Viscometry of Curdlan, a Linear (1→3)-β-D-Glucan, in DMSO or Alkaline Solutions

Hiroaki Futatsuyama, Toshifumi Yui, and Kozo Ogawa

Research Institute for Advanced Science and Technology (RIAST), Osaka Prefecture University, 1-2 Gakuen-cho, Sakai, Osaka 599-8570, Japan

*Faculty of Engineering, Miyazaki University, Miyazaki 889-2192, Japan

Received February 17, 1999; Accepted April 21, 1999

A simple method to obtain the molecular weight of curdlan was found by viscometry in its alkaline or DMSO solution. DMSO was found to be the most appropriate solvent for measuring $M_\theta$ of curdlan. Based on two Sakurada-Houvink equations, $[\eta] = KM^a$, which have been obtained so far in alkaline solutions, two sets of parameters of the equation in the DMSO solution, that is, $K = 3.5 \times 10^{-4}$, $a = 0.65$ and $K = 1.6 \times 10^{-4}$, $a = 0.74$ were introduced, respectively. Both parameter sets seemed to serve practically to measure the molecular weight of curdlan. In addition, using the equation obtained with several NaOH concentrations, the previous speculation that curdlan conformation changes from a rigid rod to random coil with alkaline concentration was confirmed.

Key words: curdlan; DMSO; viscosity; molecular weight

Curdlan is a microbial linear polysaccharide consisting of (1→3)-linked β-D-glucose residues. It has been extensively studied because it is not only a gel-forming polysaccharide but also a basic type of (1→3)-β-D-glucan. However, it was not easy to estimate the molecular weight of curdlan, one of the basically most important parameters. Aqueous NaOH, dimethyl sulfoxide (DMSO), and a cadoxen-water mixture are known as solvents for this polysaccharide. Hirano et al. had measured light scattering and viscosity on fractionated samples of curdlan in the 1:1 cadoxen-water mixture, and they obtained $K = 2.5 \times 10^{-4}$ and $a = 0.65$ in the Sakurada-Houvink equation, $[\eta] = KM^a$ where $[\eta]$ is intrinsic viscosity and $M$ is molecular weight. Recently, $K = 7.9 \times 10^{-3}$ and $a = 0.78$ in 0.3 M aqueous NaOH were reported by Nakata et al. However, in these alkali solutions the chain length of curdlan is shortened gradually because the alkali decomposition is known to occur from the reducing end of the glucan (peeling reaction). So, a prompt measurement is necessary for viscometry. In addition, it is not easy to dispose of the waste of cadoxen safely since it contains cadmium. Moreover, since the viscosity of curdlan changes greatly with alkaline concentration, particularly, when measured at lower NaOH concentration than 0.24 M owing to a drastic conformational change of the glucan, it is required to keep the concentration constant during the measurement. Thus, in order to measure the molecular weight of curdlan simply and easily, these alkali solutions are not recommended for viscometry. However, DMSO seems to be a suitable solvent because the glucan is not decomposed in the solution (although DMSO is not suitable for light scattering measurements since it has a high refractive index). In addition, curdlan seems to take up a flexible disordered conformation in the DMSO solution. In this report we got the parameters, $K$ and $a$, of Sakurada-Houvink equation for curdlan in DMSO solution by viscometry on 7 samples of curdlan. These parameters seem to serve for a simple measurement of molecular weight of a linear (1→3)-β-D-glucan for practical use. We also measured the parameters on these curdlan samples in NaOH solutions of various alkaline concentrations to confirm the previous speculation that curdlan conformation changes from a rigid rod to random coil with alkaline concentration.

Four fractionated and three unfractonated samples of curdlan were supplied from Takeda Chemical Industries Ltd., Osaka, Japan. They have measured molecular weights of the fractionated samples (1.0, 0.50, 0.20, and 0.05 $\times 10^6$, respectively) in the 0.25 M NaOH solutions, where the glucan is considered to take up a randomly coiled conformation, by high performance liquid chromatography (HPLC) using pullulan as a standard. All the unfractonated curdlans were washed with water, methanol, and diethyl ether to obtain desalinated and dried samples. Viscometry was done at 25°C using a Ubbelohde dilution type viscometer and, in the case of alkaline solutions, under nitrogen atmosphere to prevent the change in the alkaline concentration induced by the presence of carbon dioxide in air.

To get information on the molecular weight of curdlan, the polysaccharide should not be aggregated in the solvent. As shown in the Fig. 1 plot of reduced viscosity, $\eta_\infty/c$, against curdlan concentration, $c$, in DMSO solution was linear, indicating that curdlan is completely dissolved with DMSO and that the solvent is appropriate for getting intrinsic viscosities, $[\eta]$, of this polysaccharide (Table 1). The linear relationships between $\eta_\infty/c$ and $c$ were also observed in all the curdlan-alkaline solutions studied including the cadoxen-water mixture (1:1). The resultant values of $[\eta]$ of all the curdlan samples in the cadoxen-water mixture or in 0.3 M NaOH solution, and their viscosity average molecular weights, $M_\eta$, calculated through the Sakurada-Houvink equation using parameters, $K$ and $a$, reported by Hirano

---

1 To whom correspondence should be addressed. Kozo Ogawa, FAX: +81-722-51-7439; E-mail: kozo@riast.osakafu-u.ac.jp
Table 1. Intrinsic Viscosities, $[\eta]$, and Molecular Weights of Curdlan Samples in Various Solutions at 25°C

<table>
<thead>
<tr>
<th>Curdlan$^a$</th>
<th>$M_0$ ($\times 10^6$)</th>
<th>DMSO</th>
<th>Cadoxen-water (1:1)</th>
<th>0.3 M NaOH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>[\eta] (dl/g)</td>
<td>[\eta] (dl/g)</td>
<td>$M_r$ ($\times 10^6$)$^a$</td>
</tr>
<tr>
<td>A</td>
<td>8.13</td>
<td>5.19</td>
<td>4.38</td>
<td>7.05</td>
</tr>
<tr>
<td>B</td>
<td>5.70</td>
<td>4.07</td>
<td>3.02</td>
<td>4.96</td>
</tr>
<tr>
<td>F-1</td>
<td>1.00</td>
<td>3.85</td>
<td>2.95</td>
<td>1.84</td>
</tr>
<tr>
<td>C</td>
<td>3.35</td>
<td>2.26</td>
<td>1.22</td>
<td>2.87</td>
</tr>
<tr>
<td>F-2</td>
<td>0.50</td>
<td>2.81</td>
<td>1.04</td>
<td>2.32</td>
</tr>
<tr>
<td>F-3</td>
<td>0.20</td>
<td>1.32</td>
<td>0.94</td>
<td>0.32</td>
</tr>
<tr>
<td>F-4</td>
<td>0.05</td>
<td>0.58</td>
<td>0.44</td>
<td>0.098</td>
</tr>
</tbody>
</table>

$^a$ A, B, C: Unfractionated curdlan samples, and F-1 – F-4: Fractionated.

$^b$ Molecular weight obtained by a HPLC measurement.

$^c$ Viscosity average molecular weight calculated using Hirano’s parameters.$^{29}$

$^d$ Viscosity average molecular weight calculated using Nakata’s parameters.$^{30}$

---

Fig. 1. Plot of Reduced Viscosity, $\eta_r/\eta$, versus Curdlan Concentration, $C$, in DMSO Solution at 25°C.


For the former solvent and Nakata et al.$^{30}$ for the latter are shown in Table 1. These results indicate that molecular weights of fractionated curdlan samples obtained by HPLC using pullulan as standard are practically in agreement with those obtained using both Hirano's and Nakata's parameters. It may be attributed to the similarity of molecular conformation (a random coil) between pullulan and curdlan in 0.25 M NaOH solution.

When $M_r$ of the curdlan fractions obtained using Hirano’s parameters of cadoxen-water solutions, a linear relation between logarithmic values of intrinsic viscosity, log [\eta], and that of the molecular weight, log $M_r$, of 4 fractions in the DMSO solutions was observed (Fig. 2, closed circles). The straight line led to the values of $K=3.5 \times 10^{-4}$ and $a=0.65$ in DMSO solution at 25°C. Data on the unfractionated samples (open circles) were fitted on this line. Log [\eta], versus that of the molecular weight, log $M_r$, of 4 fractions in the DMSO solutions of curdlan fractions, the molecular weights of which were calculated using Nakata's parameters of 0.3 M NaOH solutions, were also in a linear relationship (Fig. 3, open circles). The line led the values of $K=1.6 \times 10^{-4}$ and $a=0.74$ in DMSO solution at 25°C. The unfractionated samples (open circles) were well fitted on this line, as well.

The parameters, $K$ and $a$ are essentially obtained by viscometry using fractionated glucan specimens the molecular weights of which have been obtained by a
method to measure the weight average molecular weight, such as a light scattering measurement. Although such samples were not used in this study we believe that our values of \( K \) and \( a \) are valid to calculate the \( M_w \) of curdlan since the viscosity average molecular weight is between the weight average and the number average but is generally much closer to the former than the latter.\(^7\) The Sakurada-Houwink equations, \([\eta] = 3.5 \times 10^{-4} M^{0.65}\) or \([\eta] = 1.6 \times 10^{-4} M^{0.74}\) obtained by this study in DMSO solution can be used to measure the molecular weight of not only curdlan but also other linear \((1\rightarrow 3)\)-\(\beta\)-d-glucan samples in the molecular weight region studied now, that is, between approx. 4 and \(0.05 \times 10^6\) simply and conveniently by viscometry in their DMSO solutions.

Ogawa et al. have found that the intrinsic viscosity of a curdlan NaOH solution decreased with the increase of the alkali concentration from 0.005 to 0.19 M, and at higher concentrations the viscosity fell abruptly between 0.19 and 0.22 M, then rose sharply at 0.24 M, finally decreased gradually with the increase of the concentration. Based on this viscosity behavior coupled with changes in the rotation angle or the extinction angle of flow birefringence, they have speculated that the curdlan molecule took up an ordered rod-like conformation at lower alkaline concentrations than 0.22 M NaOH, but at higher alkaline concentrations it was a random coil.\(^6\)\(^\text{a}\) The intrinsic viscosities were obtained for these 7 curdlan samples in its 0.1, 0.22, 0.3, or 0.6 M NaOH solutions to examine the previous speculation. Double-logarithmic plots of \([\eta]\) versus molecular weight obtained using Nakata’s parameters\(^6\)\(^\text{a}\) at each alkaline concentration are shown in Fig. 4. At 0.1 M NaOH, the conformation of curdlan molecule is nearly a rigid rod since values of \([\eta]\) are the highest at all alkaline concentrations studied and the value of \(a\) was 0.85. At 0.22 M, where the conformation may change, values of \([\eta]\) were the lowest and \(a\) was 0.66, which suggest that the curdlan molecule seems to take up a compact conformation although, at this concentration, the largest deviation was observed in the double logarithmic plots because the viscosity of curdlan was the most sensitive with the change in alkaline concentration. At 0.3 M NaOH, values of \([\eta]\) and \(a\) (0.78) were higher than those at 0.22 M. This suggests that the curdlan molecule takes up an expanded conformation to some extent according to ionic dissociation of the hydroxyl groups of the molecule. The value of \(a\) was 0.76 at 0.6 M NaOH suggesting that the conformation of curdlan is similar with that at 0.3 M NaOH. These results coincided with the previous speculation on the behavior of the curdlan molecule in NaOH solution.

Acknowledgment

We are indebted to Takeda Chemical Industries Ltd., Osaka, Japan for their generous gifts of curdlan samples.

References


* When the molecular weights of curdlan specimen were calculated using Hirano’s parameters\(^2\) the resultant \(a\) value at each concentration of NaOH was a little different from that obtained using Nakata’s parameters, but dependence of the value on NaOH concentration was similar.