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HUMAN SPECTRIN

VI. A VISCOMETRIC STUDY

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Summary

Employing viscometry, human spectrin heterodimers and heterotetramers were analyzed in aqueous solution containing different amounts of salt.

(1) In aqueous 0.1 M NaCl, pH 7.5, at 4°C, the intrinsic viscosity of isolated human spectrin heterodimers and heterotetramers was found to be 40 ± 6 and 79 ± 7 ml/g, respectively.

(2) The intrinsic viscosity of isolated human spectrin heterodimers and heterotetramers increased to 78 ± 8 and 180 ± 10 ml/g, respectively, as the ionic strength of the solution was reduced to about 2 mM.

(3) This viscometric study indicates that isolated human spectrin heterodimers and heterotetramers are flexible molecules with a contour length of at least 110 and 200 nm, respectively.

Introduction

Previously reported values the intrinsic viscosity of spectrin range from 10 to 139 ml/g [1—3]. All these studies were conducted using standard capillary viscometers. We report here on a systematic study of the intrinsic viscosity of human spectrin heterodimers and heterotetramers in aqueous solution containing different amounts of salt. In our study we have employed a Cartesian diver low shear-rate viscometer [4,5]. In the case of highly asymmetric molecules, low shear-rate measurements may be important in order to correct for non-Newtonian viscosity behavior [6,7]. Since the size and structure of human spectrin have been subject to controversy [2,3,8—13], it could not in advance be ruled out that the previously reported values the intrinsic viscosity of spectrin were affected by the relatively high shear rates of the viscometers used.

Shotton et al. [13] presented electron microscopic evidence indicating that spectrin heterotetramers are formed by end-to-end association of heterodimers. The intrinsic viscosity of rigid molecules is sensitive to the axial ratio of the molecules. For rigid prolate ellipsoids, the intrinsic viscosity is approximately proportional to the square of the axial ratio when the axial ratios are larger than about 10 [7,14].

The intrinsic viscosity of polyelectrolytes often increases as the concentration of monovalent ions in the solution is reduced. The relative increase in intrinsic viscosity for a given reduction in ionic strength is related to the flexibility/expansibility of the polyelectrolyte [15—18].

Materials and Methods

Preparation of human spectrin. Human spectrin heterodimers and heterotetramers were prepared according to the method of Mikkelsen and Elgsaeter [19] except for the following modifications. (a) The final dialysis was carried out at 2—4°C for 3—48 h against two 1 l portions of the desired salt solution. The shortest dialysis period was performed against solutions containing 100 mM NaCl and the longest against solutions containing 1 mM NaCl. (b) The spectrin preparations were centrifuged at 200 000 × g (50 000 rev./min in a Beckman 50 Ti rotor) for 60 min (heterodimers) or 45 min (heterotetramers) at 0—4°C prior to viscosity measurements.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis. Aliquots for sodium dodecyl sulfate polyacrylamide gel electrophoresis [11] were taken from the spectrin preparations just prior to the viscosity measurements. Viscosity measurements on spectrin preparations which did not exhibit normal electrophoretic behavior were ignored.

Spectrin concentration measurements. Spectrin concentrations were determined by measuring the absorbance at 280 nm using the specific absorbance for human spectrin, $A^{1\,cm}_{1\%}$ (280 nm) = 10.1 [2,11].

Viscometry. The viscosity measurements were conducted employing a Cartesian diver viscometer constructed mainly as described by Klotz and Zimm [5]. The instrument essentially is a Couette viscometer utilizing a Cartesian diver as rotor. A dedicated servoregulator keeping the Cartesian diver in the proper vertical position is an integral part of our instrument. A Motorola MEK 6800 D2 microprocessor system with added memory and input/output circuits is used to record the rotor movement resulting from the external torque applied to the rotor. The relative viscosity, η_{rel}, for spectrin was obtained by measuring the time per revolution at steady state, t_o and t_s, when the rotor was placed in the solvent and the spectrin solution, respectively, and using the relationship $\eta_{rel} = t_s/t_o$ [5].

Preliminary studies indicated that just taking the rotor out and putting it back into the solution in question often resulted in a change of up to 1% in the time of rotation at steady state for a given applied torque. The viscometer was therefore modified so that a change of solution could be carried out without having to open the rotor chamber (Fig. 1). This allowed repeated changes of the same solution obtaining to within ±0.15% the same time of rotation at steady state for a given external torque on the rotor.

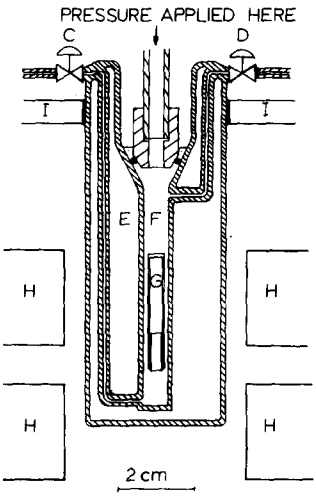


Fig. 1. A schematic diagram illustrating the new modified rotor chamber of the Cartesian diver viscometer. A and B, solution inlet and outlet tube, respectively; C and D, glass/Teflon inlet and outlet valve, respectively; E, thermostat-regulated circulating water; F, spectrin solution; G, Cartesian diver; H, electromagnets to give the Cartesian diver the external torque.

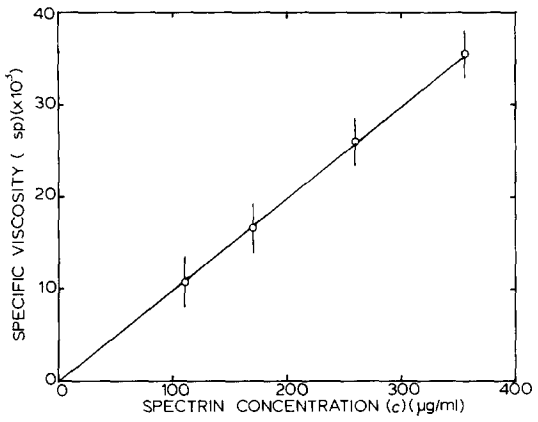
The mean shear rate of the viscometer is easily altered by changing the external torque of the rotor [4]. The employed mean shear rates, σ, in the spectrin solutions ranged from 0.7 to 11 s^{-1}.

The performance of the viscometer was checked by measuring the viscous properties of bovine serum albumin (Sigma, fraction V) in 145 mM NaCl, 7 mM phosphate buffer, pH 7.5, at 4°C. Prior to the viscosity measurement, the albumin solution was centrifuged at 200 000 × g (50 000 rev./min in a Beckman 50 Ti rotor) for 1 h at 0—4°C, the pellet discarded and the centrifugation repeated. The bovine serum albumin concentrations were determined by measuring the absorbance at 280 nm and employing the specific absorbance, $A^{1cm}_{1\%}$ (280 nm) = 6.66 [20]. At a mean shear rate of 8.5 s^{-1}, we measured an intrinsic viscosity of 3.9 ± 0.2 ml/g for bovine serum albumin. This is within the range reported by others [16,22,23].

Results

The intrinsic viscosity of human spectrin heterodimers and heterotetramers was calculated by first plotting the specific viscosity, $\eta_{sp} = \eta_{rel} - 1$, vs. concentration, c, and then obtaining the slope of the curve at infinite dilution (Fig. 2). The shortest t_o and t_s values were determined as the mean of approx. 60 independent measurements of t_o and t_s. Approx. 20 independent measurements were used to determine the larger t_o and t_s values. The relative viscosity was determined for three to four different concentrations in the range 100—450 μg/ml for heterodimers and 70—250 μg/ml for heterotetramers. We observed no significant difference in intrinsic viscosity between the parallel spectrin preparations.

The spectrin intrinsic viscosity was determined at three to four different



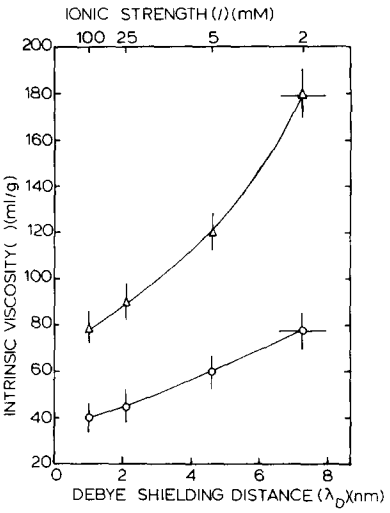


Fig. 2. Specific viscosity, η_{sp}, vs. spectrin concentration, c, for spectrin heterodimers in aqueous solution containing 0.1 M NaCl, 0.1 mM EDTA, and 0.05 mM dithiothreitol, pH 7.5, at $T = 3.65 \pm 0.04°C$, and employed mean shear rate of 8.9 s^{-1}.

Fig. 3. Intrinsic viscosity, η, extrapolated to zero shear rate of human spectrin heterodimers (○) and heterotetramers (△) vs. Debye shielding distance, λ_D, in aqueous solution, pH 7.5, at $T = 3.65 \pm 0.04°C$.

mean shear rates in the range 0.7—11 s^{-1}. Neither heterodimers nor heterotetramers showed any significant dependence on the shear rate for any of the ionic strengths used.

The intrinsic viscosity for human spectrin heterodimers and heterotetramers extrapolated to zero shear rate in aqueous solutions at different ionic strengths, I, is shown in Fig. 3.

Discussion

The intrinsic viscosity extrapolated to zero shear rate for both spectrin heterodimers and spectrin heterotetramers is approximately doubled by reducing the ionic strength from 0.1 M to 2 mM (Fig. 3). Smidsrød and Haug [18] have been shown that such an increment in intrinsic viscosity by reducing ionic strength is characteristic of flexible/expansible polyelectrolytes in aqueous solution. Spectrin heterodimers and heterotetramers exhibit approximately the same relative increase in intrinsic viscosity with reduction of ionic strength as carboxymethyl cellulose which has a Kuhn length of about 8.2 nm [18]. Our viscometric study therefore strongly indicates that spectrin heterodimers and heterotetramers are flexible molecules. This is in agreement with our previous results [11,12,19].

Flexible polyelectrolytes in aqueous solution are extended or rod-like at low ionic strength. The intrinsic viscosity of compact rigid macromolecules is well accounted for theoretically [7,14] provide the intermolecular interactions can be neglected. These interactions can be ignored when the Debye shielding distance, λ_D, is substantially less than the macromolecule nearest

neighbor distance. In a 70 μg/ml spectrin heterodimer solution at an ionic strength of 3 mM, the average nearest neighbor center-to-center distance is approx. 250 nm whereas λ_D is only 5 nm. Using the equations of Simha [14] and the intrinsic viscosity of spectrin at an ionic strength of 1—5 mM, an estimate of the minimum contour length of the extended molecules can be made. Assuming (a) that the molecular weights of heterodimers and heterotetramers are 480 000 and 960 000, respectively [2,11,24], (b) that spectrin hydration is 0.25 g solvent per g protein and (c) that the density of spectrin is 1.37 g/ml [10], our results indicate that spectrin heterodimers and heterotetramers have axial ratios of about 31 and 50 and lengths of about 110 and 200 nm, respectively. These lengths are in reasonable agreement with the electron microscopic results of Shotton et al. [13]. The spectrin heterodimer contour length obtained in our study equals that obtained previously at an ionic strength of 2 mM by static light-scattering measurements [11]. These lengths are at variance with those reported by Tilney and Detmer [9] and Kam et al. [10].

If the spectrin heterodimers and heterotetramers were rigid rod-like molecules, the theory of Saito [6] and Broersma [25] could be used to predict the shear rate needed to obtain a 50% reduction of the intrinsic viscosity obtained at zero shear rate. Using the data for spectrin listed above, these shear rates are about 6000 and 13 000 s^{-1}, respectively. Since the spectrin molecules are flexible, even higher shear rates would be needed to observe a 50% reduction in intrinsic viscosity [7]. This is consistent with the observed lack of shear rate dependence.

From light-scattering and electron-optical studies [11,12,19], we have previously concluded that spectrin heterodimers and heterotetramers are flexible molecules. Our viscometric study collaborates this conclusion and points to a contour length of spectrin heterodimers and heterotetramers of at least about 110 and 200 nm, respectively.

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