Physical gelation of lysozyme in dimethylsulfoxide aqueous solution

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Introduction
Protein physical gelation as a response to solvent microenvironment is a topic of interest due to its relevance in understanding protein aggregation phenomenon.⁴ Protein aggregation plays a central role in many instances of medical relevance, such as in neurodegenerative diseases, as well as in fields of biotechnological importance, like in the food and cosmetic areas. In the present work, the lysozyme/DMSO/water system was investigated in both dilute and concentrated protein regimes.

Results and Discussion
The aggregation of lysozyme in DMSO/water, eventually leading to the formation of physical gels, was monitored through diffuse wave spectroscopic experiments. The effects of protein and solvent concentrations were investigated in the protein concentrated regime (Figure 1). Information on the conformational state of the protein as a function of the concentration of the binary liquid solvent was gathered from circular dichroism data obtained for the protein in the dilute regime² (Figure 2). That allowed for conformational features of the protein, not affected by aggregation effects, to be revealed. The flow behaviour of systems investigated was studied by rotational and oscillatory rheology (Table 1).

![Figure 1](image1)

![Figure 2](image2)

Table 1. Relative elastic and viscous compliances ($J'/J_{\text{max}}$, $J'/J_{\text{max}}$, resp.) as a function of sample composition

<table>
<thead>
<tr>
<th>Samples</th>
<th>$J'/J_{\text{max}}$ (%)</th>
<th>$J'/J_{\text{max}}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5mM lysozyme in DMSO 0.8</td>
<td>6.6</td>
<td>93.4</td>
</tr>
<tr>
<td>5mM lysozyme in DMSO 0.9</td>
<td>61.0</td>
<td>39.0</td>
</tr>
<tr>
<td>6mM lysozyme in DMSO 0.8</td>
<td>88.8</td>
<td>11.2</td>
</tr>
<tr>
<td>6mM lysozyme in DMSO 0.9</td>
<td>89.9</td>
<td>10.1</td>
</tr>
</tbody>
</table>

Conclusions
a) Lysozyme gels are formed in DMSO/H₂O mixtures at $\phi_{\text{DMSO}} > 0.7$. That condition is coincident with the onset of loss of the characteristic protein tertiary structure;

b) Gel elasticity and internal structure are enhanced with the increase in protein concentration, DMSO volume fraction and sample age;

c) The tendency for aggregation is clearly dependent both on the protein conformational states and on the liquid solvent microstructure in a complex way. Further investigations on the physico-chemical features of such events are on course.

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