Protein Instability in Medicinal Applications of Gadolinium Complexes

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Abstract

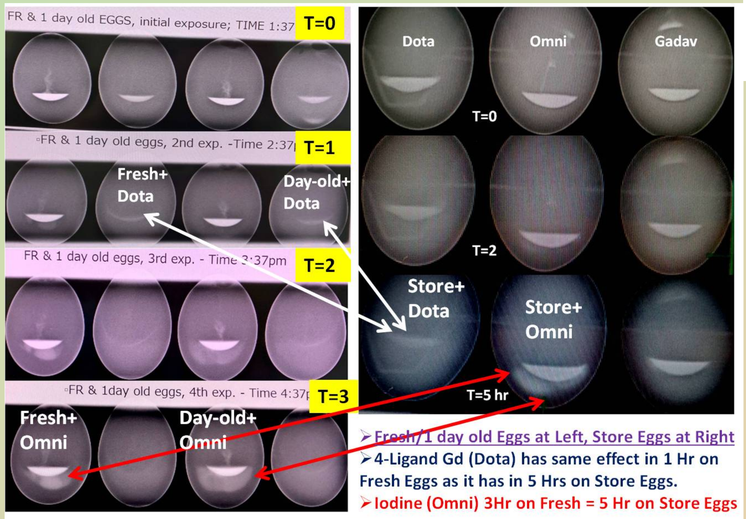
Lanthanides like Gadolinium are useful in medicinal and radiological chemistry. They can be used to eliminate free radicals and act as antioxidants in vivo. However, this affinity for reactive oxygen species also create competitive binding of lanthanides to proteins. This alters biologically important electron transfer pathways, for example in bones and in brain and renal tissues. This could cause toxicity. We have been working on the molecular interaction of Gadolinium with egg proteins. In order to understand experimental results molecular modeling is being performed for long range dispersive forces that are attractive at large ionic distances but have attractive nature at short range. It is interesting that some Gadolinium complexes can break down the 3D protein structures faster depending on ligands. Hence it is necessary to revisit the hydrogen bonding and other weak molecular interactions in such systems.

Methods

Planer X-ray: Three x-ray systems were used in Maimonides Medical Center and at City Tech Radiologic Technology department , Brooklyn, NY by 4 research students to verify reproducibility and operator reliability. Technical parameters were: low kVp range of 50-60 and high kV range at 70-80 with 1-5 mAs. Imaging was repeated serially with identical set ups for several days.
Mammography: Hologic Mamography system at Memorial Sloan Kettering Medical Center were used for 2D as well as 3D imaging at 30 kVp and 120 mAs with Rh/Al filters.
Samples: Various store brand eggs as well as fresh eggs were obtained; MR contrast agents were Dotarem and Gadavist while CT contrasts were Omnipaque and Isoview (all at 0.5mL volume). Infusions were done by creating a small hole at top of each egg and using a 23G needle to carefully reach at the yolk.

Results-1

Figure 1. Time series breakdown of fresh vs shelf-stable store eggs using Gadolinium (Dotarem, Gadavist) and Iodine (Omnipaque) Contrast Agents



Background

Water plays a significant role in protein stability via mechanical support, thermal coupling, mass and charge transport, and the competition with ligands for the binding sites. However, due to large inhomogeneity in water density, polarity and mobility around macromolecules hydration behavior is hard to assess by experiment. Nanoparticles and chelated metal ions are used in almost all medical diagnostics and therapy including cancer screening, neuroprotective medicine, tissue engineering, to name a few where protein hydration is virtually unknown . This work is based on model biosystems (chicken eggs) where the protein composition is well known, it is virtually free of heavy metals and protein degeneration due to metal toxicity could be experimentally modeled. The selected metal complexes were chosen from radiological applications that are known to be safe with minimal breakdown in vivo. However, there are safety concerns raised recently and this study was also aimed to explore such concerns as well.

Results-2

Figure 2. Relative efficiency of various MRI and CT contrast agents in breakdown of egg proteins measured by protein layer formation with time in 3D high resolution Mammography.

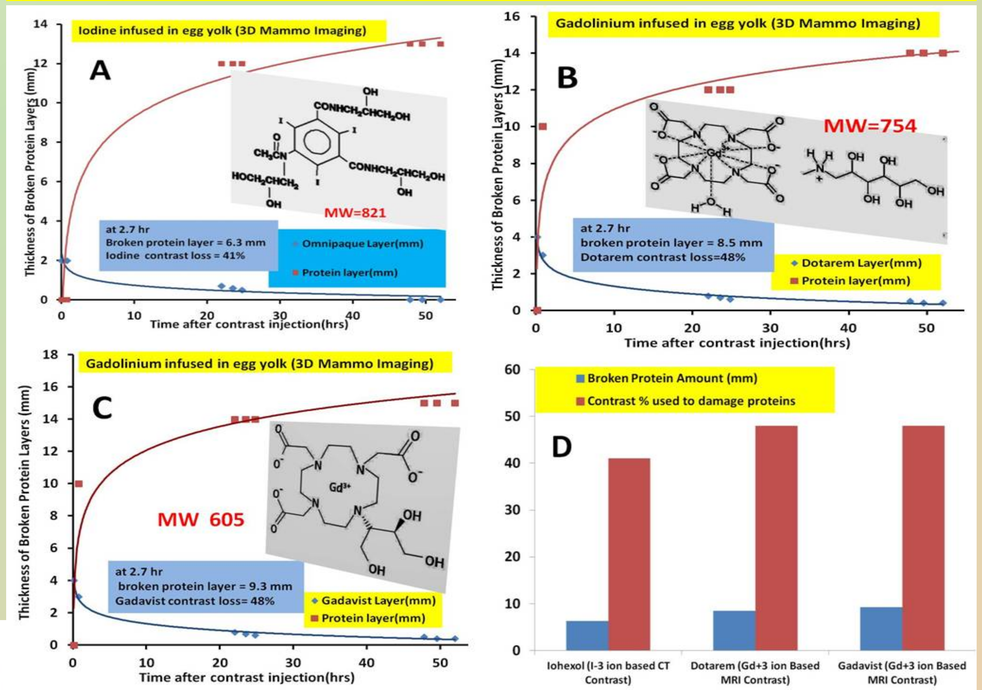
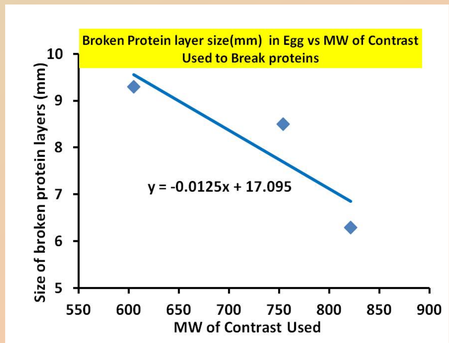


Table 1. Geometrical characteristics of contrast agents that may correlate with experiments

Contrast Agent	MW	H-Bond Donor Count	H-Bond Acceptor Count	Rotatable Bond Count	Topological Polar Surface Area (A**2)	Protein layer size in Eggs (This Work)
Dotarem	754	7	18	11	284	8.5
Omnipaque	821	8	12	12	200	6.3
Gadavist	605	3	13	7	194	9.3

Figure 3. Protein breakdown rate vs MW of contrast agents used



Protein Denaturation Model

In a first approximation, a somewhat bound water structure with the egg proteins with a pronounced slowing down of rotational motion (from 1-10ps) and an increase of H-bond lifetimes are proposed to match the contrast dynamics timelines (O(ns)), far more than the factor of 2-3 proposed so far in protein hydration.

In egg proteins, we model, the water molecules that are buried inside cavities in the protein interior are probably extracted by the Gadolinium and Iodine complexes in order to denature the proteins. These internal water molecules are an integral part of the protein structure and can only exchange with the bulk with the aid of rare protein structural fluctuations. However, in the presence of contrast agents this may not be rare events after all.

A mechanism we are yet to formulate is how does the apparently "neutral" contrast complexes find their way to exchange water with the interior of proteins. This requires a sufficient number of surface-surface interactions that may propagate in via linear chains of water molecules as a fluctuating bridge in order to perturb the inner water of egg proteins.

The more loosely bound charge structures we have used (Dotarem for example), the faster protein breakdown was observed. Hence surface-to-surface coupling seems to be the first step leading to the stripping of the loosely bound ligand in these agents. This is also argued by the larger surface area for Dotarem (284 A**2 compared to approximately 200A**2 for the other two) explaining enhanced protein breakdown.

As part of the mechanism, one may point to the larger number of H-bond acceptor counts in Dotarem (18 compared to 12 or 13 for the other two) suggestive of greater abundance of H-bond donor sites and/or slower rotational time frames for such donor pockets that could be primary targets for Dotarem.

Conclusion

Contrast media available for medical diagnostics need to be evaluated further, particularly in young and pregnant population as demonstrated here with enhanced protein breakdown in fresh, embryonically viable chicken eggs. Additionally imaging of metal ions can help in modeling of macromoleculer dynamics in vivo to complement existing knowledge.

Acknowledgment

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