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Shifts: 6 shift(s)	**Local contact(s)**: Adam Round	*Received at ESRF:*

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Report:

Cataract, eye lens clouding due to light scattering, is a leading cause of blindness and can result from protein condensation in hyperthermic and stressful conditions [i], when altered intermolecular interactions lead to dense phases that can compromise cell and organ functions. Once a lens has developed a cataract, there is no known method to make the lens clear again and cataracts can be treated only by surgically removing them.

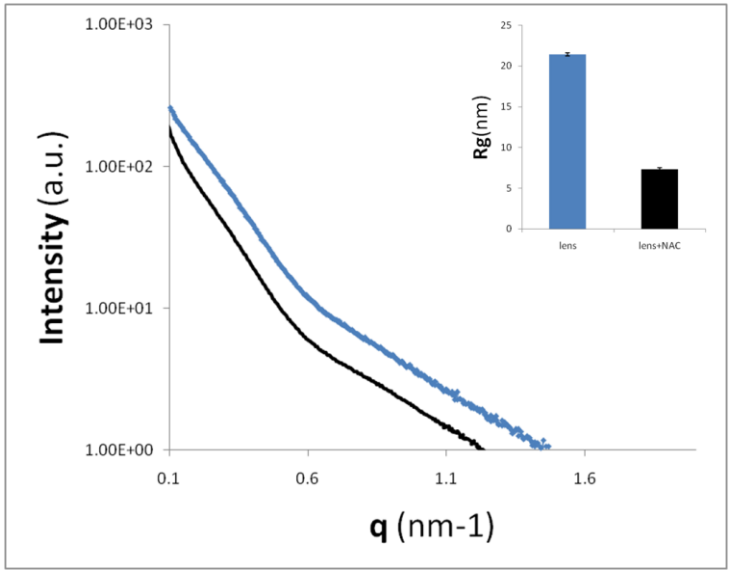

Fig. 1 SAXS curves of rat lens extracts in absence or presence of NAC antioxidant and measured Rg with Guinier extrapolation (inset).

Mammalian eye lens cells, named fibercells, lose organelles during embryonic tissue formation and cytoplasm is constituted by a concentrated solution of proteins called crystallins. Cytoplasmic lens extracts are formed by three main classes of crystallins: α, β and γ crystallins. Among these, the most abundant are the α-crystallins, which are capable of chaperon-like activity, the ability to bind unfolded proteins to avoid their aggregation and precipitation in cell. When β and γ crystallin unfold during thermal, chemical or electromagnetic stress, they become substrates of alpha crystallin[i,ii]. NAC, N-Acetyl-cysteine is an antioxidant was reported as effective in improving vision in cataract patients and reduced the appearance of cataract[iii]. As a consequence NAC represents a potential drug for cataract treatment. Hence we investigated the effect

of this antioxidant on α crystallin and cytoplasmic lens extracts to quantify the changes of structure and function of the chaperonin at low concentration and in its natural environment. Lens extracts, mainly constituted by α crystallin, contain also high-molecular-weight aggregates (HMW) that form in vivo with age and cataract progression. HMW formation can be induced with a heating stimulus [iv] and our preliminary results obtained with Dynamic Light Scattering technique showed that NAC (at concentrations higher than 10mM) can decrease the degree of aggregation of eye lens subjected to a stress stimulus (heat shock at 51°C). We measured, by means of SAXS, radius of gyration of proteins in eye lens cytoplasm extracts at 37°C both in the absence and presence of NAC (Fig.1). Interestingly the lens extracts Radius of gyration is 21,4 nm, since α crystallin has a radius of 6.3 nm at this temperature and β and γ crystallin have significantly lower radii, it is reasonable to supply that this high value of Rg is due to the presence of high molecular weight aggregates in the sample.

When NAC (10mM) is added to the lens extracts, the Rg diminishes and reaches a value of 7.29 nm, comparable to radius of α crystallin alone. Hence NAC shows an ability to reduce aggregates dimension in lens citoplasms of healthy rats.

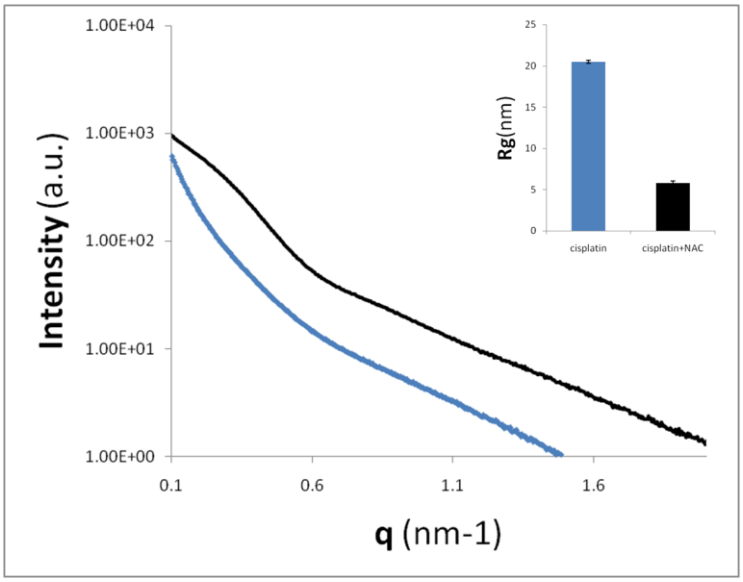

Fig. 2 SAXS curves of lens extracts from rats treated with Cisplatin in absence or presence of NAC antioxidant and measured Rg with Guinier extrapolation (inset).

Subsequently we tested NAC effect on rats who were sistemically treated with cisplatin, a chemoterapic drug whit a high oxidant capacity[v]. As a matter of fact it is known that oxidants ingested or injected have the ability to affect lens homeostasis[vi].

As expected the lens cytoplasm extract of these rats is clearly damaged by the cysplatin administration: the SAXS curve profile shows the presence of aggregates in the sample (Fig.2). The radius of gyration of this sample, is 20.5 nm. Again in presence of NAC not only the curve assumes a shape indicating the disaggregation process, but the radius of gyration reduces significantly reaching a value of 5.8 nm. Hence this antioxidant has a marked efficacy also on damaged tissues, and becomes a drug candidate for cataract removal without surgery as well as a cure for secondary cataract.

The data on this report are still preliminary and we are analyzing the effects of NAC on α-crystallin structure to verify if the antioxidant activity of NAC is mediated by the same transition of α-crystallin that we previously observed enhances its activity[vii].

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[vii] Article submitted (Journal of Physical Chemistry B)