cancer is as high as 900 000. It is a major challenge for scientists all over the world to overcome cancer. There are always some drawbacks of drugs used currently, such as side effects, drug resistance, poor solubility and narrow anti-tumor spectra, etc. In recent years, many countries have spent a lot of manpower, materials and financial resources on the research and development of the anti-tumor drugs in order to achieve a breakthrough.

N-Glycoside compounds are an important kind of glucide compounds. Natural *N*-glycoside compounds exist mainly in the form of *N*-glycoprotein peptides and

N-glycoproteins with a variety of important functions and biological activities, such as immunoregulation, anti-tumor activity, anti-oxidation activity and anti-aging activity. In the 1970s, chemists have synthesized *N*-glycoside compounds and proved that they had the anti-tumor activity. Japanese scholar Sampathkumar et al.^[1] have studied the anti-tumor activity of mannose with the hydroxyl group in *C*-2 being substituted by different amide groups. Nicolas Bridiau in France has confirmed that *N*-glycoside compounds have better stability with respect to glycosidase compared with *O*-glycoside compounds. They are also stable in neutral aqueous solutions^[2]. However, there are few reports on the anti-

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tumor mechanisms of such kind of drugs. Using laser photolysis and pulse radiolysis technology, Wang et al.^[3-5] have studied the photochemical properties and the anti-tumor mechanism of podophyllotoxin and its glycoside derivatives Etoposide (VP-16), and analyzed the relationship between the reaction mechanism and the molecular structure, providing a new idea to the further synthesis of highly efficient and low toxicity anti-tumor drugs. In this study, by using aromatic acid, hydrazine and carbohydrate, a series of *N*-glycoside compounds were synthesized and proven to have high anti-tumor activity. The photochemical properties were also studied and a series of dynamic characteristics of the transient products were obtained, providing a foundation for further exploring the redox reaction mechanism between such transient products and DNA or its components (dGMP, dTMP, etc.) in cancer cells, and finding the relationship between the molecular structure and the anti-tumor activity.

The molecular formula of the compound for the laser photolysis study is C₁₃H₁₈O₇N₂ and the molecular weight is 314.3. The molecular structure is shown in Figure 1.

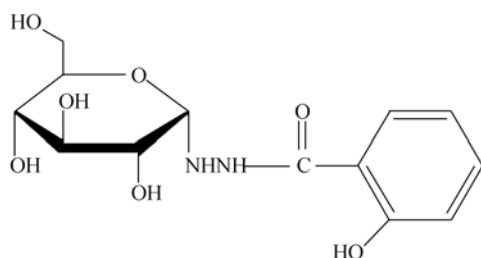


Figure 1 Molecular structure of NGSH.

1 Experimental section

1.1 Materials

NGSH was obtained from School of Chemistry and Chemical Engineering, Fuyang Normal College and was recrystallized before the experiment. Perchloric acid and sodium hydroxide, all of AR grade, were used to adjust the pH value of the solution. The solutions were prepared with triply distilled water just before the experiment and deaerated with highly pure nitrogen (99.99%) bubbling for 20 min or saturated with oxygen or nitrous oxide. All experiments were carried out at room temperature.

1.2 Experiment instruments

A nanosecond laser photolysis apparatus and a control-

lable flow ventilatory instrument were developed by School of Life Science and Technology, Tongji University. The other instruments used included an ultraviolet-visible spectrometer (VARIAN CARY 50 Probe), an electronic balance (METTLER TOLEDO AL204), a pH meter (METTLER TOLEDO DELTA320), an ultrasonic cleaning instrument (Shanghai Zhixin Instrument Company, DL-360D), a thermostatic drying chamber (Shanghai Jinghong Laboratory Instrument Co., Ltd., DHG-9140A) and an ultra pure water device (MILLIPORE Milli-Q).

1.3 Methods

A YAG laser was used as the excitation source in the laser photolysis apparatus (266 nm, 3 ns pulse width, 60 mJ per pulse). The source of analyzing light was a 500 W xenon lamp, which can be brightened 100 times during the detecting period. The laser and analyzing light beam passed perpendicularly through a quartz cell. The transmitted light entered a monochromator equipped with a photomultiplier. The signals were collected using an Agilent 54830B digital oscilloscope and then transferred to a computer.

2 Results and discussion

2.1 Laser photolysis at 266 nm

Full wave band scanning by the ultraviolet-visible spectrometer showed that the sample has strong absorption near 266 nm. This was why laser at 266 nm can be used as the excitation light source. Figure 2 shows the transient absorption spectrum obtained from 266 nm laser photolysis of an N₂-saturated neutral aqueous solution containing 1×10⁻⁴ mol/L NGSH. The absorption band shows peaks at 330 nm and 700 nm, and a photobleaching stage at 300 nm which comes from the contribution of the strong absorption of ground-state NGSH.

When the solution was saturated with N₂O or O₂, the transient absorption peaks at around 700 nm disappeared completely (Figure 3). It can be deduced that the transient species at around 700 nm is the hydrated electrons. Because of the formation of hydrated electrons, it can be confirmed that NGSH has been photo-ionized by 266 nm light^[6, 7], producing hydrated electrons at 700 nm and NGSH cation radicals at 330 nm.

Compared with the transient absorption spectra of the N₂-saturated aqueous solution containing 1×10⁻⁴ mol/L NGSH at 0.1 μs, the absorption at 330 nm of the solu-

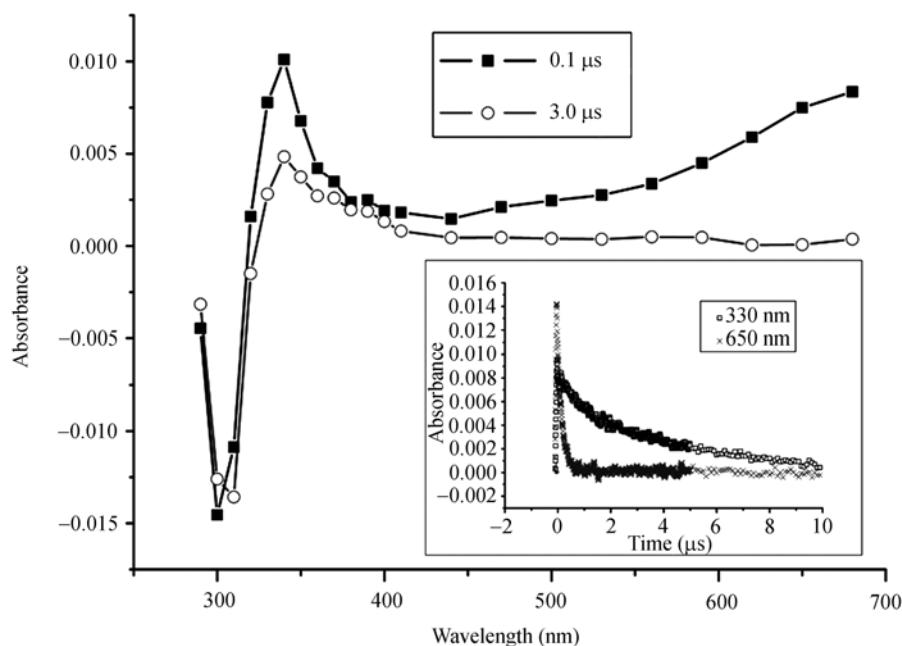


Figure 2 Transient absorption spectra from laser photolysis of an N_2 -saturated aqueous solution containing 1×10^{-4} mol/L NGSH recorded at (■) 0.1 μ s ; (○) 3.0 μ s. Insert: The forming and decay traces recorded at 330 nm (□) and 650 nm (×).

tion saturated with N_2O (containing 0.1 mol/L *t*-BuOH, which was used to eliminate the hydroxyl radicals) has obviously decreased (Figure 3). This means that there is also contribution of negative radicals at 330 nm. Compared with the spectra of the N_2O -saturated solutions, the absorptions at 330 nm do not decrease when the system was either saturated with O_2 or N_2O and contained 0.1 mol/L Mn^{2+} (used to quench the excited triplet state), and the forming and decay traces recorded at 330 nm are completely accordant with each other. It means that there is no excited triplet state coming into being when the NGSH aqueous solution interacts with laser light.

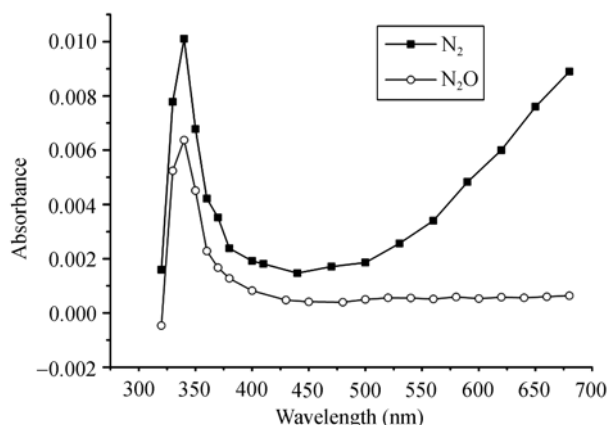


Figure 3 Transient absorption spectra from laser photolysis of the 1×10^{-4} mol/L NGSH aqueous solution at 0.1 μ s saturated with N_2 and N_2O respectively.

To obtain the decay rate constant of the cation radicals of NGSH, a series of different concentration aqueous solutions of NGSH saturated with N_2O were prepared. There should only $NGSH^+$ be generated in the reaction of these systems. The dynamic curve of different concentration aqueous solutions of NGSH at 266 nm was fitted and the K_{obs} value was obtained respectively. A linear relationship between K_{obs} and the concentration can be found. The decay rate constant of $NGSH^+$ was determined to be $2.55 \times 10^9 \text{ dm}^3 \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$.

2.2 Reaction of NGSH oxidized by

As $K_2S_2O_8$ competes with NGSH for photons, $K_2S_2O_8$ and NGSH solutions with appropriate concentrations were prepared to avoid the direct photolysis of NGSH and ensure the generation of firstly. The absorbance relationship of these two substances at 266 nm satisfies the following relationship:

Therefore, under this experimental condition, most of the laser energy is absorbed by $K_2S_2O_8$. Here anion $S_2O_8^{2-}$ is dissociated by laser light to produce radicals, which have a wide absorption spectrum from 420 nm to 550 nm and the characteristic absorptions at 460 nm and 360 nm. It could abstract an electron from NGSH. The formation process of the cation radicals of NGSH is about 1 μ s (Figure 5),

which is different from that in the case of laser photolysis (Figure 3). The mechanism can be summarized as follows:

Figure 4 shows the transient absorption spectra from laser photolysis of the N_2 -saturated aqueous solution containing 1×10^{-4} mol/L NGS H and 0.1 mol/L $K_2S_2O_8$ at different times. From formation and decay traces at 350 nm and 480 nm as shown in Figure 5, it can be seen that the transient species at 480 nm was produced immediately after laser irradiation. But there are two completely different formation processes at 350 nm: First it is an immediate process and then a slow one. Trace 3 was obtained by subtracting the trace at 480 nm from that at 350 nm. It is obvious that the transient formation process at 350 nm is synchronous with the decay of at 480 nm. In Figure 5, the P value (0.69) is the ratio of the absorbance at 350 nm to the absorbance at 480 nm at 0 μs , which was used in spectrum-subtract calculations.

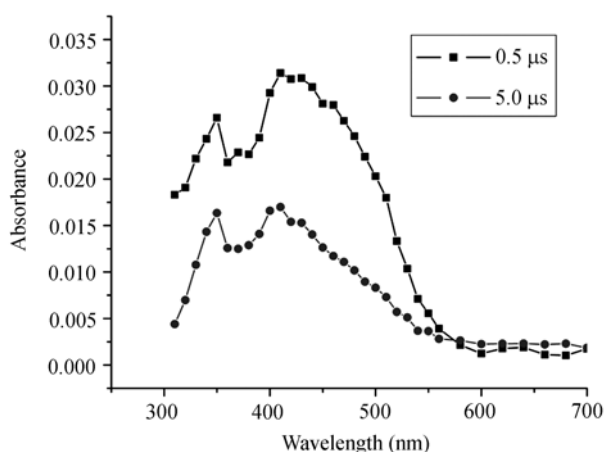


Figure 4 Transient absorption spectra from laser photolysis of the N_2 -saturated aqueous solution containing 1×10^{-4} mol/L NGS H and 0.1 mol/L $K_2S_2O_8$ recorded at 0.5 and 5.0 μs , respectively.

By changing the NGS H concentration, a series of appearance rate constants for the transient formation at 350 nm and decay at 480 nm can be obtained. The corresponding absolute rate constant can be determined by the slopes of the lines in Figure 6, which are both 1.76×10^9 $dm^3 \cdot mol^{-1} \cdot s^{-1}$. Therefore, it can be confirmed that the transient formation of NGS H at 350 nm is synchronous with the decay of at 480 nm.

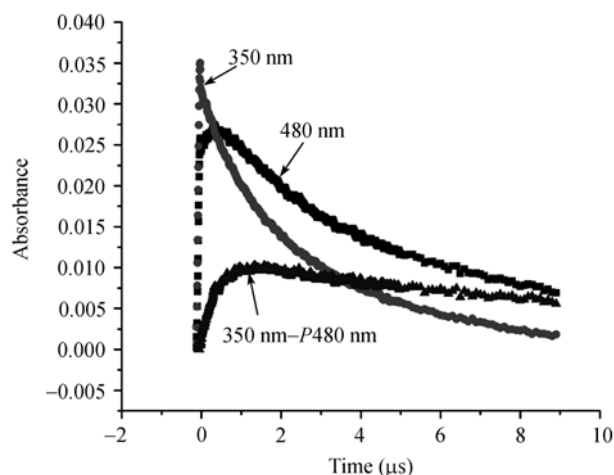


Figure 5 Transient formation and decay traces of a 1×10^{-4} mol/L NGS H and 0.1 mol/L $K_2S_2O_8$ aqueous solution saturated with N_2 at 350 nm, 480 nm and net transient formation and decay traces of NGS H^+ at 350 nm.

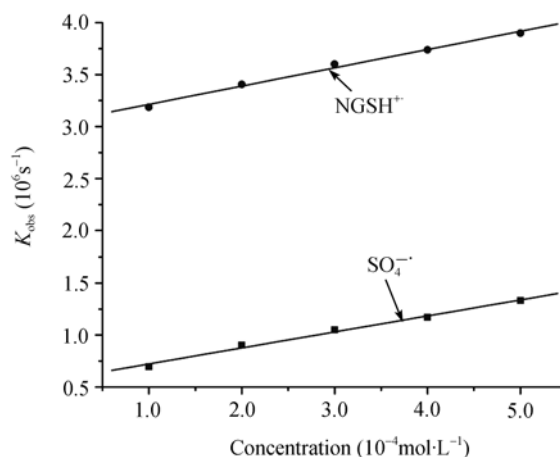


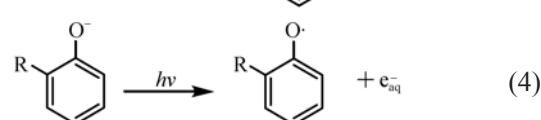
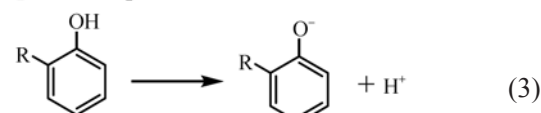
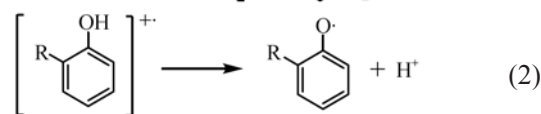
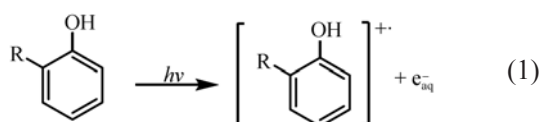
Figure 6 The apparent rate constants for the formation of NGS H^+ and decay of at different concentrations of NGS H .

2.3 Determination of pK_a value of NGS H^+

If we changed the values of pH of the N_2O -saturated aqueous solution containing 1×10^{-4} mol/L NGS H and 0.1 mol/L t -BuOH, the OD_0 values of cation radicals of NGS H observed at 330 nm in laser photolysis were found to be changed with the changes of pH values. Figure 7 shows that the pK_a value of NGS H cation radicals is 4.02.

As to phenolic compounds, the ground state molecules always keep an ionization equilibrium between deprotonation and protonation. In the course of changing the pH value, there are actually two kinds of photoionization reactions taking place after laser pulse: One is the direct photoionization of the ground state molecules and the other one is the photo-oxidation of phenoxy anions produced from proton abstraction by laser light. The

mechanism is as follows:



At first, when the pH value is at a low range, phenoxy radicals are mainly produced via reaction formulae (1) and (2). The dissociation process of ground state molecules (3) is inhibited. Therefore, the concentration of the ground state molecules is higher than that of phenoxy anions produced by formula (3). The ground state molecules are predominant in the system. The cation radicals are mainly generated from ground state molecules by direct photoionization under laser light. The cation radicals then change into neutral phenoxy radicals. The number of the phenoxy anions increases via formula (3) with the increase of the pH value. As a result, more and more phenoxy anions will change to phenoxy radicals via formula (4).

From the above discussion we can see that these two different processes present opposite trends while the pH value is changed. From the literature, we found that for a majority of the ground state molecules of phenolic compounds, the pK_a values are between 7.15 and 10, and the pK_a value of NGSH^+ is 4.02. Therefore, when the $\text{pH} > 7.15$, the reaction will take place through the path shown by eqs. (3) and (4); when the $\text{pH} < 4.02$, the reaction will take place through the path shown by eqs. (1) and (2); when the pH value is between 4.02 and 7.15, the two paths coexist.

2.4 Photoionization style and quantum yields of NGSH

To understand the style of photoionization, we changed the incident intensity of laser, and the respective absorbances of hydrated electrons at 650 nm were obtained at different incident intensities of laser. Figure 8 shows that there exists linear relationship between the incident

laser intensity and the absorption value (at 0.1 μs after the pulse), which is an indication of a monophotonic process. According to the molar absorptivity of hydrated electrons at 650 nm ($14\,900\text{ dm}^3\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$)^[8] and the formula: $OD = \epsilon c l$, the molar absorptivity of NGSH was determined to be $10683\text{ dm}^3\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$.

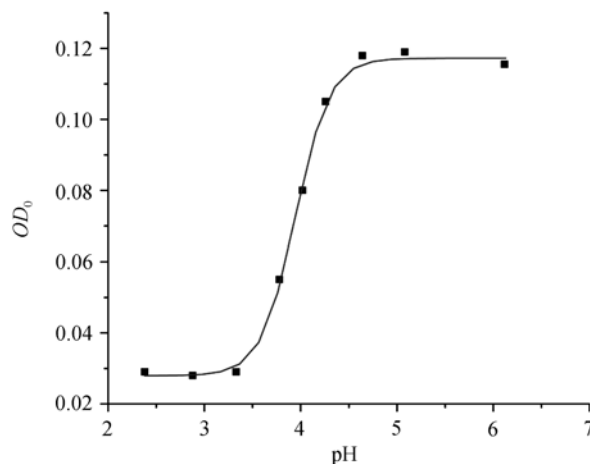


Figure 7 The OD_0 values after laser photolysis of the N_2O -saturated aqueous solution containing 1×10^{-4} mol/L NGSH and 0.1 mol/L *t*-BuOH at different pH values at 330 nm.

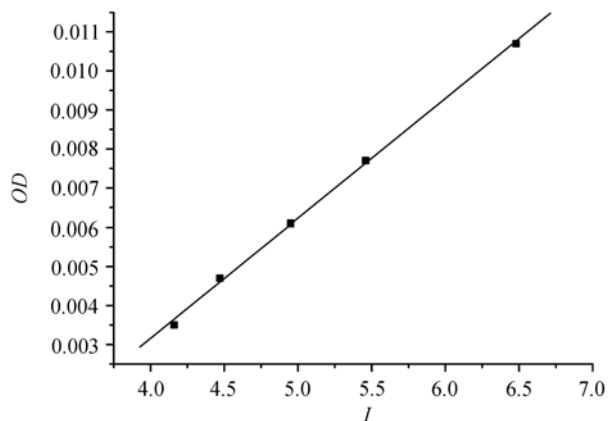


Figure 8 The OD values after laser photolysis of the N_2 -saturated aqueous solution containing 1×10^{-4} mol/L NGSH at different laser intensities (I) at 650 nm.

The quantum yield of the NGSH photoionization process was determined in a comparative experiment using potassium iodide ($\phi = 0.23$)^[9]. Before laser excitation, the absorbance of the KI aqueous solution was adjusted to be identical to that of the NGSH aqueous solution. Then two linear relationships of KI and NGSH between the incident laser intensity and the absorption value of hydrated electrons at 650 nm were obtained. Thus, the quantum yield of NGSH photoionization was

determined to be 0.02 according to the formula:

3 Conclusion

By the use of the laser photolysis technique, time-resolved spectroscopic and kinetic evidence is presented for the formation and decay of NGSH^{+} , with a maximum absorption at 330 nm. The molar absorptivity was determined to be $10683 \text{ dm}^3 \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$. At the same

time, NGSH^{+} cation radicals change to neutral radicals via deprotonation with a pK_a value of 4.02. The quantum yield of the NGSH photoionization was determined to be 0.02, which is relatively high compared with other kinds of *N*-Glycoside compounds. The results provide a preliminary theoretical foundation for further research on anti-tumor mechanisms. Besides, these findings are also expected to play a positive role for screening more efficient *N*-glycoside compounds for the anti-tumor purpose in the future.

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