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SHORT COMMUNICATION

Using C_{60} fullerenes for photodynamic inactivation of mosquito iridescent viruses

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Abstract

This article describes the photodynamic inactivation of mosquito iridescent virus (MIV) *Aedes flavescens* in the presence of water-soluble C_{60} fullerenes. It has been observed that the photodynamic inactivation of MIV for about 1 h reduces the infectious titre of the virus in large wax-moth larvae *Galleria mellonella* to 4.5 lg ID_{50}/mL. The influence of the C_{60} concentration on its anti-viral activity was tested in the concentration range from 1 to 0.001 mg/mL. It has been found that C_{60} is able to inactivate the iridovirus even in low concentrations. Consequently, the findings of this work suggest that photoexcited C_{60} fullerenes can be successfully used for the inactivation of iridoviruses in biological systems.

Keywords: Water-soluble C60 fullerenes, mosquito iridescent virus, photodynamic inactivation

Introduction

Fullerenes belong to the third natural allotropic form of carbon. C_{60} fullerene is the most studied member of fullerene family. It is a highly stable icosahedral molecule (with size of ~0.7 nm) consisting of 60 carbon atoms[1]. In recent years, much attention was paid to a comprehensive investigation of C_{60} influence in biological systems *in vitro* and *in vivo*[2,3]. It was shown that the addition of fullerenes in some gel-like medicinal forms may significantly increase their wound healing properties[4]. Fullerenes and their derivatives are able to penetrate through cell membranes[5] and they have strong anti-oxidant[6], anti-viral[3,7,8] as well as anti-microbial[9–11] properties. It is also interesting that under irradiation, C_{60} can be activated and reactive oxygen species are generated, which can cause destruction of nucleic acids, proteins and lipids[3,12,13]. There are several reports in literature regarding studies on photodynamic inactivation of viruses from *Orthomyxoviridae*, *Rhabdoviridae*, *Togaviridae* and *Leviviridae* families. It has been reported that the viruses lose their infectivity by more than 7 lg ID_{50} during a few hours of photoinactivation with C_{60} fullerenes[14–16]. A general concept of applying fullerenes in the photodynamic therapy is discussed more detail in paper[17].

Iridoviruses are large DNA viruses (with sizes of ~180–200 nm) that can be replicated in the cytoplasm of infected cells. Iridoviruses have been found to infect invertebrates and poikilothermic vertebrates, including amphibians, reptiles and fishes[18]. Iridoviruses that infect mosquitoes and midges belong to the genus *Chloriridovirus*[19]. Mosquito iridescent viruses (MIVs) are widely distributed in natural reservoirs and play role as regulators of the insect quantity.

Iridoviruses not only infect mosquitoes and other insects but also cause mass mortality of fish that leads to significant economic losses in industrial aquaculture. Consequently, the isolation of these emerging iridoviruses is closely associated with an increase of the world aquaculture production rates[20].

Thus, the purpose of this work was to study the effect of photoactivated C_{60} on infectious titre of iridoviruses. For this study, virions of MIV, isolated from the mosquito larvae *Aedes flavescens* in natural reservoirs of the Kyiv region (Ukraine) were used.

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Materials and methods

Highly stable water colloid suspension of C_{60} fullerenes (maximum concentration 1 mg/mL) was prepared by transfer of C_{60} (purity >99.5%) from toluene to water using ultrasound sonication[21].

It is important to note that the considered water solution of C_{60} fullerenes does not show a cytotoxic effect at concentrations below 1 mg/mL[22].

For the characterisation of C_{60} films deposited from water solutions (C_{60} concentration in water was 1 mg/mL) on Au(111) surface, scanning tunnelling microscopy (STM; NT-MDT Moscow, Russia) images using a low-current scanning head were taken. The Pt-Ir (80:20) tip was mechanically cut from a wire (0.25 mm in diameter). Typical imaging conditions were in a range from 0.01 to 0.1 nA and from 0.1 to 0.8 V. Reconstructed Au(111) substrates of 150-nm thickness were prepared by vacuum deposition onto a freshly cleaved mica surfaces heated at ~600 K followed by a careful annealing in a gas flame (propane). During ~10 min after flaming, the substrate revealed reconstruction lines in air. Exposition in air during 1-h lifted reconstruction. A droplet of the water solution of C_{60} fullerenes was deposited on these gold substrates.

MIV *Ae. flavescens* was propagated in multi-host wax-moth larvae *G. mellonella*. The wax-moth larvaes were infected by intraperitoneal injection and incubated at room temperature (20–22°C).

Fourteen days after injection of the MIV *Ae. flavescens,* it was purified from the infected wax-moth larvae *G. mellonella* by the differential centrifugation method. Briefly, after homogenisation of infected larvae *G. mellonella*, cell debris was separated by centrifugation at 3000*g* for 5 min at 10°C. The pellet was discarded and the supernatant centrifuged in a ultracentrifuge (Beckman L5-50B in a rotor SW-40) for 40 min at 70,500*g* at 4°C. A characteristic blue pellet confirmed the presence of the mosquito iridescent virions. The virus pellet was suspended in TNE (50-mM Tris-HCl, 150-mM NaCl, 1-mM disodium ethylene diaminetetraacetic acid [EDTA], pH 7.5) and centrifuged at 1100*g* for 5 min at 10°C. The suspension was layered onto a 10–50% (w/w) linear sucrose gradient in TNE. After centrifugation at 70,500*g* for 40 min at 4°C, one visible band near the bottom of the tube was collected, diluted in fresh TNE and centrifuged at 70,500*g* for 40 min at 4°C. The virus pellet was placed into TNE at a final protein concentration of 1.7 mg/mL. The infectious titre of MIV *Ae. flavescens* in the wax-moth larvae *G. mellonella* was determined by the Reed and Munich method[23]. The infectious titre of virus in larvae *G. mellonella* was 11.2 lg ID_{50}/mL.

For the photodynamic inactivation of MIV, a 400-W halogen lamp (intensity of light radiation was 10^{20} photons/m^2s) was used.

For the experiments, various solutions with constant concentration of virus (0.17 mg/mL) and different concentrations of C_{60} (1, 0.1, 0.01 and 0.001 mg/mL) were used. The photodynamic inactivation of MIV was performed at 400–850-nm wavelengths in a glass tube. The distance between the source of light and virus was 10 cm. The exposure time was 2.5, 10, 30 and 60 min. The MIV without C_{60} fullerenes and MIV with C_{60} fullerenes but without photodynamic inactivation were used as controls.

After exposure, the material from all variants was diluted in the sterile distilled water in an interval of 10^{-1} to 10^{-12}. The wax-moth larvae *G. mellonella* were infected by the material from every dilution. The infected larvae were incubated at temperature 20–22°C. Fourteen days after injection, the titers of MIV were determined by the methods of blue pellets.

Results and discussion

The STM images of a submonolayer C_{60} film deposited from water solution on Au(111) surface are shown in Figure 1. An almost random arrangement of C_{60} clusters (with sizes up to ~2.8 nm) (Figure 1A) can be seen. It is well known that C_{60} molecules form highly ordered hexagonal structures on reconstructed Au(111) in vacuum[24]. The absence of such ordering in our case can be attributed to the degradation in water solution. However, nucleation of islands occurs along the preferential direction <112> (see Figure 1A). Despite of the high mobility of C_{60} molecules on Au(111) at room temperature, we were able to observe single C_{60} molecules (Figure 1B). The immobilisation of the C_{60} fullerene is due to the lifting of the reconstruction by water.

The above-obtained results are in a good agreement with previously predicted theoretical calculations[25,26], which show that the water colloid solution of C_{60} contains single C_{60} molecules, their clusters and crystalline phase (with sizes of ~0.7–4 nm in dependence of C_{60} concentration in water) in the hydrated state. Analysis of the UV/VIS spectroscopic data[21] showed that C_{60} fullerenes in water are characterised by two intense broad absorption bands with maximums at 265 and 345 nm and also by narrow spectral lines at 450 and 622 nm.

The most interesting and important finding of this study is possibility for the photodynamic inactivation of MIV *Ae. flavescens* by using an aqueous solution of C_{60} (concentration 1 mg/mL). It has been found that after a treatment of about 2.5 min, the infectious titre of virus can be decreased by 2 lg ID_{50}/mL units (Figure 2). In the control group, the infectious titre of MIV was 11.2 lg ID_{50}/mL. The infectious titre of MIV in the *G. mellonella* after handling for about 2.5 min was decreased to 9.0 lg ID_{50}/mL. Further handling for 10 and 30 min leads to decrease of the infectious titre of the virus by 2.93 and 3.88 lg ID_{50}/mL units, respectively. Interestingly, the indexes of infectious titre of MIV after the photodynamic inactivation for 1 h were not significantly different from those obtained from photodynamic inactivation for 30 min. Infectious titre of MIV *Ae. flavescens* after the photodynamic inactivation during 1 h was 7.15 lg ID_{50}/mL.

Our results showed that after photodynamic inactivation for 30 min, C_{60} in a concentration of 0.1 mg/mL

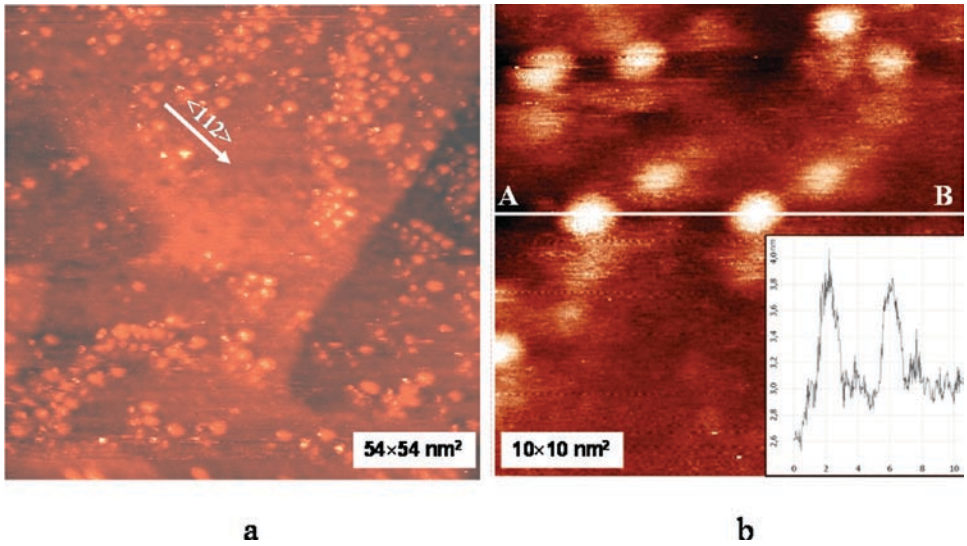

Figure 1. (A) Large-scale scanning tunnelling microscopy image of submonolayer C_{60} fullerene film deposited from water solution (C_{60} fullerene concentration in water was 1 mg/mL) on Au(111) surface. Some C_{60} fullerene clusters are aligned along preferential direction. (B) Single C_{60} fullerenes. Lateral size is increased because of shape of the tip. Inset: Cross-section along line AB. Scanning parameters: It = 40 pA, Ut = 0.7 V.

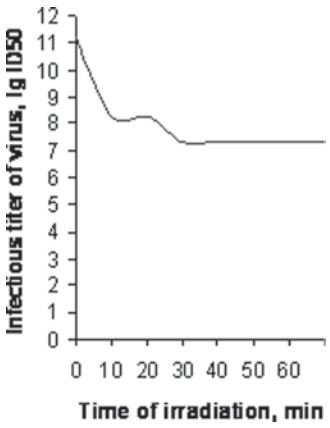

Figure 2. Photodynamic inactivation of mosquito iridescent virus *Ae. flavescens* by C_{60} fullerenes (maximum concentration 1 mg/mL).

reduced the infectious titre of MIV *Ae. flavescens* by 4.0 lg ID_{50}/mL units. In a concentration of 0.01 mg/mL, C_{60} reduced the infectious titre of the MIV by 4.5 lg ID_{50}/mL units. The use of C_{60} in a concentration of 0.001 mg/mL actually did not influence the infectious titre of MIV *Ae. flavescens*. The infectious titre of MIV *Ae. flavescens* was 10.7 lg ID_{50}/mL, that is only 0.5 lg ID_{50}/mL less than in the control group (Table 1). The presence of C_{60} in a viral suspension, but without the photodynamic inactivation, as well as irradiation of viral suspension in the absence of C_{60} did not influence the infectious titre of MIV.

It is important to note that the effectiveness of reducing the infectious titre of MIV on the C_{60} concentration and exposure time significantly related to fullerene aggregation in water[26]: formation of fullerene clusters (aggregates) with single molecules leads to a significant restructuring of their electronic structure, which determines the mechanism of their photoactivation[17].

Table 1. Photodynamic inactivation of mosquito iridoviruses *Ae. flavescens* by C_{60}.

Time of irradiation, min	30				
Concentration of C_{60} fullerenes, mg/mL	1	0.1	0.01	0.001	Control
Infectious titre of virus, lg ID_{50}/mL	7.32	7.2	6.7	10.7	11.2

Conclusion

The photodynamic inactivation of viruses by using C_{60} and their derivatives can be considered as a promising treatment, and thus, it can be successfully applied in medicine. Recently, a lot of works involved in investigation of anti-viral and anti-bacterial properties of C_{60} were reported in the literature[9,27]. In spite of the fact that the most of the investigations were involved in interactions of fullerenes with either the human immunodeficiency virus[28] or hepatitis C virus[29], in this work we studied for first time the interaction of C_{60} and MIV *Ae. flavescens*. The obtained results demonstrated the anti-viral properties of C_{60} fullerenes during photoactivation. Namely, during the photodynamic inactivation the C_{60} fullerenes (30 min) in a most efficient concentration of 0.01 mg/mL interact with the virions of MIV *Ae. flavescens* causing a reduce of the infectious titre of virus by 4.5 lg ID_{50}/mL units.

Furthermore, the effect of the concentration of C_{60} fullerenes on the infectious titre of virus was also investigated. In photodynamic inactivation, C_{60} fullerenes were found to be active against virions of MIV *Ae. flavescens* even at very low concentration (in the concentration range from 0.1 to 0.01 mg/mL). Our results are in agreement with previous published reports[27,30].

Declaration of interest

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