Radiation Physics and Chemistry 78 (2009) 177–183

Contents lists available at ScienceDirect

Radiation Physics and Chemistry

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

Gold nanoparticles enhance the X-ray-induced degradation of human centrin 2 protein

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ARTICLE INFO

Article history:
Received 15 September 2008
Accepted 3 November 2008

Keywords:
Gold nanoparticles
Human centrin 2
Radio-sensitization
X-rays

ABSTRACT

In the war against cancer, radiotherapy is a prominent tool but counterbalanced by the fact that it also induces damages in healthy tissues. Nanotechnologies could open a new possibility to decrease these side effects. In particular, gold nanoparticles (GNPs) could be used as radio-sensitizers. As the role of proteins in the processes leading to cell death cannot be neglected, their radio-sensitization by GNPs is of great interest. This is particularly true in the case of the human centrin 2 protein, which has been proposed to be involved in DNA repair processes. To investigate this effect, we quantified for the first time the degradation of this protein in a gold colloidal solution when submitted to X-rays. We showed that the X-ray-induced degradation of the human centrin 2 protein is enhanced 1.5-fold in the presence of GNPs, even though no covalent bond exists between protein and GNPs. Among the conditions tested, the maximum enhancement was found with the higher GNP:protein ratio of 2×10^{-4} and with the higher X-ray energy of 49 keV.

1. Introduction

Thanks to their unique properties, nanomaterials are currently opening new horizons in biology and medicine. Among them, gold nanoparticles (GNPs) are promising tools. The interest they raised dates back to the Roman times, the most famous example being the Lycurgus Cup, manufactured in the 5th to 4th century B.C., and keeps going on through Middle Ages with the first description of their medical uses in 1618. Nowadays, their biocompatibility (Lewinski et al., 2008), their rich surface chemistry that enables various functionalizations (Sanvicens and Marco, 2008) and their optical properties (Myroshnychenko et al., 2008) make them useful for imaging (Nagesha et al., 2007), biosensing and diagnosis (Liu et al., 2008) and gene and drug delivery (Han et al., 2007; Rosi et al., 2006).

Another field of investigation with regards to GNPs is radiotherapy. Radiotherapy is a prominent tool in oncology but it can be held responsible for important biological damages, as ionizing radiations also induce degradation of healthy tissues. For example, following pelvic radiotherapy, 20–40% of patients report that gastrointestinal symptoms severely affect their quality of life (Andreyev, 2007). It is the reason why since more than five decades, great efforts have been devoted to increase its efficiency and tolerance, such as better dose fractionation schedules or tomotherapy. In spite of these advances, normal tissue toxicity remains a dose-limiting factor in clinical radiation therapy. The dose enhancement by high-*Z* materials has been known for long (Spiers, 1949), but with GNPs, it now seems possible to draw benefits from this radio-sensitization to increase the dose to cancerous cells specifically. Herold et al. (2000) first showed that gold microspheres, suspended in cell cultures or distributed in tumour tissues, can produce an increased biologically effective dose when exposed to kilovoltage photon beams. Later, Hainfeld et al. (2004) demonstrated that GNPs injected intravenously to mice bearing subcutaneous EMT-6 mammary carcinomas enhance 250 kV p X-rays radiotherapy. They even observed a complete eradication of tumours within 30 days. More recently, a study from Chang et al. (2008) with murine B16F10 melanoma cells suggested that GNPs combined with clinical electron-beam irradiation could result in an increase of apoptotic signals. This radio-sensitization was also predicted by theoretical calculations (Cho, 2005): it is generally agreed that a dose enhancement of ~2 could be obtained in the presence of GNPs for conventional 80–140 kV p X-rays. These results open an exciting perspective in

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doi:10.1016/j.radphyschem.2008.11.003

cancer therapy because a more efficient and specific radiotherapy treatment can be envisaged. For this reason, it is necessary to confirm the role of nanoparticles on their potential partners in the cell, which constitutes an essential step in the understanding of the mechanism of radio-sensitization and its optimization in therapy. Dose enhancement was reported for supercoiled DNA in solution (Carter et al., 2007; Foley et al., 2005) or bacteria (Simon-Deckers et al., 2008) irradiated with X-rays and for DNA in thin films with high-energy electrons (Zheng et al., 2008). But up to now, no study deals with radio-sensitization of proteins by GNPs. Of course, DNA, as the genome integrity keeper, is a vital molecule but it was found not to be the initial site of damage in several cell lines (Caraceni et al., 1997; Du and Gebicki, 2004). On the contrary, there is increasing evidence that proteins are major targets of reactive oxygen species, that they could transfer the damages to other cell constituents (Du and Gebicki, 2002) and that they could protect DNA when complexed to it (Eon et al., 2001). Thus the role of proteins in processes leading to cell damage cannot be neglected.

Our model to test the radio-sensitizing effect of GNPs on protein at the molecular level is human centrin 2 (Hscen2). Centrins are small acidic proteins, highly conserved in eukaryotes, from algae and yeast to humans (Gogendeau et al., 2007). They belong to the calmodulin super-family whose members share the common property to bind calcium ions with high affinity. Centrin 2 consists of 172 amino acids, organized in two structurally independent domains, N- and C-terminal, each containing two potential calcium-binding sites. It has been reported that in humans, the nuclear fraction of centrin 2 is a functional part of the xeroderma pigmentosum group C (XPC) complex which initiates DNA nucleotide excision repair (NER) (Nishi et al., 2005). Moreover, it has been shown that Hscen2 is highly sensitive to oxidizing radicals (Blouquit et al., 2007). At doses of a few grays, that is to say in the dose range used in therapy, these radicals induce the formation of a covalent dimer with a high proportion.

The experiments reported here examine the radio-sensitizing effect of GNPs on this molecular component of the cell: the protein Hscen2.

2. Experimental section

2.1. Chemicals

Potassium tetrachloroaurate(III) (98%), tri-sodium citrate (99% min) and phosphate buffer were from Acros Organics. Water was purified with an Elga Maxima system (18.2 $M\Omega\,cm^{-1}$).

2.2. Protein expression and purification

The recombinant protein Hscen2 was produced by high-cell-density fermentation as described previously (Blouquit et al., 2007). Briefly, it was overexpressed in *E. coli* host strain BL21(DE3) (Novagen, San Diego, USA), carrying the kanamycin antibiotic resistance gene. Cell culture was achieved in a 3 L Infors-Labfors fermentor. IPTG (1 mM) initiated protein overexpression, the duration of which was 3–5 h. Bacterial pellet was lysed using a one-shot desintegrator (CellD) and the protein supernatant underwent purification. It consists of three steps: an ion-exchange chromatography on a DEAE-TSK column, followed by a Phenyl-TSK purification and a last desalting step with a Sephadex G25 column. Protein concentration was determined assuming a molar absorption coefficient at 280 nm of 1490 $L\,mol^{-1}\,cm^{-1}$.

2.3. GNP

2.3.1. Synthesis

All glassware used for GNP synthesis were thoroughly washed with aqua regia (3:1 HNO_3–HCl), rinsed extensively with water and oven-dried at 60 °C. The colloidal GNPs were prepared by the Turkevitch method (Turkevitch et al., 1951) i.e. by citrate thermal reduction. Typically, tri-sodium citrate (4.2 mL, 1% (w/v) aqueous solution) was added to $KAuCl_4$ aqueous solution (100 mL, 10^{-3} $mol\,L^{-1}$). The mixture was heated under moderate stirring up to 10 min after the solution had turned purple. It was then subjected to centrifugation (2300*g*, 20 min) and the pellet of concentrated GNPs was resuspended in water. The process was repeated twice in order to eliminate the free citrate. For each batch, the GNPs size and concentration were, respectively, determined by transmission electronic microscopy and UV–vis spectroscopy (Evolution 500, Thermo Electron Corporation). The value of $4\times10^9\,L\,mol^{-1}\,cm^{-1}$ at 450 nm was taken as the molar absorption coefficient (Haiss et al., 2007).

2.3.2. Electron microscopy

Aliquots of GNPs were adsorbed on formvar/carbon-coated copper grids (400 mesh). The grid was placed in contact with a 10 μL droplet of the colloidal dispersion for a few minutes; excess solution was removed with a filter paper and later the sample was dried in air. Samples were imaged on a Philips CM120 electron microscope operating at 80 kV. The diameter distribution of GNPs was analysed with ImageJ software (Abramoff et al., 2004): 480 particles were analysed.

2.4. Flocculation assays

Typically, GNPs solution (700 μL, 2.1 nM) was added to 100 μL of protein in phosphate buffer (10 mM, pH 7.0) with concentrations ranging from 0.14 to 14 μM. The mixture was incubated overnight. The resulting pH was 7.0. Then, NaCl (10% (w/v) aqueous solution, i.e. 1.7 $mol\,L^{-1}$, final concentration 0.15 $mol\,L^{-1}$) was added to the solution to test for flocculation and absorbance was measured at 530 nm.

2.5. Analysis

SDS-PAGE was performed with 10% acrylamide–bisacrylamide gels with a Tris–tricine buffer. As Hscen2 does not contain any cystein, the SDS-PAGE analyses were performed under non-reducing conditions. Samples containing GNPs were loaded without any special treatment. GNPs did not migrate through the gel and stayed at the bottom of the well. Gels were stained with Coomassie blue R-250 according to standard procedures. HPLC experiments were performed on a Beckman Coulter Gold 168 (Beckman Coulter) with a diode array detector. A Superdex 75 10/300 GL column was used with isocratic elution with 100 mM ammonium bicarbonate pH 8.1 with a flow rate of 0.5 $mL\,min^{-1}$. Samples containing GNPs were subjected to centrifugation (5500*g*, 10 min) and only supernatants were injected.

2.6. Irradiations

Gamma-ray irradiation was performed with a panoramic ^{60}Co source (IL60PL Cis-Bio International).

X-ray irradiations were performed at three different energies (8, 24.4 and 49 keV) with two generators.

- X-rays of 24.4 and 49 keV were generated with a superficial therapy X-ray unit (Pantak Therapax 3 series) at the Université