Endogenous Metal Nanoparticles in Biological Systems

Subjects: Biophysics

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The blood and tissues of vertebrate animals and mammals contain small endogenous metal nanoparticles. These nanoparticles were observed to be composed of individual atoms of iron, copper, zinc, silver, gold, platinum, and other metals. Metal nanoparticles can bind proteins and produce proteinaceous particles called proteons. A small fraction of the entire pool of nanoparticles is usually linked with proteins to form proteons. These endogenous metal nanoparticles, along with engineered zinc and copper nanoparticles at subnanomolar levels, were shown to be lethal to cultured cancer cells. These nanoparticles appear to be elemental crystalline metal nanoparticles. It was discovered that zinc nanoparticles produce no odor response but increase the odor reaction if mixed with an odorant. Some other metal nanoparticles, including copper, silver, gold, and platinum nanoparticles, do not affect the responses to odorants. The sources of metal nanoparticles in animal blood and tissues may include dietary plants and gut microorganisms. The solid physiological and biochemical properties of metal nanoparticles reflect their importance in cell homeostasis and disease.

Keywords: proteons ; blood ; olfaction ; cancer ; prions

1. Introduction

Scientists of the 18th century observed and described particles found in human blood and other liquids [1][2]. Some of those forms could move and multiply. Recently, it was found that the blood and tissues of vertebrate animals and mammals contain endogenous metal nanoparticles comprising 30–400 atoms [3]. The metal nanoparticles were not oxidized or heterometallic but were composed of individual atoms of iron, copper, zinc, silver, gold, platinum, and other metals. Metal nanoparticles can bind proteins and produce proteinaceous particles of 0.05–5 µm in size called proteons. Some proteons exhibit a cell-like form with an external membrane-like structure, although their appearance is distinct from that of bacterial walls or the mammalian plasma membrane. Nanoparticles bind misfolded proteins and help to remove them from the blood. Endogenous metal nanoparticles obtained from the blood of humans, rabbits, or dogs and engineered zinc and copper nanoparticles at subnanomolar levels were shown to kill cultured cancer cells [3][4]. Naturally occurring zinc nanoparticles were found to exist in olfactory and nasal respiratory epithelia and cilia in animals [5]. Studies of these nanoparticles by transmission electron microscopy and the selected area electron diffraction method revealed the existence of metal elemental crystalline zinc nanoparticles 2–4 nm in diameter.

2. Metal Nanoparticles in Blood and Tissues of Vertebrates and Mammals

The plasma of freshly drawn blood from humans, rabbits, dogs, or sharks was subjected to 120 °C at 140 kPa pressure for 2 h, revealing particles named proteons [3]. Healthy human blood contained ~3 × 10^8 proteons/mL. Proteons 0.05–5 µm in size were visible by transmission and scanning electron microscopy as disks of 50 to 250 nm or coconut-shaped structures with one or more nuclei of the size of 1–5 µm (**Figure 1**). The larger proteons showed a cell-like form with a peripheral membrane-like structure, although their appearance was distinct from bacterial walls or the mammalian cell membrane. Instead, this external structure appeared as a fibrous shell with a thickness of 10–12 nm. Similar cell-like structures with the inner mineral core and the external simulated membrane were observed in calcified human arteries, cardiac valves [6], pathologic renal calcification [7], fetal bovine serum [8][9], and human atherosclerotic plaques [10]. Proteons from human blood are primarily composed of carbon, hydrogen, oxygen, nitrogen, sulfur, and minute levels of potassium, sodium, chlorine, zinc, and copper. Though proteons contain fragments of hemoglobin protein, they do not have nucleic acids [3]. Kajander and Ciftcioglu utilized the PCR technique to demonstrate the presence of DNA in nanobacteria from fetal bovine serum [8]. However, succeeding studies by Cisar and colleagues [9] reported that DNA found by Kajander and Ciftcioglu more likely belonged to *Phyllobacterium umyrsinacearum,* depicted as a typical contaminant in PCR studies [11].

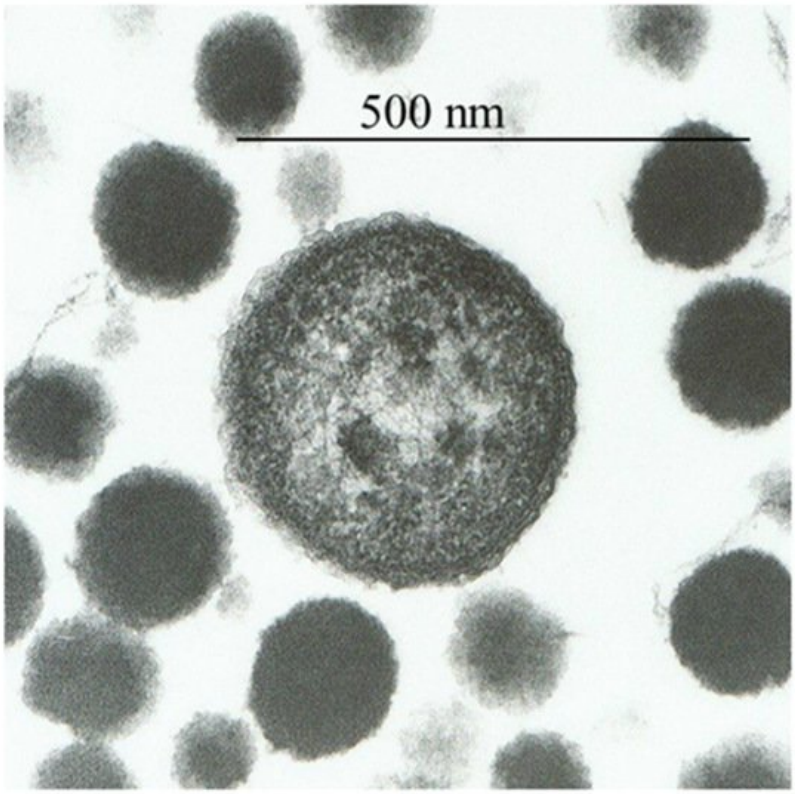

Figure 1. Electron micrographs of proteons from human blood.

Proteons were incubated in culture medium proliferate, reaching saturation after 13 days [3]. The culture medium alone contained no proteons and served as a negative control (**Figure 2**). The half-time of proliferation is eight days.

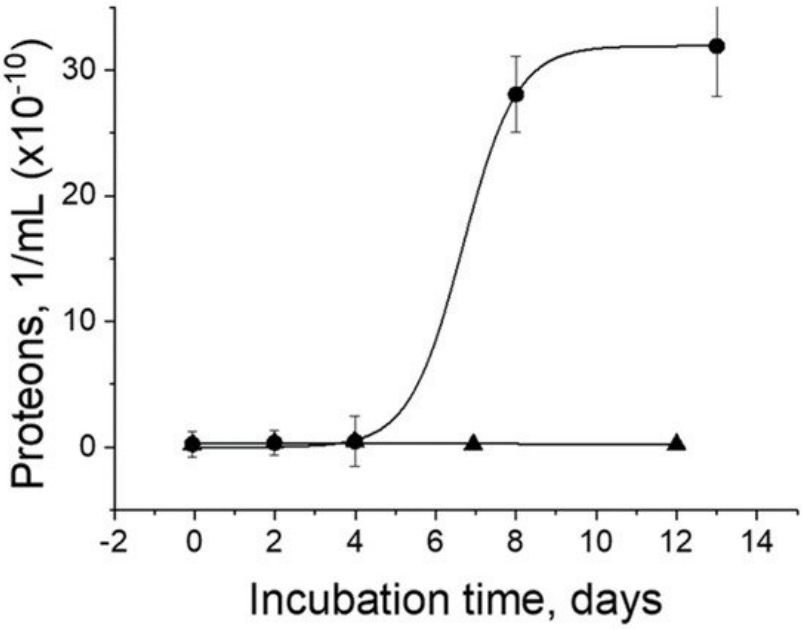

Figure 2. Proliferation of proteons. Proteons (●) were incubated in the tissue culture media (▲).

The proliferation of proteons agrees well with the multiplication of the nanoforms obtained from fetal bovine serum in the culture medium, showing an S-shaped growth curve saturated in approximately 16 days [12].

Transmission electron microscopy and the selected area electron diffraction method revealed metal nanoparticles in blood plasma and inside proteons. Free metal nanoparticles of copper, zinc, and iron serve as nucleating centers for the nucleation and growth of proteons. Each milliliter of healthy human blood contains approximately 7×10^{13} crystalline non-oxidized metal nanoparticles, and only approximately 0.004% of the entire pool of nanoparticles is bound to form proteons. These nanoparticles are 1 to 2 nm in diameter and contain 30–400 atoms [3]. Similar nucleation and growth of nanoforms were described in fetal bovine serum [12].

The proliferation of proteons was observed under a high-resolution light microscope [13][14]. Early in the proliferation process, only a few motile proteons were observed, and the rest of the space around these proteons appeared clear and void. As time passed, even though there was no change in the number of proteons in the observation field, the average size of proteons slightly increased. The clarity of the solution was progressively reduced, and empty space was gradually filled with milky clouds that slowly turned into myriads of tiny particles that were faintly visible. These were not produced by division of original proteons—the numbers of those at this point were not changed—but instead originated from a large number of small particles invisible through light microscopy, becoming observable when their size reached the limit of detection of the microscope system. Gradually, tiny particles grew larger and completely filled the observation field. Following this, proteons started combining in groups, aggregating, and producing large proteons [15].

I was able to find an interesting description of particle formation in blood plasma, which was observed about 150 years ago. H. Charlton Bastian described new particles formed in blood plasma under continuous microscope observation: “It seems almost equally certain that they did not originate from particles which were recognizable by microscopic power employed, since the fluids were at first, to all appearance, perfectly homogeneous. Either, therefore, the minute particles which were seen at a later stage must have originated owing to some primitive formative process taking place in a really homogeneous organic solution, or else the fluid, seemingly homogeneous, in reality, contained the most minute particles (microscopically invisible), derived in some unknown way from the previously existing protoplasmic elements” [2]. While further describing particles of different sizes, Professor Bastian wrote: “The corpuscles also presented different aspects, the largest of them appeared to possess a cellular structure with slight evidence of a boundary wall, and numerous large protein granules within, more or less completely concealing a faint ovoid nuclear-looking body. This granular appearance seemed to become more and more marked as the corpuscles become larger, and the nucleus also becomes more and more distinct, though only appearing as a space free from granules. The corpuscles, which were about 10 microns in diameter and those that were of smaller size, presented none of these characters. There was no break whatever in the continuity of the series; all graduations in size could be and were measured from mere plastid particle of 250 nm in diameter up to fully developed corpuscle. But in those corpuscles which exceeded 10 microns, the protoplasm gradually become granular, and they then began to exhibit changes which appear characteristic of the age and approaching degeneration.” [2]. Professor Bastian also described particles similar to those observed in this study via electron microscopy (**Figure 1**) as particles that “had assumed a most distinctly cellular appearance—each cell containing one or perhaps two well-defined ovoid nuclei…” [2].

The protein scavenging properties of endogenous metal nanoparticles are critical for intravascular hemolysis, resulting in the release of erythrocyte contents like hemoglobin into the general blood circulation [16]. Hemolytic conditions with substantial intravascular hemolysis occur in many diseases, including sickle-cell disease and malaria [17]. Released hemoglobin is normally filtered in the renal glomeruli. A high hemoglobin load can cause kidney dysfunction [18]. Released hemoglobin is additionally captured by haptoglobin, which is then recognized by hemoglobin scavenger receptors and endocytosed by macrophages [19]. When haptoglobin is depleted during critically elevated hemolysis [17], the released hemoglobin is collected by endogenous metal nanoparticles. The scavenging capabilities of metal nanoparticles are very high. On average, a 160-nm diameter proteon can bind approximately 10,000 protein molecules that are the size of hemoglobin [3].

The process of misfolded protein scavenging may provide an understanding of the mechanisms of some blood conditions related to intravascular hemolysis, which causes hemoglobin aggregation [20][21][22]. Certainly, metals have vital functions in conformation-based disorders such as prion disease, Parkinson’s disease, Alzheimer’s disease, and familial amyotrophic lateral sclerosis [23].

3. Metal Nanoparticles and Prions

Prions are misfolded proteins capable of transmitting their misfolded shape onto normal variants of the same protein [24]. The infectious prion PrP^{Sc} converts surrounding normal prion proteins (PrP^{C}) by stimulating them to assume this abnormal conformation. PrP^{Sc} clusters accumulate within the cell and exit the cell into neighboring tissues. As aggregate-containing cells die, they form the ‘spongiform’ manifestation of transmissible spongiform encephalopathies [25]. Trace minerals, including manganese and zinc, have been shown to interact with PrPC and have been found in abnormal concentrations in prion diseases [26][27][28][29]. To examine the effect of metal nanoparticles on the crystallization of prion protein, recombinant human PrP(23–230) (Alicon, Schlieren, Switzerland) was dissolved in purified water at a concentration of 1 mg/mL at 25 °C and heated at 120 °C and 20 psi for 20 min. Then, 3 µL of metal nanoparticle suspension from shark blood at a concentration of 5×10^{13} 1/mL was added to 7 µL of PrP solution, and the sample was subjected to the same temperature and pressure treatment (**Figure 3**).

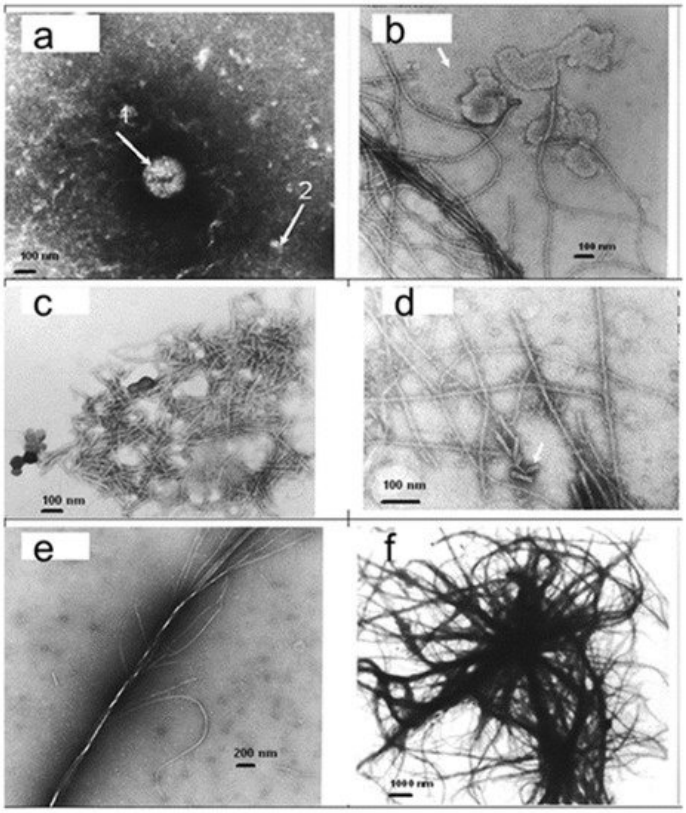

Figure 3. Effect of metal nanoparticles on crystallization of prion protein. Transmission electron microscopy. (**a**) Sample without metal nanoparticles. Arrow 1: A small number of relatively large round ball-type particles of ~300 nm. Arrow 2: A limited number of ~30 nm particles. (**b**) A large number of small particles (arrow) of 10–15 nm and filamentous structures were observed in the presence of metal nanoparticles. (**c**) Thin short rodlets (40–50 nm long). (**d**) Rodlets interconnected into longer thin filaments. (**e**) Thin filaments are interwoven into the thicker fibril. (**f**) Large fibrils create a system of fibrils and plaques.

Transmission electron microscopy showed a few types of structures in PrP samples. In samples without metal nanoparticles, a small number of particles were observed. In the presence of metal nanoparticles, a dramatic change in the protein structure was observed. The structure changes from small particles to small rodlets, filaments, and thick fibrils, and finally, large fibrils create a complicated system of fibrils and plaques (observed in my laboratory, **Figure 3**). It took 160 h to grow fibrillary structures from recombinant PrP (89–231) at the University of California [30], while it took us only 15 min to produce all structures shown in **Figure 3**. I suggest that the difference in the rate of growth is explained by the presence of metal nanoparticles, so that those prion particles are formed by the nucleation and growth of proteins around nucleating centers [3].

4. Metal Nanoparticles Are Lethal to Cancer Cells

Cancer is a disease that should not affect us if our defense systems are intact. All healthy animals and humans have small metal nanoparticles in their blood that may be dominant components of the body's defense mechanisms against cancer. Small concentrations of metal nanoparticles of size 1–2 nm isolated from animal blood were observed to be toxic to cultured cancer cells. [3][4]. After incubation with these nanoparticles, the viability of two rat glioma cell lines (F98 and RG2) decreased by 90% and 75%, respectively, while that of normal rat astrocytes (CTX) was reduced by only 25%. Total suppression of growth in-vitro required ≈ 1×10^{12} metal nanoparticles/mL (i.e., a few nmol/L), a concentration smaller than what is typically found in a healthy animal [3] (**Figure 4**A). Engineered zinc and copper metal nanoparticles of size 1 nm–2 nm were lethal to cultured RG2 glioma cancer cells. Cell death was confirmed by a colorimetric assay to assess cell metabolic activity, showing that the relative viability of RG2 glioma cells was reduced in a dose-dependent manner at subnanomolar concentrations of the nanoparticles. Noncancerous astrocytes were not affected under the same conditions [4] (**Figure 4**B). Synthetic copper nanoparticles have been found to be toxic to cultured cancer cells, including U937 (human histiocytic lymphoma) and HeLa cells (human cervical cancer origin), at concentrations of 1–500 µmol/L, [31][32][33][34], which are a few orders of magnitude higher than those of engineered zinc and copper nanoparticles [4].

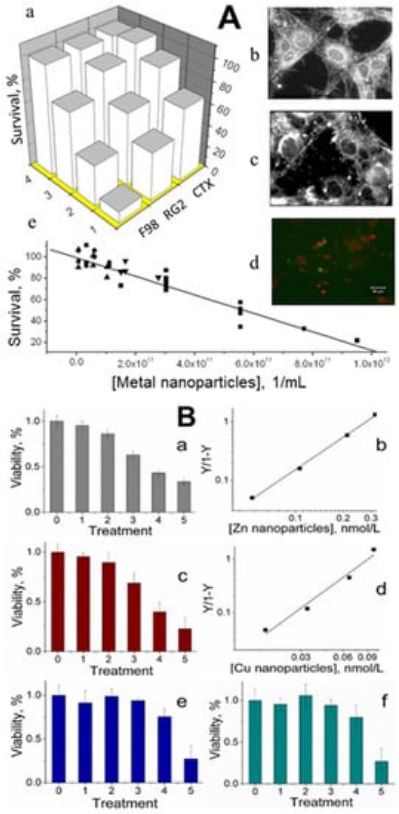

Figure 4. Panel (**A**). Effect of PNCs on cell viability. (**a**)—Viability of cultured cells after a 20-h exposure to different concentrations of metal nanoparticles (obtained from shark blood). Row 1: 1.7×10^{11} metal nanoparticles/mL; row 2: 9.1×10^{10}; row 3: 9.9×10^{9}; row 4: no metal nanoparticles. F98 and RG2 represent rat brain glioma cells, and CTX represents rat astrocytes. (**b**)—Darkfield microscope images of rat glioma cells. (**c**)—Cells after exposure to 7.7×10^{11} metal nanoparticles/mL. (**d**)—Fluorescence photomicrograph (400×) of RG2 glioma cells exposed to 7.7×10^{11} PNCs/mL for 30 min and stained with Annexin V and propidium iodide. (**e**)—Viability of cultured RG2 after 20 h of exposure to different concentrations of Pmetal nanoparticles obtained from the blood of blue sharks (Prionace; ■), dogs (●), humans (▲), or New Zealand white rabbits (Harland Sprague-Dawley) (▼). $R^2 = 0.95$; $p < 0.0001$. Scale bars: 40 µm. Panel (**B**). Viability and mortality of glioma cancer cells treated with metal nanoparticles (error bars are standard deviations). (**a**)—Viability of glioma cells under six experimental conditions: 0, no treatment; 1, 2, 3, and 4, zinc nanoparticles (all expressed in nmol/L) 0.053, 0.106, 0.212, and 0.318, respectively; 5, 1 µmol/L of staurosporine. (**b**)—Hill representation of glioma cell mortality caused by zinc nanoparticles. (**c**)—Viability of glioma cells under six experimental conditions: 0, no treatment; 1, 2, 3, and 4, copper nanoparticles (all expressed in nmol/L) 0.017, 0.033, 0.066, and 0.1, respectively; 5, 1 µmol/L of staurosporine. (**d**)—Hill representation of glioma cell mortality caused by copper nanoparticles. (**e**)—Viability of astrocytes under six experimental conditions: 0, no treatment; 1, 2, 3, and 4, zinc nanoparticles (all expressed in nmol/L) 0.053, 0.106, 0.212, and 0.318, respectively; 5, 1 µmol/L of staurosporine. (**f**)—Viability of astrocytes under six experimental conditions: 0, no treatment; 1, 2, 3, and 4, copper nanoparticles (all expressed in nmol/L) 0.017, 0.033, 0.066, and 0.1, respectively; 5, 1 µmol/L of staurosporine. (Adopted from [4]).

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