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Glycation-assisted synthesized gold nanoparticles inhibit growth of bone cancer cells

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ABSTRACT

This study presents a novel approach to synthesize glycogenic gold nanoparticles (glycogenic GNps) capped with glycated products (Schiff's base, Heyns products, fructosylamine etc.). These glycogenic GNps have been found to be active against human osteosarcoma cell line (Saos-2) with an IC_{50} of 0.187 mM, while the normal human embryonic lung cell line (L-132) remained unaffected up to 1 mM concentration. The size of glycogenic GNps can also be controlled by varying the time of incubation of gold solution. Glycation reactions involving a combination of fructose and HSA (Human Serum Albumin) were found to be effective in the reduction of gold to glycogenic GNps whereas glucose in combination with HSA did not result in the reduction of gold. The progress of the reaction was followed using UV–visible spectroscopy and NBT (Nitroblue tetrazolium) assay. The glycogenic GNps were found to be spherical in shape with an average size of 24.3 nm, in a stable emulsion. These GNps were characterized using UV–visible spectroscopy, zeta potential analysis, transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

1. Introduction

Non-enzymatic glycation of proteins by reducing saccharides such as glucose and fructose results in the formation of fructosamine [1] and AGEs (Advanced Glycated End products) [2,3]. The implication of glycation in a number of diseases has increased the focus on glycation since the last decade. Glycation alters the biological activities of proteins, leading to protein dysfunction. The effects of glycation, in particular, are more pronounced in several age-related diseases such as diabetes [4], cancer [5], cataracts [6], atherosclerosis [7], Alzheimer's [8], renal failure [9], etc. Thus, the study of glycation has become one of the most important areas of biomedical research. Glycation by glucose, among the reducing sugars, has been extensively studied. In addition to glucose, other reducing sugars such as galactose [10], sialic acid [11], mannose [12], glucose-6-phosphate [13], glyceraldehydes, and fucose [12] have been used as glycating agents in vitro. The phenomenon of glucose glycation is a slow process, although it is associated with numerous cellular metabolic processes [14]. There is a great deal of information available on glycation by glucose, on the contrary, only a handful of studies have investigated the role of fructose in glycation. In vitro studies suggest that fructose is a more potent initiator of Maillard reaction than glucose [15]. Elevated levels of fructose in blood can lead to glycation of biomolecules, affecting their structure and function, through the formation of AGEs [16]. Although the level of fructose in human plasma is relatively less as compared to that of glucose, due to the increasing use of fructose as a dietary sweetener and its highly reactive nature, the study of the effect of fructose mediated glycation gains significance.

Human serum albumin (HSA), being prone to glycation, has a relatively long half life (about 21 days) in comparison to other proteins [17]. In addition, proteins such as collagen [18], lens crystallins [19], red cell membrane [20], peripheral nerve proteins [21], ferritin [12], Apolipoprotein, hemoglobin [22], insulin, immunoglobins, laminin and fibronectin [23] are also susceptible to glycation and have been widely studied. Even though, human serum albumin is the major serum transport protein, very little information is available on the effect of glycation of this protein [24]. The glycation reaction, also known as Maillard reaction, is a slow, non-enzymatic reaction involving the attachment of carbonyl group of reducing sugars (Glucose, fructose, etc.) to free amino groups of Human serum albumin to form glycated HSA (Schiff's base), followed by amadori rearrangement to form more stable aminomethyl ketone [1,25]. This is the early glycation reaction wherein, Schiff's base and fructosamine are the early glycation adducts [26]. Subsequent

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modifications in early glycated end products such as rearrangement, oxidation, polymerization and cleavage, give rise to irreversible advanced glycated end products [27]. In the case of fructose, the rearrangement of Schiff base is known as Heyns rearrangement. The study of AGEs is important, since the loss of function of proteins and tissue damage in vivo, is directly related to the formation of AGEs. Early glycation reactions themselves can alter the function of a number of proteins. There are certain reports which establish the role of glycated products against cancer with probable mechanism of causing apoptosis, through activation of ROS, MAP kinases, the FOXO1 transcription factor [28], inhibition of formation of nitrosamine, direct scavenging of carcinogenic species and performing anti-promoter activity [29].

This study focuses on the synthesis of gold nanoparticles (glycogenic GNps) using fructose and HSA, as the glycating agents and the utility of these nanoparticles as anticancer agent against bone cancer.

2. Materials and methods

2.1. Chemicals and reagents

D-glucose, D-fructose, human serum albumin, NBT (Nitroblue tetrazolium) reagent were purchased from Merck. Tetrachloroauric [III] acid ($HAuCl_4$) and HEPES (4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid) were purchased from Sigma–Aldrich. Unless otherwise indicated, all solvents and chemicals were of analytical grade and were used as received.

2.2. Glycation assay

Glucose glycated or fructose glycated HSAs were prepared by incubating HSA (150 μg, 300 μg, 450 μg) with 80 mM of glucose or fructose (80 mM, 150 mM, 250 mM) for 33 days at 37 °C in 10 mM phosphate buffered saline (pH 7.4) in 3 ml reaction volume, under sterile conditions.

All the solutions were filter sterilized using PuradiscTM 0.2 μm syringe filter (Whatman, GE Healthcare UK limited, UK) and were dispensed into culture tubes, under aseptic conditions. HSA under identical conditions except for the addition of reducing sugars, served as the control.

2.3. Absorbance spectroscopy

The absorption spectra of native and glycated samples incubated for 33 days were recorded on Shimadzu dual-beam spectrophotometer (model UV-1601 PC) in the wavelength range of 220–800 nm in quartz cuvette of 1 cm path length. Individual aliquots of the reaction mixtures were analyzed for absorbance at 279 nm. Absorption spectra in the range of 250–700 nm were found to be optimal for detecting AGEs.

2.4. Analysis of glycated albumin

The keto-amine moieties resulting from glycation of HSA were determined by a modified form of NBT reduction assay [30]. Absorbance spectra were measured each day by removing aliquots from the reaction mixture to determine the presence of keto-amine moieties. 20 μl of native and glycated HSA samples were mixed with 200 μl of 100 mM carbonate buffer (pH 10.8) containing 0.25 mM NBT and incubated for 10 min at 37 °C and absorbance measured at 525 nm. The presence of keto-amine moieties in nmol/ml was determined based on an extinction coefficient of 12,640 M^{-1} cm^{-1} for monoformazan [31].

2.5. Glycation-assisted synthesis of gold nanoparticles (glycogenic GNps)

For the in vitro synthesis of gold nanoparticles (glycogenic GNPs), 3 ml of reaction mixture containing glycated HSA (prepared by incubating 300 μg human serum albumin with 250 mM fructose for one, two and three days, respectively, in three separate reactions) and 1.0 mM freshly prepared $H[AuCl_4]$ was incubated at 37 °C for 24 h. Reactions performed with native HSA and gold solution as well as with native fructose and gold solution, were used as control. The synthesis of nanoparticles was followed by measuring the absorbance spectra of the reaction mixture at regular intervals. On completion of the reaction, gold nanoparticles were collected by centrifugation (30,000 × *g*, 30 min) and washed twice with Milli-Q water. The unbound proteins were removed by extracting with 50% (v/v) of ethanol. Glycogenic GNPs were separated from unused HSA and fructose by passing the reaction mixture through Biogel P-30 gel filtration column pre-equilibrated with 20 mM HEPES buffer (pH 6.0) containing 150 mM NaCl. The fractions were scanned between 220 nm and 800 nm and the fractions that exhibited absorbance at 520 nm were pooled together. The pooled samples were dialyzed against distilled water and used for further characterization.

2.6. Characterization of gold nanoparticles

The glycogenic GNPs were characterized by absorbance spectroscopy, zeta potential analysis, TEM and SEM. Absorbance measurements were performed on a Shimadzu dual beam spectrophotometer (model UV-1601 PC) operated at a resolution of 1 nm. Zeta potential was measured on Zetasizer Nano-ZS, Model ZEN3600 (Malvern Instrument Ltd, Malvern, UK). Scanning electron microscopy was performed by drying a drop of suspension of glycogenic GNPs on glass slides and coated with gold. The morphology of nanoparticles was examined under a scanning electron microscope (JEOL JSM 5200). Transmission electron microscopy (TEM) was performed on TecnaiTM G2 Spirit BioTWIN, FEI Company, by drop coating gold nanoparticles suspension on carbon coated copper grids.

2.7. In vitro anticancer studies of glycogenic GNps

2.7.1. Cell culture

Human osteosarcoma cell line (Saos-2) and normal human embryonic lung cell line (L-132) were obtained from National Centre for Cell Science (NCCS), Pune, India. Saos-2 and L-132 cells were grown as monolayer in Mac Coy's and EMEM medium, respectively, supplemented with 10% fetal bovine serum and 1% antibiotic containing 10,000 units of penicillin, 10 mg streptomycin and 25 μg amphotericin B, in a humidified atmosphere in a 5% CO_2 incubator at 37 °C. Stocks were maintained in 75 cm^2 tissue culture flasks.

2.7.2. Cell viability assay

Saos-2 and L-132 cells were plated in 96-well plates at a density of 1×10^4 cells per well and incubated for 24 h in a humidified 5% CO_2 incubator at 37 °C. After 24 h, the cells were treated with glycogenic GNps at concentrations of 1, 0.5, 0.25, 0.125 and 0.062 mM, in triplicates, and incubated for 48 h. After incubation, media was discarded and 50 μl MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] (5 mg/ml in PBS), was added to each well and incubated for 4 h in 5% CO_2 incubator. The resulting formazan crystals were solubilized in 150 μl DMSO (Dimethyl sulfoxide). The reduced MTT was quantified by measuring the optical densities at a wavelength of 570 nm with reference filter of 655 nm using an ELISA reader (Microplate Reader (BIORAD-680)). Percentage inhibition of the cells was calculated using the