triple helix,[12] presumed to occur during gelation, are under investigation.

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Vacuum Ultraviolet Circular Dichroism of Dextran

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ABSTRACT: Vacuum ultraviolet circular dichroism (VUCD) spectra were recorded for a series of native dextrans [(1→6)-α-D-glucans] ranging in $\bar{M}_n$ from 410 to 303 000. Films exhibit a positive CD band near 167 nm with [Θ] = +1630 deg cm^2 dmol^{-1}. Evidence was obtained for a positive band of greater magnitude in solution at approximately the same wavelength. The variation in molar ellipticity with molecular weight is equal to or less than 10% over the $\bar{M}_n$ range studied.

Vacuum ultraviolet circular dichroism (VUCD) spectroscopy has recently been used to characterize carbohydrates and polysaccharides.[1-8] This technique is potentially of special importance for those molecules with no electronic transitions above 190 nm, such as dextran, a slightly branched (1→6)-α-D-glucan.

One of us[9-11] has previously studied the intrinsic viscosity and other physicochemical properties of dextrans as a function of molecular weight. The purpose of this study was to measure the molecular weight dependence of dextran chiroptical properties.

Experimental Section

Dextran samples ranging in molecular weight ($\bar{M}_n$) from 410 to 44 500 were obtained by fractional precipitation of acid-hydrolyzed native dextran (Meito Sangyo Co., Ltd.) estimated to contain 96% (1→6)-α-linkages.[10] Pharmacia dextrans T10 ($\bar{M}_n$ = 5200) and T500 ($\bar{M}_n$ = 303 000) were also examined. All samples were readily soluble in water at 20–25 °C.

The VUCD spectrometer and typical operating conditions are described elsewhere.[3] Each sample was examined in aqueous solution (both H_2O and D_2O) and as an amorphous film. Solution measurements were made in fused-silica cells of 0.100- and/or 0.054-mm path lengths, with concentrations ranging from 10–20 mg/mL. Moisture content corrections were made for each solution based on elemental analysis data (Galbraith Laboratories). Films were prepared by allowing a 0.1-mL drop of the above solutions to air-dry on a 1.9-mm-diameter CaF_2 disk at 80 °C. Films cast in this manner did not crystallize or adopt a preferred orientation. Molar ellipticities, [Θ], were calculated on the basis of a monosaccharide molecular weight of 162.

Results

Figure 1 shows actual tracings for a solution (top) and film (bottom) of $\bar{M}_n$ = 4500 dextran; those spectra are typical of all solution and film spectra obtained in this study. The 175-nm cutoff for aqueous solutions in 50-μm

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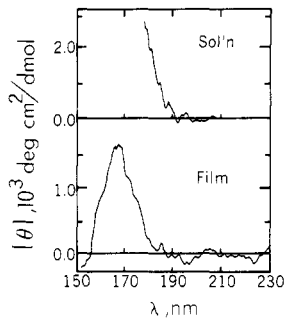

Figure 1. VUCD spectra of $\bar{M}_n$ = 4500 dextran solution (15 mg/mL) and film (0.1 mL, 10 mg/mL, on CaF_2).

Table I
Molar Ellipticities for Aqueous Solutions and Films of Dextrans

			[Θ], 10^3 deg cm² dmol⁻¹	
$\bar{M}_n$	$[\eta]^{25\,°C}$	wt % H_2O	soln 177 nm	film 167 nm
410	0.0260	6.94	2.45	1.38
630	0.0277	7.17	2.66	1.61
998	0.0337	8.14	2.42	1.71
1300	0.0375	5.95	2.49	1.74
1550	0.0395	5.91	2.49	1.66
1900	0.0450	8.90	2.66	
4500	0.0746	4.42	2.85	1.57
5200[a]	0.10	3.52	2.37	1.42
9300	0.112	12.14	2.62	1.47
21400	0.1663	10.52	2.70	1.73
44500	0.283	10.75	2.95	1.82
303000[a]	0.53	6.99	2.78	1.54

[a] Pharmacia T10, T500.

cells results from intense solvent absorption. Spectra can be extended 3–9 nm further with D_2O in a 50-μm cell or with H_2O in a 25-μm cell. Those spectra indicate an extremum in solution at 170 ± 3 nm, but we were not able to achieve satisfactory signal-to-noise ratios at that wavelength. We, therefore, used the molar ellipticity at 177 nm for purposes of comparing dextran samples. Since this value varied no more than 10% over the molecular weight range studied (see below) and since all indications were that the position of the extremum and the band shape were the same for all molecular weights, the molar ellipticity at 177 nm, a wavelength near the extremum, will be an acceptable indicator of band intensity for purposes of comparing different molecular weight samples. Table I shows molar ellipticities in solution at 177 nm corrected for water content. Each value of ellipticity represents an average of two to seven determinations.

Film spectra could be measured to 150 nm (Figure 1, bottom). In all cases a positive band is observed near 167 ± 2 nm, followed by a decrease in dichroism until a crossover is reached at approximately 155 nm. Satisfactory signal-to-noise ratios could not be obtained below 150 nm, but measurements on very thin films indicated qualitatively that there is a negative band below 150 nm. Molar ellipticities at 167 nm are listed in Table I.

Figures 2 and 3 show the molar ellipticities plotted as a function of molecular weight for solutions and films, respectively.

Discussion

A log $[\eta]$–log $\bar{M}_n$ plot for these dextran fractions is linear[9] only for molecular weights greater than about 2000 (Table I). (See ref 9 for a complete discussion of the observed relationship between intrinsic viscosity and molecular

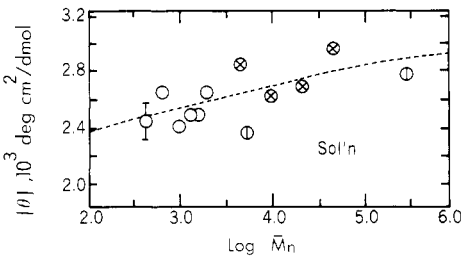

Figure 2. Molar ellipticity chain-length dependence of dextran in aqueous solution: (○) $\bar{M}_n$ < 2000; (⊗) $\bar{M}_n$ > 2000; Pharmacia T10 and T500 are also shown. The error bar indicates the estimated experimental uncertainty, and the dashed curve indicates the best least-squares fit.

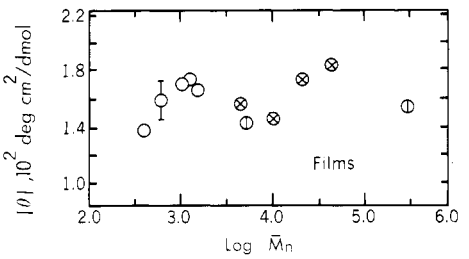

Figure 3. Chain-length dependence of molar ellipticity in dextran films: (○) $\bar{M}_n$ < 2000; (⊗) $\bar{M}_n$ > 2000; Pharmacia T10 and T500 are also shown. The error bar indicates the estimated experimental uncertainty.

weight.) We were interested in determining whether there is any corresponding change in the chiroptical properties.

The dashed line in Figure 2 shows the best least-squares fit of the experimental data, corresponding to

$$[\Theta] = 1904 + 253 \log M_n - 14.8(\log M_n)^2$$

The standard deviation for that fit is ±139 deg cm² dmol⁻¹, which is virtually the same as the uncertainty in any single determination estimated by us to be 186 deg cm² dmol⁻¹.

On the other hand, one marginally significant feature of the data for the ten fractionated samples should be pointed out. The six fractions with molecular weight less than 2000 have an average molar ellipticity of 2530 deg cm² dmol⁻¹, with a standard deviation of 102 deg cm² dmol⁻¹; the average for the four fractionated samples with molecular weight greater than 2000 is 2780 deg cm² dmol⁻¹, with a standard deviation of 146 deg cm² dmol⁻¹. The second average is 10% higher than the first. That difference is greater than the experimental uncertainty of 7%, but only at a marginal level of significance.

The film data (Figure 3) show even less dependence of ellipticity on molecular weight than the solution data. The average molar ellipticity for the films is 1632 deg cm² dmol⁻¹, with a standard deviation of 139 deg cm² dmol⁻¹, or 8.5%. We estimate the experimental uncertainty for each determination as 186 deg cm² dmol⁻¹ (11%). A 10% increase in ellipticity in the higher molecular weight fractions over the lower molecular weight fractions would amount to 163 deg cm² dmol⁻¹, which is outside the range of detection of our instrument, given its current sensitivity.

In summary, we can place an upper limit of 10% variation over a $\bar{M}_n$ range of 410 to 303 000. The result is not necessarily inconsistent with the more substantial molecular weight dependence in viscosity and degree of hydration in solution.[9-11] Factors such as molecular coiling, which profoundly affect hydrodynamic properties, may have a lesser effect on the local order to which circular dichroism is sensitive. Furthermore, it is not unreasonable that the observed molecular weight dependence in circular dichroism results entirely from a greater extent of

branching in the higher molecular weight fractions.[12]

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Dextran-Induced Changes in Fibrin Fiber Size and Density Based on Wavelength Dependence of Gel Turbidity

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ABSTRACT: The effect of Dextran T70 on the polymerization, size, and density of fibrin fibers was evaluated both by monitoring the kinetics of the turbidity increase during the thrombin-induced gelation of fibrinogen and by measuring the wavelength dependence of the gel turbidity. The presence of Dextran T70 in the clotting solution increased the rate of fibrin polymerization and resulted in increased fiber cross-sectional size through enhancement of the side-to-side association. At a constant fibrinogen concentration of 1 mg/mL, the addition of greater than 4.00 mg/mL dextran resulted in the subsequent production of fibers whose density was decreased relative to fibers formed in the absence of dextran. Above 4.00 mg/mL dextran, measurable amounts of dextran were associated with the fibrin network. The previously proposed mechanism for dextran–fibrin interaction, steric exclusion, is not sufficient to explain the results reported here.

Introduction

Dextran has been used as a plasma expander for more than 30 years;[1,2] however, its antithrombogenic properties were not initially appreciated until 1954 when bleeding complications were first recognized.[3] The antithrombogenic effect of dextran is puzzling since dextran has been shown to accelerate thrombin-initiated fibrin clotting.[4] Dextran has subsequently been shown to interact with fibrinogen, Factor VIII, and numerous other blood proteins,[5-13] as well as platelets.[12,14-17]

Dextran interacts both with fibrinogen, reducing its solubility,[18,19] and with fibrin, accelerating its polymerization.[4] The interaction between fibrin and dextran has been studied by a variety of techniques, all of which indicate that fibrin fibers formed in the presence of dextran are coarser, producing fibrin fibers with larger cross-sectional areas than those formed in the absence of dextran.[20-25] These coarse gels also have an increased turbidity.[22-25]

While some of the above studies have utilized the measurement of gel turbidity to study the effect of dextran on fibrin gel formation, none have yielded quantitative information about the dimensions of the fibrin fibers. We recently reported a technique for calculating the mass per unit length and radius of fibrin fibers based on a measurement of the wavelength dependence of gel turbidity.[26] This technique assumes that the fibrin fiber is a long rod. This assumption is supported by numerous reports utilizing a diverse array of investigational techniques, including electron microscopy and gel perfusion. Fiber dimensions derived from the turbidity technique are in excellent agreement with values obtained from light scattering and gel perfusion procedures and furthermore allow one to calculate fiber density.[27] In this report, we show that the previously observed dextran-induced turbidity changes can be quantitatively interpreted in terms of the fiber structure. The previously proposed mechanism of dextran effect on fibrin polymerization, based on steric exclusion, is not sufficient to explain the observed changes in the fiber structure described in the report.

Materials

Human fibrinogen (Grade L, A.B., Kabi, Stockholm, Sweden) was dissolved in 0.3 M NaCl at 25 °C. The solution was cleared by centrifugation at 30000*g* for 20 min. The fibrinogen solution was dialyzed against 0.3 M NaCl for 18 h to remove any free calcium. The solution was then frozen at −70 °C. Clottability was greater than 95%. Fibrinogen concentration was determined from absorbance at 280 nm, using an extinction coefficient of 1.6 mL/(mg cm).

Thrombin solutions were prepared by dissolving Parke-Davis bovine thrombin in water to reach a concentration of 130 NIH units/mL. Free calcium was removed by dialysis against 0.3 M NaCl. The thrombin was also stored in small aliquots at −70 °C.

Dextran (T70, MW = 70 000; Pharmacia Corp.) solutions were prepared by dissolving powdered dextran in deionized distilled water to the desired concentration. The solutions were used fresh, stored at 2 °C, and never frozen.

Methods

Fibrin Gel Formation. All gels for this study were formed by the addition of thrombin in a final concentration of 1.25 NIH

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