International Journal of Mass Spectrometry xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

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

Photoinduced dissociation mass spectroscopy of firefly oxyluciferin anions

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ARTICLE INFO

Article history:
Received 5 August 2013
Received in revised form 7 November 2013
Accepted 7 November 2013
Available online xxx

Keywords:
Oxyluciferin
Photoinduced dissociation
Isotope labeling
DFT calculations
Bioluminescence
Scrambling

ABSTRACT

The oxyluciferin molecule in its anionic form is responsible for light emission from fireflies and some railroad worms and click beetles. Here we have studied the breakdown of the ions after photoexcitation by 550-nm light, and identified the atom composition of eight fragment ions based on mass spectrometric experiments on isotope-labeled compounds. A sector instrument with an electrospray ion source and a pulsed laser system was used for the experiments. After photoexcitation the time for dissociation was up to about 15 μs, which is much shorter than the 100-μs time constant for dissociation after one-photon absorption. The laser power was therefore kept high to allow the oxyluciferin anions to absorb two photons to produce enough fragment ions on the instrumental relevant time scale. The reaction energies leading to these ions were obtained from density functional theory calculations. The dominant fragment ion was deprotonated 2-cyano-6-hydroxybenzothiazole. Interestingly this behavior mirrors that of oxyluciferin both *in vivo* in insects, where the same nitrile is an intermediate in the postulated regeneration of D-luciferin from oxyluciferin or *in vitro* in near-neutral aqueous buffer. Dissociation of the oxyluciferin anion into this fragment ion was calculated to require 1.86 eV, which is less than the energy of one photon (2.25 eV). Experiments done on 5,5-dimethyloxyluciferin revealed a similar fragmentation pattern.

1. Introduction

In fireflies and other light-emitting insects, the D-luciferin substrate is converted biochemically to the oxyluciferin anion (Fig. 1) in its electronically excited state by the luciferase enzyme [1–5]. The energy-rich ATP (adenosine triphosphate) is involved in the oxidative decarboxylation of luciferin together with molecular oxygen and divalent metal ions. Electronically excited oxyluciferin anions emit light (bioluminescence) in the yellow-green, orange, and red dependent on the insect species [1–6] and with a high quantum yield of 41 ± 7.4% [4,6]. After light emission the oxyluciferin anion is enzymatically broken down to thioglycolic acid and 2-cyano-6-hydroxybenzothiazole, and the latter in the presence of cysteine is recycled to D-luciferin thereby allowing for a new light flash [7–9].

Still, a significant fraction of ions undergo internal conversion to the electronic ground state instead of light emission, resulting in vibrationally excited ions. These ions dissipate their heat in interactions with the protein environment (typical time scale is tens of picoseconds) though it cannot be excluded that a few would have time to dissociate prior to cooling. An environment can provide an efficient way of protection by energy dissipation [10]. The dissociation time of the isolated photoexcited ions *in vacuo* is about 100 μs based on previous storage-ring experiments [11], which results in a dissociation yield of 10^{-6}–10^{-8} assuming a time constant for vibrational cooling between 1 ps and 100 ps. Each flash from a firefly contains about 10^{13} photons [12], and the same order of magnitude of hot ions is therefore produced (59% yield *versus* 41%). Hence for each flash, a rough estimate of the number of ions that dissociate is 10^5–10^7. Considering that the flashing rate can be about ten per minute [13], this number will quickly multiply. For the atom economy of the firefly (and maybe also its safety) these fragments should as far as it is possible be recycled to D-luciferin.

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http://dx.doi.org/10.1016/j.ijms.2013.11.012

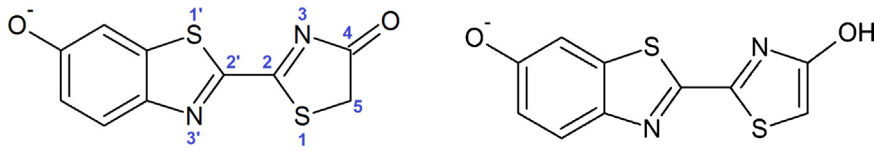

Fig. 1. Two tautomeric structures of the oxyluciferin anion. Left: keto form with atom numbering. Right: enol form. Atoms that are isotope labeled in this work are numbered 2, 2′, 3, 3′, 4, and 5, either as C-13 or N-15. Experiments were either done with both sulphurs (atoms 1 and 1′) being S-32 or one being S-32 and the other S-34, randomly.

To shed light on the fragment ions that potentially could be produced from hot oxyluciferin anions we have in this work measured photoinduced dissociation (PID) mass spectra. Isotope-labeled compounds were subjects for study (anions shown in Fig. 2) to get detailed information on the actual atoms in the fragment ions and to identify possible scrambling reactions. A wavelength of 550 nm was used as we have earlier found that this is the maximum of the absorption band [11]. For each of the identified fragments, we calculated the reaction energy associated with the dissociation channel to see if the reaction would be possible or not for vibrationally excited (hot) oxyluciferin ions within the luciferase enzyme. The lowest-energy structure of oxyluciferin is the keto form [11] but the enol form (Fig. 1) may also be present in the ion beam. We therefore also did experiments for 5,5-dimethyloxyluciferin that is locked in the keto form (Fig. 2).

2. Materials and methods

Experiments were done with a sector instrument equipped with an electrospray ion source [14,15]. Oxyluciferin was dissolved in methanol and electrosprayed to produce the anions. After a heated capillary and tube-lens skimmer region, the ions were stored in an RF-only octopole trap. The trap was emptied every 25 ms, and all ions were accelerated to 50-keV energies. Those of interest according to their mass-to-charge ratio (m/z) were selected by an electromagnet. In a field free region, the ions were photoexcited by a nanosecond laser pulse. The laser is a Nd:YAG laser where the third harmonic is led into an optical parametric oscillator to generate visible light at 550 nm. The repetition rate of the laser is 20 Hz so only every second ion bunch was irradiated to subtract a background signal due to collision-induced dissociation from residual gas in the beam line (laser on–laser off). After photoexcitation, the time for dissociation was a few microseconds. Daughter ions were selected by a hemispherical electrostatic analyzer and counted by a channeltron detector. The detection efficiency is high as the ions have high velocities.

The synthesis of both 5,5-dimethyloxyluciferin and the isotope-labeled oxyluciferins was performed following established synthetic pathways toward firefly luciferin [16], but using appropriately ^{13}C- and ^{15}N-labeled precursors. Eventually, the labeled oxyluciferins were obtained according to Goto's method by condensation of 2-cyano-6-hydroxybenzothiazole with ethyl mercaptoacetate [17,18], taking advantage of recent improvements for this capricious key step [19]. The samples displayed a chemical purity of >98%, and the labeling in the respective positions were close to 99% according to NMR. Full experimental details will be reported elsewhere.

Theoretical calculations were done with the Gaussian03 program package [20]. Geometries were first optimized at the B3LYP/6-31+G(d) level of theory and vibrational frequencies calculated to verify that the structures are local minima and not transition states. Single-point energies were calculated at the higher B3LYP/6-311++G(2d,p) level of theory, and these were corrected for zero-point kinetic energies. All structures, energies and zero-point corrections can be found as supporting information.

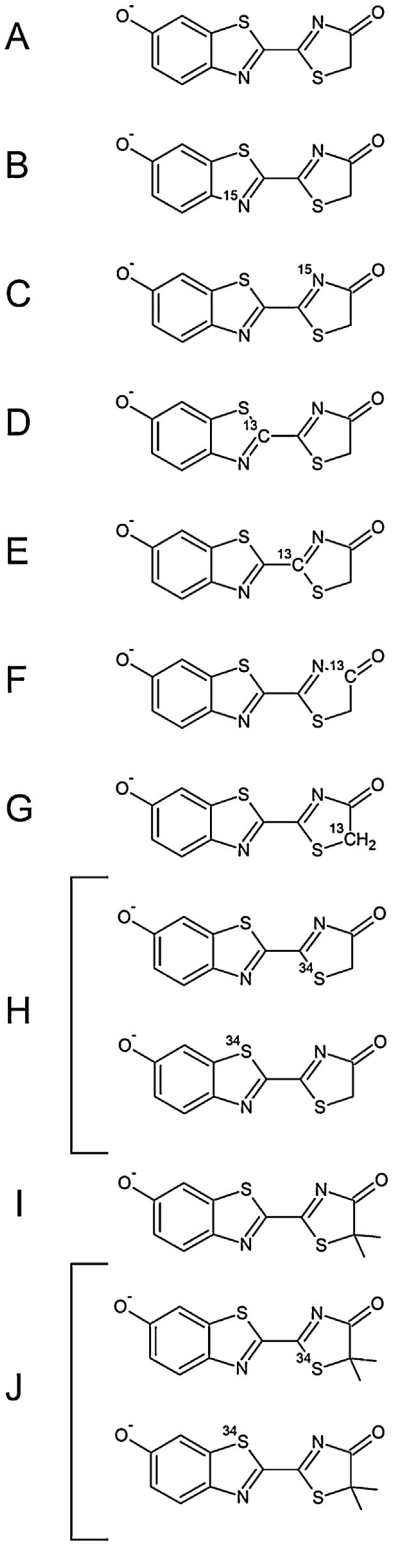

Fig. 2. Oxyluciferin and 5,5-dimethyl oxyluciferin anions subject for study.