Functionality of egg proteins: formation of long fibrils and consequent gel properties

E. VAN DER LINDEN

Food Physics Group, Department of Agrotechnology and Food Sciences, Wageningen University, Bomenweg 2, 6703 HD Wageningen, The Netherlands
erik.vanderlinden@wur.nl

Keywords: ovalbumin; fibrils; gel elasticity; minimal gelation concentration; flow, innovation

Proteins in solutions exhibit fascinating assembly properties. One particular phenomenon is the assembly into long fibrils. Many different proteins show this behaviour under the appropriate conditions. We will review various proteins that show this behaviour and address the according circumstances, and then focus on the protein ovalbumin. We subsequently discuss gel elasticity and minimal gelation concentration of systems containing such fibrils in terms of the properties of the fibrils. We will also briefly address the properties of such systems under flow, an important aspect for processing.

Introduction

Exploration of the functionality of egg proteins has received interest over many decades. Applications originating from these explorations find their way in emulsions, foams and gel materials, as, for example, in mayonnaises (McGee 2003), egg-white foams (McGee 1985) and in the control of the hardness of a cooked egg (Kimball 1999), respectively. In this paper we will focus on the latter area, i.e. gel materials based on egg protein.

Since this area has been actively explored for decades, one immediate question is why it would be an area with innovation potential (the paper is, after all, in the session on “Egg proteins and Innovations”) Let’s assume that an innovation refers to a (usually unexpected) step forward in technological development from which new products can arise. Then indeed the above question seems justified since gel properties have been investigated for already a great number of different conditions and different protein mixtures and one therefore can ask whether one actually still can expect really new and breakthrough type of things?

In this paper a few insights will be given in one specific area of protein research, i.e. gelation of protein solutions, with reference also to a particular egg white protein, i.e. ovalbumin. The paper is not intended to be a full review of gelation of egg proteins. It is merely intended to give the reader an appreciation of the innovation potential of protein gelation research and zooming in on egg proteins.

In order to define the area of interest we start with considering a solution of proteins. This solution can become a gel when the proteins aggregate into structures that (efficiently) fill up space. See Figure 1.

![Figure 1](image-url) Proteins that aggregate into clusters (branched or fibrillar) can cause gelation.

In the middle part of Figure 1 one sees clusters of proteins that are relatively small (red circles) and thus fill up space or at least form a closed packed system, leading to gel characteristics. In the right...
hand part of Figure 1 one sees very elongated protein aggregates, which entangle with one another in solution and this situation also leads to gel characteristics. In principle, both situations are characterised by the existence of an overall system structure which can transfer deformations that are initiated at one end of the system to another end of the system (one typical gel characteristic). The structure in the middle has a concentration of monomeric protein which is considerable higher than the concentration of monomeric protein at the right hand site. Still both situations are gel like. The necessary concentration of monomeric protein to make a gel also will be considerable higher for the middle than for the right hand site situation. In other words, elongated structures are much more weight efficient to make a gel than isotropic, dense, spherical clusters.

It is known that the various types of aggregate structures that proteins can form in solution depend on temperature, salt concentration, concentration of protein, and presence of co-solutes like alcohol. One thus should be able to determine the specific circumstances for a specific protein which can lead to the formation of e.g. elongated structures, i.e. which circumstances yield a weight efficient gel. Vice versa, the desire for controlling the preparation of weight efficient protein gels, requires understanding of the aggregation mechanisms and according thermodynamic conditions in relation to the formation of elongated structures. It turns out that many different proteins actually are able to form elongated structures, up to micron size (see e.g. Aymard et al. 1996, Aymard et al. 1999, Veerman et al. 2002, Veerman 2003a, Kavanagh et al. 2000). The occurrence of more elongated aggregates in general lies within a relatively large and easily practically accessible formulation window. This all applies to many different food proteins like beta-lactoglobulin, soy-protein, BSA, ovalbumin, pea protein etc.

The extreme case of long and stiff fibrils is a relatively difficult structure to end up with, and only a relatively narrow formulation window is suitable for arriving at these structures. The activity within the science community to come up with possible mechanisms for protein fibril formation where the fibrils are stiff has been very extensive during the past few years, not to the least because also several brain tissue diseases exhibit the presence of fibrillar protein aggregates. Despite the many efforts in the direction of understanding the aggregation mechanisms and according aggregate structures as a function of solution properties, the area still seems not be matured at all.

Although the understanding of the formation mechanism for stiff and long protein fibrils has not come to a level that allows predictions, this does not hamper the investigations on the consequences regarding properties of systems which contain such long protein aggregates. From an innovation perspective, it allows for instance to prepare extremely low weight fraction gels, because of the considerations already outlined above (gelation efficiency due to elongated structures). Furthermore, foods that contain much of such elongated and stiff structures may give rise to unexpected novel material properties, possibly including sensory related properties.

In the following we will address examples of food proteins that form fibrils, and discuss some prerequisites for fibril formation. We also treat ovalbumin as one of these food proteins. Subsequently we address gel properties of protein based fibrillar systems in general. We also address how these fibril containing systems behave under flow, which has relevance to processing conditions. Lastly, we show how one can make use of certain properties of fibrils to prepare extremely low weight fraction gels with relatively high brittleness.

**Examples of food protein based fibrils**

In the Figure below one observes three examples of fibrils formed by food proteins. Respectively beta-lactoglobulin, BSA and ovalbumin, under specific conditions.

![Figure 2](image.png) Electron microscopic evidence of fibril formation for (left to right) β-lactoglobulin, BSA and ovalbumin (pictures taken from Veerman 2004 for which the reader is referred to for details).
The fibrils are different in terms of length of the fibrils, stiffness, and in terms of the conditions necessary to make them (pH, salt concentration, temperature). The reasons for these differences in terms of protein characteristics are at the moment far from clarified. Interestingly however, within a certain range of salt concentration, the average fibril length remains constant for a specific protein, provided one prepares all samples of that protein in the same way. For another protein, one finds a different salt concentration range within which the average fibril length remains constant. This allows one to establish the effect of salt concentration on characteristics of gels based on fibrillar protein assemblies.

**Minimum gel concentration for fibrillar protein gels**

One interesting gel characteristic is the minimal (or critical) concentration of material to yield a gel. In the regime near this particular concentration (also referred to as percolation concentration) the gel elasticity, $G'$, can be written as

$$G' \sim (c - c_p)^t$$

with $c_p$ the critical or percolation concentration and $t$ a parameter which is constant for a specific type of gel, and depending on the properties on the gel structures and the type of their mutual interaction (van der Linden and Sagis (2001)). This implies for a certain protein that once $c_p$ is known, one can predict various characteristics for gels based on that protein, provided one deals with the same type of protein assembly structure. As we saw in the paragraph above, the average fibril length remains constant within a specific range of salt concentration. One may determine $c_p$ for different proteins as a function of salt concentration. Within the range of constant average fibril length, one then determines the effect of salt on the mutual fibril interactions as a function of salt. It should be noted that it should be checked that within the range in which the average fibril length remains constant the conversion of proteins into fibrils also is constant, since otherwise one cannot directly relate the fibril length with the experimentally determined overall protein content. This has been checked (Veerman 2004). A convenient way of taking into account the salt concentration is the length over which the electrostatic interaction decreases by a factor $1/e$. This length becomes shorter with higher salt concentration. The length is referred to as $D_{\text{eff}}$, a so called electrostatic (effective) thickness of the fibril.

![Figure 3](image-url)  
**Figure 3** The minimal gel concentration, $\phi_{p,m}$ as a function of $D_{\text{eff}}$ (see text). Taken from Veerman 2004.

One finds that the minimal gel concentration depends linear on this effective thickness, and the dependence is different for each protein. An explanation is offered in terms of the fibril stiffness (which indeed is different for each type of protein) (see e.g. Sagis et al. 2004, Veerman et al. 2004). The slope in Figure 3 for each protein is directly related to the stiffness of the fibrils formed by that protein.
Reversibility and irreversibility of fibrillar protein assembly

It has been found (Veerman 2003a) that depending on the type of protein one may encounter assembly which is not reversible (after waiting for a few hours), or assembly that is reversible upon dilution. For betalactoglobulin (Veerman et al. 2002) and ovalbumin (Veerman et al. 2003c) it was found that the assembly was irreversible, while for BSA it was found to be reversible (Veerman et al., 2003b). For a short review on this matter see Veerman et al. 2003a. This may be due to irreversible (or very unlikely to reverse within the experimental time frame) conformational changes of the protein once assembled, much like the so called skin formation of proteins that reside at for example an air-water interface. Irreversibility can be deduced partially for ovalbumin from the results depicted in Figure 4. For a complete argument see Veerman 2003c.

Once assembly has taken place, and it would be irreversible upon dilution, one may use this (preparing at high concentration and subsequent dilution). In other words, the assembly is robust against dilution. Of course, robustness of the fibrillar assembly against other treatments may add to its applicability. One important fact is that for example beta-lactoglobulin exhibits robustness against detoration by pH change (Veerman et al. 2003a, Veerman 2003 d). The fibrils are being prepared at low pH (pH=2) and the fibrils do not show any dissociation or dissolution upon a pH change towards pH=7. Confirm Figure 5. Note also that the sequence of heat treatment and pH adjustments yields a different result, due to the fact that at different pH, the assembly process is different (related to different conformations of the protein among other things).

Innovation potential of fibrillar assembly

One may use the findings of the section above to ones advantage. The idea is how to realise an extremely low weight fraction gel. One realisation is that one needs a low pH for fibrillisation. The second is that one needs efficient interaction between the fibrils once formed.

Figure 4  Ovalbumin fibrils prepared in a 7% solution, diluted to 0.01% and (at times indicated) observed using electron microscopy (taken from Veerman 2004, see for details Veerman 2004).
The interaction may be induced by for example Ca bridges, which however do not act at the low pH under which the fibrils are being made. Now one can use the fact that the fibrils remain intact after adjusting the pH to 7. This yields an opposite charge to the fibrils and thus enables Ca bridging to occur at that pH. Indeed, formation of a solution of fibrils below the gel point at low pH yields, after pH adjustment to 7, a gel, induced by Ca bridging (Veerman et al. 2003a, Veerman et al 2003d). This multi-step process is illustrated in Figure 6 (described in more detail in Veerman et al. 2003d). The result of this new multi-step process is that a gel can be formed at 0.07% (weight) of protein using the new multi-step method which is a significant advantage over the conventional method of heating, cooling and subsequent additional addition of salt, all at pH 7, which yields a minimum gel concentration 0.5%.

**Figure 5** Electron microscopy of beta-lactoglobulin samples with different treatment (Taken from Veerman 2004).

**Figure 6** Schematic of a new multi-step gelation process for weight efficient gels. Taken from Veerman et al. 2003a.
Effect of flow on minimum gel concentration

Above, a new multi-step gelation process was described. One of the issues for making gels (based on fibrils) is how the systems that will become a gel will behave under flow while being prepared. Here only the result will be presented, and the interested reader is referred to the relevant reference for more details (Veerman et al. 2005). The picture which arises from the experiments can be given in simple terms. The gel point is determined by how much the fibrils interfere with one another. Without flow, the fibrils orient themselves isotropically, i.e. without any preferred symmetry. Once flow is exerted on the fibril containing system, there will exist a preferred orientation of the fibrils along the direction of the flow (we disregard here for reasons of argument sake the type of flow). This preferred orientation implies that one needs more fibrils per unit of volume to have them interfere to the same extent as in the case for the gel point at rest. Thus, the minimal gel concentration will increase with flow, as depicted in Figure 7. This Figure results from a combination of experiments on ovalbumin and theoretical analysis (Veerman et al. 2005). It implies that one might prepare a fibril solution under flow, at concentrations that will not lead to gelification as the systems remains under flow, but which will yield gelification as soon as the systems stops flowing (i.e. processing and putting afterwards in a bottle).

![Figure 7](image)

Figure 7  Effect of flow strength (proportional to Pe [-]) on the minimal gel concentration, c_p. Modified after Veerman et al. 2005.

References

Aymard, P., Nicolai, T. Durand, D. Macromolecules 1999, 32, 2542-2552
Kimball, C. et al. (the editors of “Cook’s illustrated Magazine), The best recipe, 1999, Boston
Common Press, Massachusetts, USA
Linden, E. van der, Sagis, L.M.C. Langmuir 2001, 17, 5821-5824
Sagis, L.M.C., Veerman, C., Linden, E. van der, Langmuir 2004, 20, 924-927
Veerman, C., Ruis, H., Sagis, L.M.C., Linden, E. van der, Biomacromolecules 2002, 3, 869-873
Veerman, C., Sagis, L.M.C., Linden, E. van der, Macromol. Biosci. 2003 a, 3, 243-247
Veerman C., Schiffaert, G. de, Sagis, L.M.C., Linden, E. van der, Int. J. Biol. Macromolecules 2003 c, 33, 121-127
Veerman, C., Sagis, L.M.C., Venema, P., Linden, E. van der, Macromolecules 2005, to appear