Review Article

Synuclein Aggregation: Critical Role of Water in Entropy-Driven, Temperature-Responsive Aggregations of Macromolecules and Cells

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The change from monomeric α-synuclein to aggregated forms plays a fundamental role in the initiation of Parkinson's diseases that include its genetic subtypes. The biophysics of the homo-aggregation of whole cells (erythrocyte rouleaux) shares many features of the homo-aggregation of macromolecules such as α-synuclein. Evidence from both reveals that the aggregation pressure is determined as much by concentrations of surrounding macromolecules and water, as that of α-synuclein. Such entropic, rather than enthalmic, considerations predict that aggregation pressures would be less if measures were taken to prevent transient small increases in body temperature.

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Highlights

- Aggregation of α-synuclein is fundamental to Parkinson's and related disorders.
- Similar principles govern the aggregation of macromolecules and red blood cells.
- Thus, α-synuclein aggregation is influenced by other macromolecules and water.
- Pyrexia is postulated to support immune defenses, both intra- and extra-cellularly.
- By avoiding pyrexia, α-synuclein aggregation tipping points might be avoided.

1. Introduction

An editorial recently heralded a leading medical journal's first ever series of papers that address the question "what is next for Parkinson's disease?" No immediate solution seems on the horizon and deeper

probings into the underlying science are encouraged[1]. Elsewhere, another editorial regrets "the alpha synuclein tug of war" between experts in both Parkinson's disease (PD) and its related disorders[2] (such as Lewy body Parkinsonism;). However, this refers to different α-synuclein states that can distinguish various *post-initiation* disease stages when genetical and environmental factors could have grown in number and influence. Thus, there remains general agreement that *initiating* mechanisms are likely to involve "transformation of healthy α-synuclein" (monomeric) into an aggregated "pathological form"[2] (oligomeric that can grow to polymeric;). And growing stronger is the case that such higher aggregates can activate autoimmune processes that damage neurons[3][4].

Nevertheless, in certain genetic subtypes involving a leucine rich repeat kinase (LRRK2), no pathological forms of α-synuclein were detectable[5]. Despite clear Parkinson symptomatology, this correlated with absence of microscopically visible Lewy bodies (LBs) in autopsy examinations[6]. A "causal role" of α-synuclein aggregates in generating symptoms was questioned[5]. However, a more sensitive assay has now made the aggregates detectable in those subtypes. Hence, it is concluded that "Lewy-body negative LRRK2-related PD is not associated with a lack of α-synuclein aggregation in neurons but rather a deficiency in the formation of inclusions." Thus, it is the *last step* in the progression – monomeric to oligomeric to polymeric to Lewy body inclusion – that seems to be missing[7].

When considering the biophysics, it is understandable that some would regard the monomeric form as an initial "catalyst" that enhances ("seeds") the phase transition to less-soluble aggregated forms. It would then seem experimentally reasonable to simulate this in the laboratory by dissolving increasing quantities of purified α-synuclein monomers to define a saturation point. Going beyond this to "supersaturation" should result in the appearance of aggregates.

Surprise was expressed when it was found that the saturating concentration was much greater than that observed in the tissues of PD patients. Thus, "to trigger aggregation through seeding in the laboratory, at least a ten-fold increase above the physiologic concentration of α-synuclein in neurons must be used." It was concluded: "This is why seeding is unlikely to operate as a source of protein aggregation in the human brain"[5]. The present paper proposes that the underlying biophysics is likely to be more complex.

We begin by showing that, when entropic factors are considered, α-synuclein is far from alone in operating "as a source of protein aggregation." The surrounding macromolecules and water play important roles (section 2). We then deal with an extensive history, not of PD itself[8], or of temperature effects[9], or of the relationships of biochemical changes to specific brain structures[10]. Rather, we deal

with an aggregation studies that, prior to electron microscopy, involved intact cells whose aggregations could be followed by conventional light microscopy (section 3). This leads to consideration of both normal and pathological roles of aggregation tendencies (section 4). The fortunate conclusion is that, although cures appear distant, there are some actions PD patients themselves could take that might slow aggregation and hence disease progression (section 5).

2. Water, entropy and tipping points

We are here primarily concerned with the like-with-like homoaggregation of *one* macromolecular species, rather than with the heteroaggregation of two or more. However, depending on the stage, minor degrees of heteroaggregation can occur. We are also concerned with the energetic power of changes brought about entropically – namely creating order in one area *at the expense of* creating disorder in another.

A superficial understanding of the biophysics of protein aggregation would suggest that, by lowering temperature, free-moving individual proteins would be slowed, so making it easier for them to gather together (an organized aggregation). Conversely, by increasing temperature they would tend to fly apart. Eventually, a denaturation temperature would be attained. They would then form an unorganized precipitate. This scenario sometimes applies, in accordance with the enthalpic biophysical principles that some espouse[5].

Less intuitive, but more probable, is that aggregation restricts free proteins by bringing them to order (i.e., moving less). The energetics of the order imposed on proteins by homoaggregation is usually far less than the energetics of the disorder imposed on water molecules by liberating them from entrapment at proteins surfaces. This is a major factor in biological systems where proteins are crowded and entropic principles prevail. With around 30% of intracellular volume taken up with macromolecules, nonideal solution behavior is the rule[11]. In this case, raising temperature tends to liberate these water molecules and the energy associated with this *disordering* of water more than compensates for the energy associated with the *ordering* of protein. This entropy-driven aggregation can confer on susceptible proteins an exquisite sensitivity to small *increases* in temperature over a narrow physiological range[12][13].

In the context of progressive global warming, the term “tipping point” signals a rapid and irreversible further increase in planetary temperature. However, the term tends not to be used in the context of the progressive increase in the severity of the symptoms of certain neurological diseases, such as PD. Yet,

characteristically, in each disease there is a progressive increase in the aggregation of a specific brain protein[14]. For such pathological proteins, there may be tipping points – potentially temperature-dependent – that precede rapid increases in aggregation[15]. Thus, in PD the normally crowded cytoplasm consists of alpha-synuclein and many other macromolecules, that collectively compete for water.

3. Fåhraeus and rouleaux formation

In infections there are changes in plasma proteins that cause disc-shaped erythrocytes to aggregate into structures that resemble piles of coins. These "rouleaux" sediment more rapidly than single erythrocytes. This is the basis of the erythrocyte sedimentation rate (ESR) test. In detailed historical studies, Robin Fåhraeus (1888-1958) outlined possible underlying biophysical principles of such cellular aggregations[16][17][18], which can today be seen as similar to those of macromolecular aggregations[15][19].

Of note was that the changes rendering plasma rouleaugenic likely resided in the plasma protein *collective*, rather than in individual proteins. A small change in acidity[20] (pH;), or mild concentration by removing water[21] can suffice. Only in the case of the rouleaugenicity of heated serum[22], could the phenomenon be attributed to one protein, namely albumin – the most abundant serum protein – when it was aggregated by heating to 62˚C[21].

Among possible explanations for changes in ESR values, Fåhraeus,[17] considered that "dehydration of the [red blood] corpuscle surface layer may be the predominant factor." At that time, the liberation of weakly bound water molecules from the surface of macromolecules (such as albumin) was discussed in entropic terms mainly by chemists and physicists. Fåhraeus did not use the term in his work. However, studies of the aggregation of tobacco mosaic virus (TMV) coat proteins initiated in the 1930s established the powerful influence of entropic factors in biochemical reactions, which include its *increase* with temperature[23][13].

As with the aggregation of *individual* erythrocytes (RBCs), aggregation restricts *individual* macromolecules (e.g. proteins) by bringing them to order. Sometimes, this loss of entropy is driven by energy-yielding chemical reactions (exothermic). However, more often aggregation is endothermic, achieved at the expense of the water molecules that are trapped at protein surfaces. Raising the temperature tends to liberate these water molecules and the energy associated with this *disordering* of water more than compensates for the energy associated with the *ordering* of protein[24][25][26]. Referred to

as entropy-driven aggregation, this confers on susceptible proteins an exquisite sensitivity to small increases in temperature that can occur within a narrow physiological range, so permitting fine discriminations.

Indeed, when added to normal blood, certain agents that aggregate proteins (e.g., polyethylene glycol) also aggregate RBCs[27]. In this respect, RBCs differ from large proteins only in that their aggregation can be easily observed microscopically. The self-aggregation of macromolecules (TMV, serum albumin) is referred to as their "polymerization," although this term is not applied to aggregated cells. Yet there is specificity. Just as proteins can form individual like-with-like homoaggregates, so RBCs from different species, when mixed, form distinct rouleaux homoaggregates[28][29][30]. Although independent, homoaggregates collectively summate to form a total aggregate. Intriguingly, in rare individuals with two independent brain diseases, each associated with a distinct protein aggregate, there may also be a degree of summation[31].

4. Normal and pathological roles of aggregation phenomena

While this paper is primarily concerned with pathology, a positive function of macromolecular aggregations in immunology has been proposed[32][19][33][29][30]. Given the need for the dosage-compensation of proteins encoded by female X chromosomes[34], and the greater conservation of intracellular protein concentrations relative to that of the mRNAs that encode them[35][36], a case has been made that a *fine-tuning* of self-protein concentrations, aided by heat-shock protein (HSP) chaperones, would be relevant to the problem of distinguishing peptides derived from foreign proteins from those derived from self-proteins. Somehow, alerted by selective aggregation of proteins deemed "foreign" (Fig.1b), proteosomes would cluster around the aggregate[14] and prepare peptides for binding to MHC proteins.

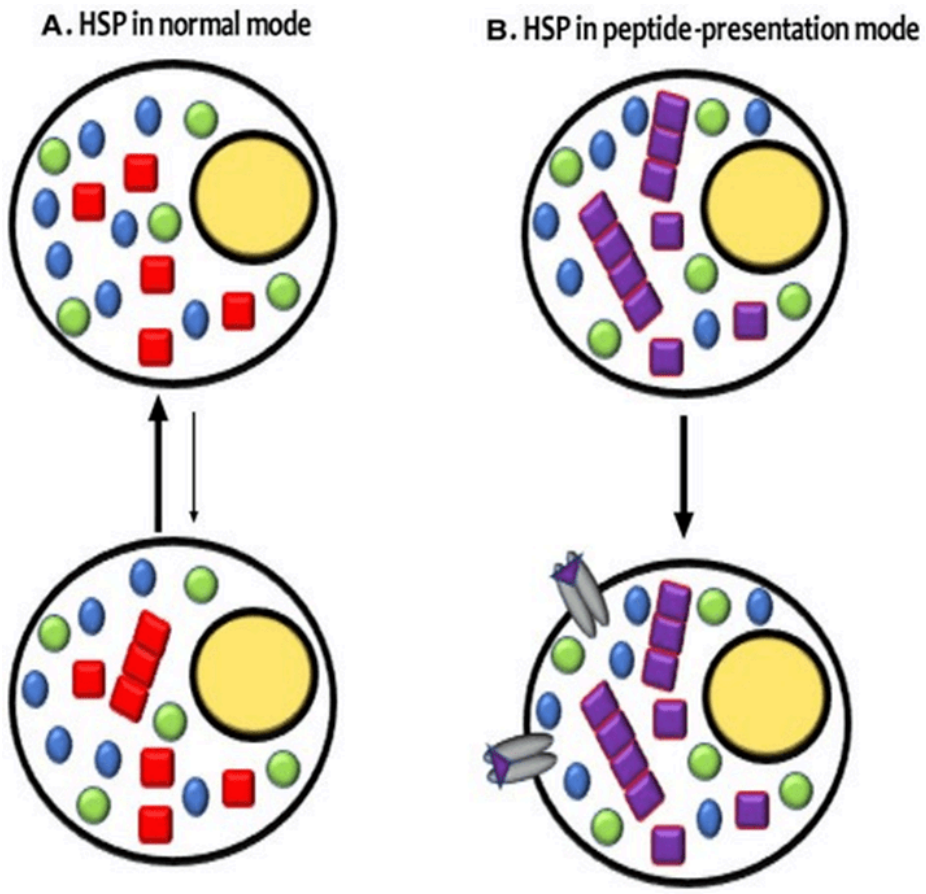

Figure 1. Homoaggregation hypothesis. Heat shock proteins (HSPs) are in either normal chaperone mode (A) or peptide-presentation mode (B). In A, 'self' proteins (green circles, blue ovals or red squares) approach the limits of their solubility in the crowded cytosol (area between the perimeters of the large circle representing the cell wall and the medium-sized yellow circle representing the cell nucleus). Here HSPs (not shown) in 'normal mode' act as molecular chaperones to disassemble incipient red square aggregates. In B, there are 'self' proteins in autoimmune states (purple squares). Like foreign 'non-self' viral proteins, these more readily cross the aggregation threshold than most 'self' proteins, and form stable aggregates. This self/not-self discriminatory signal causes an HSP switch to peptide presentation mode and the triggering of various intracellular alarms, including the upregulation of MHC protein expression (grey ovals). Aggregates are processed to form peptides (small purple triangles) that are displayed at the cell surface as peptide-MHC complexes (adapted from Forsdyke[32]).

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Cell surface display of peptide-MHC complexes would be recognized by cytotoxic T-lymphocytes[3][4]. Whether this role provides insight into how α-synuclein might function negatively in PD is an open issue. One scenario would be that aberrant MHC display of alpha-synuclein peptides by neurons triggers autoimmune phenomena.

5. A role for PD patients?

Like low-hanging fruit, cures for some diseases are there for the taking. Long before the content of vitamin C in limes and other fruit was elucidated, sailors were able to remain free of scurvy on long voyages provided that their ships were appropriately provisioned. Any such sailors struck with what is now known as PD were not so lucky. And, despite scientific advances, centuries later no immediate solution is on the horizon[1]. Reflecting the Parkinson's literature in general, among therapeutic approaches there is little mention of the possibility of lifestyle changes that patients *themselves* might make to control the aggregation of the α-synuclein protein.

From the entropic perspective outlined here and elsewhere[19], the possibility of restraining possible increases of body temperature above normal, in order to reduce entropy-driven aggregations (Fig. 2), might be considered[37][38].

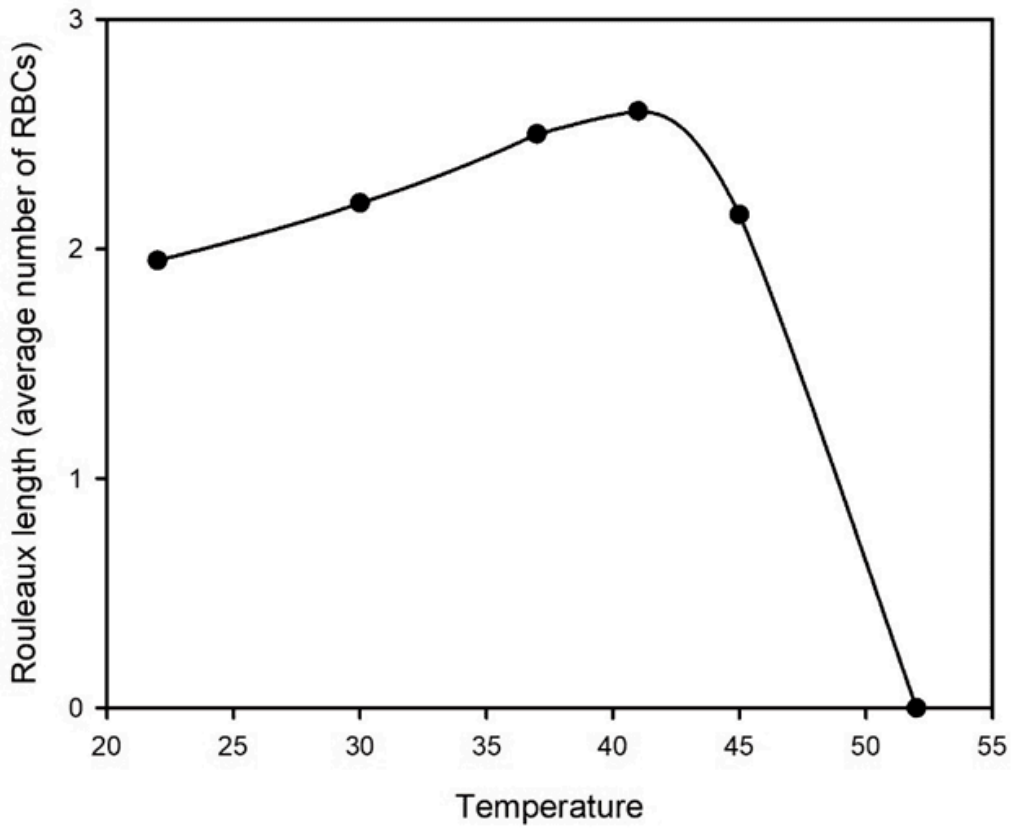

Figure 2. Data consistent with Fåhraeus's entropic (water molecule liberation) hypothesis. Rouleaux were formed by human RBCs when incubated in plasma for 10 minutes. Over the range of a physiological pyrexia (37˚C – 41˚C), rouleaux length increased. In this time, at temperatures above 41˚C RBC discs can begin rounding up, so impeding their aggregation. Data are adapted from Kernick et al.,[37]. In shorter times the rounding tendency is less, and length can increase until at least 45˚C[38].

Some simple measures come to mind. No long baths in hot water. No saunas. When an infection is sensed (e, g., sore throat, malaise), take an antipyretic drug (e.g., aspirin) sooner rather than later. Allow hot food and drinks to cool a little before consumption. Keep cool in hot weather and when exercising.

Hopefully, at some future point there will be formal retrospective and prospective studies of the effectiveness of these measures. Given the high incidence of protein aggregation diseases, it is likely that there will be many patients with a scientific background who can make informed decisions and amass useful data[39].

Statements and Declarations

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Contribution

Forsdyke is responsible for writing, etc.. Background contributions are acknowledged in Forsdyke[19].

Data availability

No personal data was used for the research described in the article and no AI was employed.

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Queen's University hosts my neurobiology webpages.

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