Immunogenicity of Therapeutic Proteins by QED Induced EM Radiation

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***Abstract*—Protein therapeutics is used in the treatment of diabetes and various forms of cancer. A major concern is that repeated administration to patients often leads to undesirable antidrug antibodies (ADAs) with a wide range of consequences that may be life – threatening by inducing immunogenicity or an adverse response of the immune system. It is generally thought the ADAs are triggered by the tendency of monomer protein molecules to aggregate, although why immunogenicity occurs is not known. In protein deposition diseases such as Alzheimer and Parkinson, protein aggregates have stronger immunogenicity. Typically, the aggregates known to elicit ADA response are globular proteins having molecular weights from 6 -100 kDa and diameters from 3 – 10 nm comparable to inorganic nanoparticles (NPs) that based on QM have been linked to DNA damage by the natural emission of low-level QED induced EM radiation. QM stands for quantum mechanics, QED for quantum electrodynamics, and EM for electromagnetic. Similarity suggests the toxicity to the immune system is the EM radiation from the protein aggregates themselves. How immunogenicity may be reduced is discussed.**

Keywords - immunogenicity, toxicity, therapeutic proteins, cancer, quantum mchanics, quantum electrodynamics

I. INTRODUCTION

Aggregation of proteins activate their rejection by the immune system that otherwise are beneficial for the treatment of disease. However, the mechanisms by which aggregates trigger unwanted immunogenicity of therapeutic proteins are still unknown. For over a half century, the high molecular weight (MW) of aggregates compared to their monomeric form is thought [1,2] to be the main cause for their increased immunogenicity. Protein aggregates are believed to trigger immunogenicity based on MW that enhance their recognition and rejection by the immune system.

Currently, the formation of repetitive arrays of epitopes or sites on the aggregate are thought [1,2] to provide sites for the antibody to react. However, conformation of the aggregate protein constituents to high MW cannot be dismissed in related immunogenicity. Aggregates formed by native - like proteins are expected [2] to pose a higher threat for immunogenicity. The immune system has evolved to make antibodies to conformational rather than to linear determinants, i.e., independent of spatial arrangement. Arrays of epitopes are therefore less likely to be significant in aggregate - related immunogenicity than high MW.

Aggregates formed from already immunogenic proteins are also expected to be more efficient in triggering immune responses. Thus, aggregates of a foreign origin protein are expected [1] to be more immunogenic than self - protein aggregates. The number of constituent monomers in an aggregate necessary to trigger immune responses based on pure crystalline dimers of human insulin was shown [3] to be dose dependent. But this differs from the minimum number of epitopes necessary [4] to illicit Ti type 2 antibody responses, i.e., at least 20 repetitive epitopes was required to successfully trigger an immune response. Indeed, the minimum number of was quantized in that the epitopes must be connected together in a spatially contiguous cluster. The polymers required a MW higher than 100 kDa and a minimum of 20 epitope repeats in order to induce antibody formation. By this theory, aggregates that present more than 20 repetitive epitopes on their surface and have an MW bigger than 100 kDa are immunogenic.

Hydrophobicity of protein aggregates has also been suggested to play a role in their immunogenicity. It has been proposed [5] that hydrophobic portions of biological molecules act as universal damage - associated molecular patterns to initiate immunity. More hydrophobic aggregates of therapeutic proteins therefore represent a bigger threat for unwanted immunogenicity.

Another factor that may affect aggregate - related immunogenicity is if the aggregates are formed only by the protein itself or by a mixture of protein and impurities. Protein adsorption to inorganic NPs may create aggregates with epitopes other than those in self – aggregates. Regardless, immunogenicity based on MW should apply to any aggregate.

II. BACKGOUND

Immunogenicity by aggregates finds similarity with DNA damage [6] by QED induced EM radiation that claims NPs naturally emit low-levels of EM radiation and if not repaired by the immune system may lead to cancer. Cancer research is only beginning to recognize the remarkable fact that natural and manufactured NPs in body fluids [7] damage DNA by the same reaction pathways as conventional sources of UV radiation. But biological NPs < 100 nm [6] also emit UV provided the NPs have a refractive index greater than the water surroundings. For cancer cells, the index varies from 1.34 to 1.38 compared to 1.33 for water. Sources [6] of biological NPs within the human body are described as follows.

A. *Disorganization of Epithelial Tissue*

Epithelial tissue forming the outer layers of the skin protect exterior surfaces of the body, but also provide protection for hollow organs and glands including the breast, prostate, colon, and lung from body fluids. Epithelial tissue is organized by a submicron thick < 100 nm basement membrane (BM) that provides the structural scaffold template for the extracellular matrix (ECM). Breakdown of the BM is associated with the spread of tumors.

Loss of integrity in the ECM is triggered [8] by enzymes called matrix metalloproteinases (MMPs). Indeed, MMPs induce the epithelial-mesenchymal transition (EMT) that fragment the BM and move through the body. In breast cancer, EMT allows tumor cells more mobility to penetrate barriers like the walls of lymph and blood vessels, facilitating metastasis, e.g., the MMP-3 enzyme causes normal cells to produce a protein called Rac1b that is found only in cancers.

Current thought is the Rac1b protein found in most cancers itself damages the DNA. Contrarily, the ROS are not induced by Rac1b to stimulate the development of cancer by mutation of genomic DNA. Rather, the ROS are formed in a side reaction from the QED radiation emitted from the NPs of fragmented BM. Nevertheless, the Rac1b is still a NP that emits UV and can damage DNA.

B. *Exocytosis of Small Proteins*

The exocytosis or release of fusion products into the extracellular fluid through the tumor cell membrane is known [9] to produce onco proteins. Indeed, the release of fusion products is required for the initiation and growth of malignancy. It should come as no surprise that exocytosis is linked to tumor growth. Hence, QED induced radiation at UV levels from submicron fusion products as biological NPs during exocytosis is consistent with malignancy.

C. *Molecular Markers in Cancer Detection*

Changes that occur in cancer cells compared with normal tissue can be detected in body fluids and used as molecular markers of cancer. As an epithelial tumor grows, cancer cells fragment from the organ epithelium and enter the body fluid as NPs making it possible to detect molecular markers such as DNA mutations.

Protein markers are of interest in QED induced radiation whether by BM fragmentation or exocytosis because the UV radiation produced is damaging to DNA. One such marker is the telomerase enzyme [10] expressed by almost every cancer type: head and neck, lung, breast, colon, pancreas, bladder, and prostate cancers.

III. THEORY

Similar to DNA damage [6] by NPs, immunogenicity by aggregates is proposed to occur by the theory of QED induced EM radiation

A. *QM Restrictions*

Unlike statistical mechanics, QM restricts the heat capacity of atoms in nanostructures. The Einstein-Hopf relation [11] for the harmonic oscillator giving the dispersion of Planck energy E with the EM confinement wavelength λ is the measure of the heat capacity of the atom to absorb EM energy. QM in relation to the classical oscillator by statistical mechanics is shown in Fig. 1.

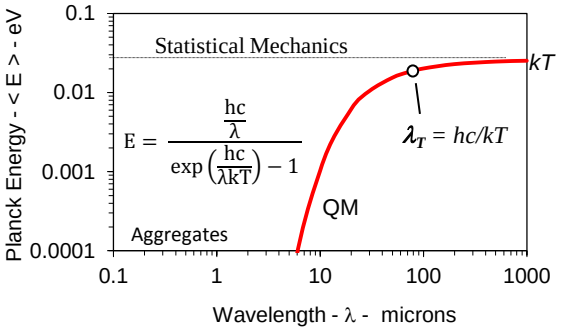


Figure 1. Heat Capacity of the Atom at 300K

By the equipartition theorem of statistical mechanics, the classical oscillator allows the atom to have the same heat capacity in aggregates as the macroscale. QM oscillators differ in that kT energy is only available for $\lambda > \lambda_T$ while heat capacity is restricted for $\lambda < \lambda_T$. At ambient temperature, $\lambda_T \sim$ 50 microns. Fig. 1 shows the heat capacity of the atom is less than kT for λ < 50 microns with full kT energy available only for λ > 50 microns. By QM, atoms in aggregates having λ < 1 micron have virtually no heat capacity to conserve heat from any EM source by an increase in temperature.

B. *TIR Confinement*

Lack of heat capacity by QM precludes heat from EM sources to be conserved in protein aggregates by an increase in temperature. Still, the EM energy must be conserved, and therefore conservation by QED proceeds during TIR confinement by creating EM radiation *inside* the aggregate. TIR stands for total internal reflection. TIR has a long history beginning with Tyndall in 1870 who discovered if the refractive index of a body is greater than that of the surroundings, absorbed light is trapped at its surface. In nanostructures, TIR has an important significance [12] and need not be limited to light absorption. Unlike macrostructures, nanostructures of aggregates have high surface to volume ratios, and therefore heat from any EM source (lasers, molecular collisions, electrical resistance, etc.) is absorbed almost totally in the aggregate surface. Since the aggregate surface corresponds to the TIR wave function, QED induces the absorbed EM energy to undergo the spontaneous creation of photons *inside* the aggregate. However, TIR confinement is not permanent, but rather sustains itself only during heat absorption, i.e., absent absorption of EM energy, there is no TIR confinement and QED radiation is not created.

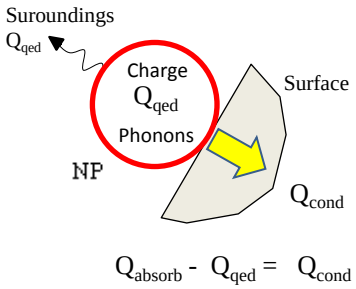
Taking the spherical geometry as the idealized shape of the typical aggregate, the TIR confinement of EM energy creates QED photons at frequency f having Planck energy E,

$$f = \frac{c/n}{\lambda}, \qquad \lambda = 2d, \qquad E = hf \tag{1}$$

where, d the diameter of the aggregate, n is the refractive index. Typically, n = 1.4 – 1.7 for biological material.

C. QED Induced Heat Transfer

QED induced heat transfer is the consequence of the QM requirement that the heat capacity of the atom vanishes in nanostructures. Consider an aggregate resting on a surface as depicted in Fig. 2.



$$Q_{absorb} - Q_{qed} = Q_{cond}$$

Figure 2 QED Induced Heat Transfer

Since absorbed EM energy Q_{absorb} cannot be conserved by an increase in aggregate temperature, conservation occurs by other paths. One path is conductive flow Q_{cond} into the surface by phonons, and the other by creation of QED radiation Q_{qed} *inside* the aggregate that in turn is conserved by the creation of charge by Einstein's photoelectric effect or by emission to the surroundings. However, phonons respond to absorbed heat at acoustic velocities while QED radiation moves at the speed of light. Hence, absorbed Q_{absorb} is promptly conserved by QED radiation well before phonons respond, and therefore conductive heat transfer does not occur, i.e., $Q_{cond} \sim 0$.

IV. Analysis

The collision power Q_C of water molecules [6] of mass m transferred to an aggregate having diameter d is,

$$Q_C = \frac{\pi}{2\sqrt{3}} pPd^2 \sqrt{\frac{kT}{m}} \qquad (2)$$

where, p is the probability of full kT energy transfer for inelastic collisions and P is ambient pressure. The mass m = MW/ N_{Avag} where MW = 18 and N_{Avag} is Avagadro's number. Taking n = 1.4, the power Q_C for p = 10^{-7} with aggregate diameter d in relation to the Planck energy E of the QED photons created is shown in Fig. 3.

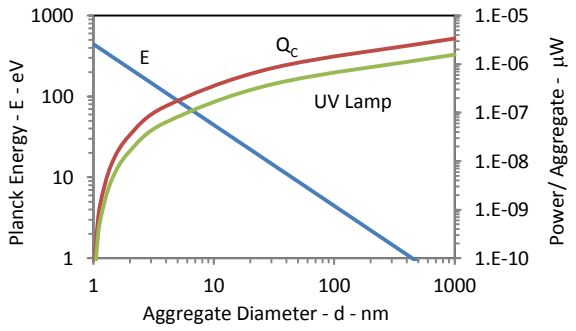


Figure 3. Aggregate Planck Energy E and Power Q_C

V. Discussion

A. UV

Unfolded chicken egg white lysozyme (CEWL) triggered by UV illumination can form [13] uniform globular aggregates. The morphology and aggregation of proteins is of great importance for the pathogenesis of the related diseases. Closely related is unfolding of CEWL observed in other disulfide-bonded proteins.

CEWL undergoes drastic conformational changes resulting in the exposure of some hydrophobic residues upon the dissociation of the native disulfide bonds by UV illumination. Subsequently, partially unfolded molecules self-assemble into small granules driven by intermolecular hydrophobic interaction. With longer UV illumination times, the granules self-assemble into larger globular aggregates. Similar aggregation is found in other disulfide-bonded proteins, i.e., a-lactalbumin, RNase A, and bovine serum albumin.

The UV radiation selected [13] was a UV-B lamp with wavelength centered at 285 nm given that tryptophan and tyrosine residues are excited by UV-B. Upon UV radiation at 200 µW/cm^2 at room temperature, the aggregates started to form observable aggregates of diameters 30 nm after 10 min and grew monotonically reaching diameters 80 nm and 150 nm after 30 and 50 min. The TEM image of the aggregate after 72 hours is shown in Fig. 4.

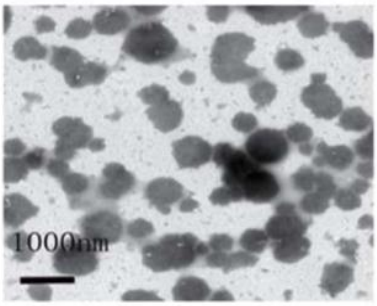


Figure 4. Spherical aggregates in the CEWL sample after 72 h continuous illumination. Scale bar = 1000 nm.

Lacking UV radiation sources within human bodies, aggregation of proteins is thought [13] to originate in the UV of sunlight.

However, UV need not be limited to sunlight. Fig. 3 shows the collisional Q_C energy from water molecules is comparable to that from the UV lamp at 200 µW/cm^2 even assuming the probability of p inelastic collisions is 10^{-7}. Hence, QED induced EM radiation appears significant in protein aggregation. More study is suggested in the probability of inelastic collisions by water molecules.

B. Simulated Sunlight

Most organic polymers undergo chemical change, or photodegradation, when exposed to visible or UV radiation. The biological activity of a protein depends on its native structure [14] as determined by the three-dimensional arrangement of amino acids at the active site. Research on the

photochemistry of proteins has demonstrated that physical and chemical alterations may be observed following irradiation by UV light. Most of the amino acids do not absorb UVA or visible radiation. Only tryptophan and tyrosine and to a lesser extent phenylalanine absorb UVB light. The enzymes that are constituted exclusively by amino acids and no other chemical groups are not affected by visible light in both their catalytic activity and their structure.

Under simulated sunlight, the significant changes in percent denaturation (i.e., % reduction in endothermic peak area) were found [14] after 1 h of light treatment for lactase, invertase, lysozyme, and BSA is duplicated here in Fig. 5.

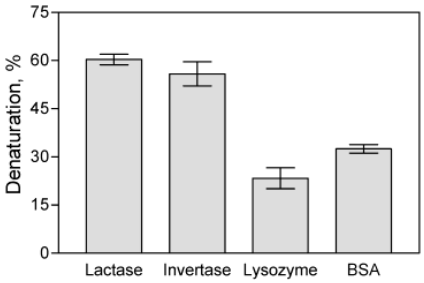


Figure 5. Denaturation after 1 h of Simulated Sunlight

Protein aggregation involves the formation of higher molecular weight complexes from the denatured protein, which then cross-link by disulfide bonding common in denatured protein. After light exposure, an increase in high molecular weight protein fractions [14] was observed. The peak corresponding to the enzyme was reduced, probably because of the aggregation of protein molecules. It is likely that the aggregation observed for samples exposed to sunlight was due to disulfide bond formation between protein molecules.

VI. CONCLUSIONS

Arrays of epitopes are less likely to be significant in aggregate - related immunogenicity compared to its high MW (> 6 kDa) of the aggregate. A minimum of 20 epitope repeats in order to induce ADA does not appear a necessary condition for aggregate formation.

Hydrophobicity of protein aggregates in enhancing immunogenicity is consistent with the QED requirement that the refractive index of the aggregate is greater than that of the surrounding water. Hydrophobicity provides a distinct change in refractive index at the surface of the aggregate. Conversely, agents that destroy hydrophobicity are recommended to reduce immunogenicity.

By QED theory, immunogenicity by protein aggregates is similar to DNA damage by inorganic or biological NPs in that both convert collisional energy by water molecules to EM radiation beyond the UV that enhances immunogenicity and DNA damage.

QED theory for protein aggregates is based on the QM requirement that the heat capacity of the atom vanishes in submicron aggregates. Instead, absorbed EM energy from the collision of surrounding water molecules is conserved by the creation of UV photons by the QED induced frequency up-conversion to the TIR resonance of the aggregate.

Experiments showing aggregation by UV of CEWL embryos and simulated sunlight of lactase, invertase, lysozyme, and BSA support EM radiation as the source of protein aggregation.

Collisional EM energy from water molecules depends on the probability of inelastic collisions. For CEWL, the 200 $\mu W/cm^2$ power from a UV lamp is found similar to the collisional power at very low probabilities 10^{-7} of inelastic collisions. Protein aggregation therefore need not depend on sunlight alone, but occurs naturally in body fluids as water molecules collide with aggregates.

The very low probability 10^{-7} of inelastic collisions necessary to be consistent with the UV lamp in the CEWL experiment suggests QED induced EM radiation is significant in aggregate formation. Typically, the probability of inelastic collisions may vary from 1 to 10^{-10} suggests QED radiation produces more intense UV than the UV lamp. More study is required to confirm this conclusion..

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