Protein Aggregates
Measuring what you can, not what you want?

Protein aggregates! Definitely not something I would want to be injected into me. The idea of injecting something containing visible particles, or even an opalescent solution, is not for me. I am not interested in medication that clogs up my blood vessels with particulates especially as the FDA website tells me that they may also trigger an adverse immune response.

Not surprisingly there is much interest in measuring protein aggregation in therapeutic proteins. What does surprise me, however, is that Size Exclusion Chromatography (SEC), almost universally performed using HPLC, is often the first line of attack taken in measuring aggregates. Don't get me wrong, I like SEC; one of the most memorable undergraduate experiments I performed was separating blue dextran, cytochrome c and ε-dinitrophenyl lysine on a Sephadex column. I used it extensively when purifying peptides as a PhD student and post-doc; at one point I even worked for Pharmacia, so no doubt Sephadex contributed towards my salary.

What concerns me is that, to my mind, SEC does not really measure protein aggregation. I must admit that when I think about aggregates I think about insoluble aggregates, the sort that would block up my smaller blood vessels, and I would welcome any feedback that readers have on the problems caused by soluble aggregates. But my first objection to SEC as a method for detection is that insoluble aggregates will be filtered out, either by the column itself, or in many laboratories by the guard column, whose job is to do exactly that. So at the very best, the method measures only soluble aggregates and in these cases what you measure is definitely not what you see.

My second objection is that even then the method may not be representative. Monoclonal antibodies are a reasonably typical example of a therapeutic protein, and these proteins have a molecular weight in the region of 150,000 Da. If I analyse this protein for aggregates on a typical SEC HPLC column, then what can I expect to see? A brief literature search for HPLC columns used for protein aggregate analysis, for example from Waters or Tosoh, shows that a typical SEC column used for this purpose will have a fractionation range of 10,000 to 500,000 Da. In the case of a monoclonal antibody, I would detect the monomer, I would detect a dimer and even a trimer. But a tetramer would not be fractionated at all, nor would any higher aggregates. I simply would not detect them. The analysis would be even more fraught in the case of a therapeutic protein which existed naturally as a dimer, or higher order multimer.

This raises the inevitable question, are the lower aggregates which will be detected by SEC HPLC representative of the higher order soluble aggregates and of any insoluble aggregates. More importantly are they representative of the aggregates that may damage my health?

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