Dynamic Light Scattering Studies of $\alpha_{IIb}\beta_3$ Solution Conformation†

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ABSTRACT: The prototypical integrin receptor, $\alpha_{IIb}\beta_3$, isolated from the membrane fraction of human blood platelets by solubilization in Triton X-100 (reduced) and affinity chromatography on lentil lectin–agarose, has been further purified by gel filtration chromatography in octyl glucoside to obtain the intact receptor complex in a form suitable for hydrodynamic measurements. The molecular weight [$(6.0 \pm 0.2) \times 10^3$] and Stokes radius ($2.3 \pm 0.1$ nm) of detergent micelles formed in 0.03 M octyl glucoside have been determined by classical light scattering intensity and dynamic light scattering measurements, respectively. An algorithm has been developed which explicitly considers the contribution of detergent micelles to the intensity autocorrelation function of particles suspended in detergent. This procedure has been validated with polystyrene particles of known radius, as well as with the soluble protein fibrinogen. Application of these procedures to dynamic light scattering data obtained with $\alpha_{IIb}\beta_3$ resulted in a translational diffusion coefficient ($D_t^{o(20,w)}$) of $(2.78 \pm 0.31) \times 10^{-7}$ cm^2 s^{-1}, corresponding to a Stokes radius (R_s) of 7.67 ± 0.85 nm for the integrin/octyl glucoside complex. Light scattering intensity measurements gave a molecular weight of $(2.26 \pm 0.22) \times 10^5$ for the polypeptide moiety of the complex, in excellent agreement with the 2.28×10^5 value calculated from primary structure data. As a spherical, hydrated $\alpha_{IIb}\beta_3$ complex, with bound detergent, would exhibit a Stokes radius of approximately 5 nm, these data indicate considerable asymmetry in the solution conformation of $\alpha_{IIb}\beta_3$.

The human blood platelet $\alpha_{IIb}\beta_3$ complex is the prototypical member of a widely distributed class of mammalian cell adhesion receptors termed the integrins (Hynes, 1987; Ruoslahti & Pierschbacher, 1987; Ginsberg et al., 1988; Albelda & Buck, 1990). The platelet integrin $\alpha_{IIb}\beta_3$ plays a central role in the hemostatic process [see recent reviews by Plow and Ginsberg (1989) and Phillips et al. (1991)], yet much remains to be learned about its structure and relationship to ligand recognition. Electron microscopic images of rotary shadowed $\alpha_{IIb}\beta_3$ have been interpreted in terms of an ellipsoidal particle 8×12 nm from which two extended regions approximately 18 nm in length make contact with the lipid bilayer (Weisel et al., 1992; Carrell et al., 1985; Parise & Phillips, 1985). However, a recent report has questioned this conclusion and emphasized the heterogeneity of molecular morphologies seen with the detergent-solubilized $\alpha_{IIb}\beta_3$ complex (Rivas et al., 1991). Furthermore, a compact model of $\alpha_{IIb}\beta_3$ solution conformation has been proposed, based on disulfide bond mapping, proteolytic digestion, and peptide cross-linking data (Calvete et al., 1992a,b).

Hydrodynamic measurements provide another route for characterizing the size and shape of detergent-solubilized polypeptides (Tanford et al., 1974). However, most available data on the $\alpha_{IIb}\beta_3$ integrin have been obtained by indirect methods. Jennings and Phillips (1982) employed sucrose density gradient ultracentrifugation and size exclusion chromatography to determine a molecular mass of 265 kDa, sedimentation coefficient of 8.6 S, and a 7.1-nm Stokes radius (R_s) for $\alpha_{IIb}\beta_3$ in 0.1% Triton X-100. Most recently, Rivas et al. (1991) reported a molecular mass of 315 kDa and a sedimentation coefficient of 8.9 S (analytical ultracentrifugation), corresponding to $R_s = 7.4$ nm for $\alpha_{IIb}\beta_3$ in 0.2% Triton X-100. Both sets of measurements indicate the $\alpha_{IIb}\beta_3$/Triton X-100 particle is highly asymmetric ($f/f_{min} = 1.5$).

Further interpretation of these results in terms of macromolecular dimensions has been hampered on one side by the experimental limitations of size exclusion chromatography for Stokes radius determination (Andrews, 1965; Tanford et al., 1974) and on the other by the large size of the Triton X-100 micelle (Robson & Dennis, 1977) used in all previous studies. Therefore, we have employed the physically rigorous technique of dynamic light scattering to determine the translational diffusion coefficient (Johnson & Gabriel, 1981; Pecora, 1983; Berne & Pecora, 1990) of the $\alpha_{IIb}\beta_3$ complex isolated in octyl glucoside.

EXPERIMENTAL PROCEDURES

Protein Purification and Characterization. $\alpha_{IIb}\beta_3$ Complex. The platelet integrin receptor, $\alpha_{IIb}\beta_3$, was isolated from human blood platelets (American Red Cross, Triad Blood Center, Winston-Salem, NC) by methods previously described in detail (Ramsamooj et al., 1990, 1991). Briefly, the membrane fraction obtained by sonication and centrifugation of the washed platelets was solubilized in a buffer containing 1% Triton X-100 (reduced) (Sigma, St. Louis, MO). Following sonication and ultracentrifugation (100000*g* for 90 min), a fraction enriched in $\alpha_{IIb}\beta_3$ was isolated by lentil lectin chromatography (Pharmacia, Piscataway, NJ). This material was concentrated (Amicon concentrator, YM30 membrane) and then eluted from a 0.9×85 cm column of Ultrogel 34 (LKB, Bromma, Sweden) at 4 °C in a buffer containing 0.13 M NaCl, 0.01 M HEPES, 0.002 M $CaCl_2$, 3×10^{-7} M basic trypsin inhibitor (Aprotinin, Sigma), 10^{-6} M leupeptin, pH 7.2, and 0.03 M *n*-octyl β-D-glucopyranoside (octyl glucoside, OG, Sigma).

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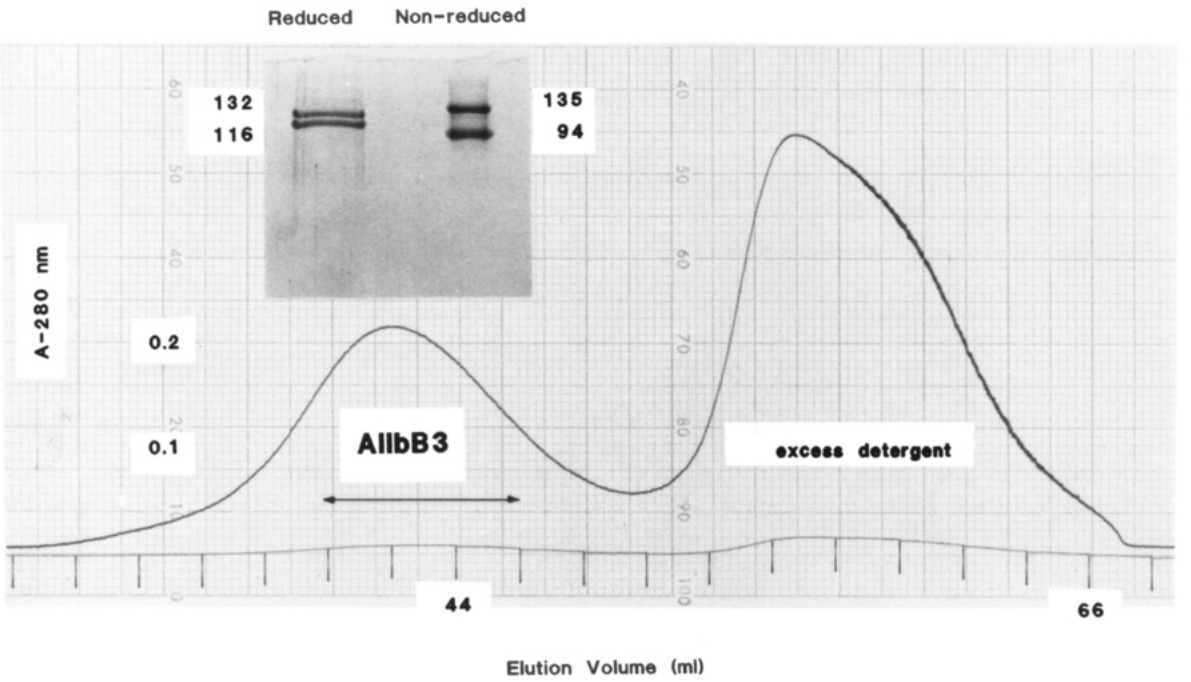

FIGURE 1: Isolation of $\alpha_{IIb}\beta_3$ for light scattering studies by gel filtration chromatography. Four milligrams of partially purified $\alpha_{IIb}\beta_3$, isolated by lentil lectin affinity chromatography in 1% Triton X-100 (reduced), was applied to a 0.9 × 85 cm column of Ultrogel 34 and eluted in a buffer containing 0.03 M octyl glucoside, 0.13 M NaCl, 0.01 M HEPES, 0.002 M $CaCl_2$, and 3×10^{-7} M trypsin inhibitor, pH 7.2. (Inset) SDS–polyacrylamide gel electrophoretic separation of a 15-μg sample (from a pool of the three peak $\alpha_{IIb}\beta_3$-containing fractions obtained by gel filtration chromatography), stained with Coomassie Brilliant Blue. Molecular masses (kDa) of the major bands are indicated.

As can be seen in Figure 1, the major protein-containing peak was clearly separated from excess detergent and was shown by SDS–PAGE followed by staining with Coomassie Brilliant Blue R-250 to contain polypeptides with electrophoretic mobilities on nonreduced and reduced gels characteristic of the α and β subunits of the platelet integrin receptor (Clemetson, 1985; Ramsamooj et al., 1990). Densitometric scans indicated that approximately 90% of the total stained material was present in the doublet corresponding to the α_{IIb} and β_3 subunits, whereas bands of higher (200–250 kDa) and lower (<60 kDa) apparent molecular masses each contributed about 5%. The peak fractions were pooled for subsequent analyses and, in some cases, concentrated with an Amicon pressure concentrator and YM-100 filter.

Additional immunological assays were performed to determine the quantity of fibrinogen and von Willebrand factor present in a sample of $\alpha_{IIb}\beta_3$ used for light scattering experiments. Results of an enzyme-linked immunosorbent assay (ELISA), which utilized fibrinogen labeled with alkaline phosphatase (kindly provided by Dr. G. Doellgast) in competition with native fibrinogen for capture by a rabbit antihuman polyclonal antibody (Cooper Biomedical, Malvern, PA), indicated a level of contamination of ~3 μg of fibrinogen/mg of $\alpha_{IIb}\beta_3$. Results obtained with an ELISA kit for von Willebrand factor (vWf; Imubind, American Diagnostica, Greenwich, CT) (Hantgan et al., 1990) indicated <3 ng of vWf/mg of $\alpha_{IIb}\beta_3$. While this level of contamination by high molecular weight adhesive ligands (<0.4%) is insufficient to exert a significant influence on the measurements reported here, the densitometric scans are consistent with the presence of some aggregated material in the $\alpha_{IIb}\beta_3$ preparations (Eirin et al., 1986; Ramsamooj et al., 1990).

Aliquots of the $\alpha_{IIb}\beta_3$ complex isolated in octyl glucoside were subjected to amino acid composition analyses as previously described (Ramsamooj et al., 1990, 1991), and the resultant protein concentration data combined with ultraviolet absorbance measurements (LKB Ultrospec II) to determine an extinction coefficient at 280 nm of 1.21 ± 0.03 mL mg^{-1} cm^{-1}, which is in excellent agreement with the value of 1.18 ± 0.11 mL mg^{-1} cm^{-1} we previously reported for the $\alpha_{IIb}\beta_3$ complex in Triton X-100 (reduced) (Ramsamooj et al., 1990).

Fibrinogen. Human fibrinogen (American Diagnostica) was further purified by gel filtration chromatography on a 0.9 × 85 cm column of Ultrogel 34 at 4 °C in a buffer containing 0.13 M NaCl, 0.01 M HEPES, 0.002 M $CaCl_2$, 3×10^{-7} M basic trypsin inhibitor, and 10^{-6} M leupeptin, pH 7.2, either with or without 0.03 M *n*-octyl β-D-glucopyranoside (octyl glucoside, Sigma). Fibrinogen concentrations were determined with ultraviolet absorbance measurements using an extinction coefficient at 280 nm of 1.6 mL mg^{-1} cm^{-1} (Carr & Hermans, 1978).

Dynamic Light Scattering/Light Scattering Intensity. Measurements of the translational diffusion coefficient and molecular weight of the $\alpha_{IIb}\beta_3$ complex, fibrinogen, and micelles of octyl glucoside were performed with a Brookhaven Instruments BI-2030AT correlator, operated in conjunction with a BI-200SM light scattering goniometer/photon counting detector and a Spectra Physics 127 He–Ne laser (35 mW, equipped with a vertical polarization rotator). Light was collected at angles from 45° to 120° from samples contained in 1-cm diameter cylindrical cuvettes or in 1-cm path length square cuvettes for 90° measurements; in each case the cuvettes were contained in a thermostated refractive index matching bath (glycerol/water, 1:1, by volume, n = 1.40) which was maintained at 25.0 ± 0.1 °C with a recirculating bath. For dynamic light scattering, the detector aperture was set at either 0.4 or 0.8 mm to optimize the signal/noise ratio (which ranged from 0.14 to 0.25) and minimize the time required for data collection (which ranged from 5 to 30 min). Intensity measurements were made as a function of angle using a motor-driven, computer-coupled goniometer to collect data in 5 × 2-s increments at each of 10 angles using a 2-mm aperture; data from triplicate runs were averaged for molecular weight determinations.

Refractive index measurements of buffered octyl glucoside were made with an Abbe refractometer. Refractive index increment (dn/dc) measurements were performed with a Brice-Phoenix differential refractometer at wavelengths of 436, 546, and 578 nm. Data obtained with 0.40 M KCl were extrapolated to 589 nm to determine the calibration constant of the instrument (Brice & Hawler, 1951). Refractive index differences obtained with octyl glucoside at 0.03, 0.06, and 0.09 M were each extrapolated to 633 nm and then plotted as a function of concentration to obtain $dn/dc = 0.140 \pm 0.001$ cm^3/g.

Dynamic Light Scattering: Data Analysis. The z-average translational diffusion coefficient, D_t, of a macromolecule can be obtained from the intensity-normalized photocount autocorrelation function determined in a dynamic light scattering experiment (Johnson & Gabriel, 1981; Berne & Pecora, 1990; Pecora, 1983). For the special case considered here, a mixture of a macromolecule (component 2) and a micellar detergent (component 1), the experimentally determined reduced first-order correlation function of the faster-moving component (1) was weighted by its fractional intensity (typically less than 0.3) and subtracted from the corresponding function determined under the same instrumental conditions for the mixture:

$$Y_2(\tau) = [g_2^{(2)}(\tau) - 1]^{1/2} = [g_{1,2}^{(2)}(\tau) - 1]^{1/2} - [g_1^{(2)}(\tau) - 1]^{1/2} = B^{1/2}[(I_1/(I_1 + I_2)) \exp(-K^2 D_{t1}\tau) + (I_2/(I_1 + I_2)) \exp(-K^2 D_{t2}\tau)] - B^{1/2}[(I_1/(I_1 + I_2)) \exp(-K^2 D_{t1}\tau)] = B^{1/2}[(I_2/(I_1 + I_2)) \exp(-K^2 D_{t2}\tau)] \quad (1)$$

The translational diffusion coefficient of component 2 was then obtained by cumulants analysis (Koppel, 1972).

$$Y_2(\tau) = (1/2)\ln B + \ln [I_2/(I_1 + I_2)] - D_t K^2 \tau + \mu_2 \frac{\tau^2}{2} \quad (2)$$

This treatment is an extension of that described by others for a two-component mixture (Patkowski et al., 1990; Koppel, 1972; Aksiyote-Benbasat & Bloomfield, 1975; Sam et al., 1990) and reduces to the classical case when $I_1 = 0$.

To test the validity of this approach, dynamic light scattering measurements were performed in both water and buffered octyl glucoside using 20-nm nominal diameter latex microspheres (Duke Scientific, Palo Alto, CA) at particle concentrations chosen to yield scattering intensities comparable to those measured with the $\alpha_{IIb}\beta_3$ complex. Particle sizes, expressed as the Stokes radius R_s, were calculated from the translational diffusion coefficient, D_t, using the Einstein–Sutherland equation and the definition of the frictional coefficient of translational diffusion (Tanford, 1961).

Viscosity determinations were kindly performed in the laboratory of Dr. J. Starita, Rheometrics, Inc. (Piscataway, NJ), using a Couette viscosimeter. Results obtained as a function of shear rate (10–50 s^{-1}) and extrapolated to zero shear indicated 0.03 M octyl glucoside increased the solvent viscosity by a factor of 1.022 over that determined with buffer alone. This result, coupled with published data on the temperature dependence of the viscosity of water (Hodgman et al., 1962), was used to correct the diffusion coefficient data to $D_t^{20,w}$ values, using standard equations (Cantor & Schimmel, 1980).

Light Scattering Intensity: Data Analysis. These data were analyzed by Rayleigh–Gans theory to obtain the weight average molecular weight, M_w, from scattering intensity data, collected as a function of detector angle and particle concentration (Johnson & Gabriel, 1981). Sample scattering intensities were expressed relative to that of a benzene standard, which has a Rayleigh ratio, $R_{std} = 1.26 \times 10^{-5}$ cm^{-2} at 633 nm (Pike et al., 1975). Corrections for refractive index differences and for the scattering intensity of the solvent were made as described by Carr et al. (1977). The accuracy of this calibration procedure was checked with a polystyrene sample (no. 25168) with nominal M_w of 2.08×10^4 and a very narrow size distribution, $M_w/M_n = 1.04$ (Waters, Inc., Framingham, MA). Right angle scattering intensity data were collected from samples of this material at concentrations of 4.0 and 11.6 mg/mL in toluene and expressed as $Kc/R(\theta)$ using a refractive index increment of 0.106 cm^3/g at 633 nm [mean of the values reported by Huglin (1989)]. Extrapolation to $c = 0$ yielded $M_w = (2.19 \pm 0.07) \times 10^4$, which agrees within 5% of the value stated by the manufacturer.

Published refractive index increment values for fibrinogen (0.192 cm^3/g at 633 nm in 0.1 M NaCl) (Carr et al., 1977) and $\alpha_{IIb}\beta_3$ (0.243 cm^3/g at 633 nm in 0.2% Triton X-100) (Rivas et al., 1991) were used for molecular weight calculations. Due to the limited solubility of $\alpha_{IIb}\beta_3$ in octyl glucoside (<2 mg/mL), determination of dn/dc for the integrin/octyl glucoside complex was not possible with the Brice-Phoenix differential refractometer. As the refractive index increment for a detergent-solubilized protein contains contributions from both the polypeptide and bound detergent (Hayashi et al., 1989), differences in detergent binding and detergent refractive index increment ($dn/dc = 0.140$ cm^3/g for octyl glucoside (this work) and 0.155 cm^3/g for Triton X-100 (Kushner and Hubbard, 1954) may introduce an uncertainty of ±20% in M_w for the $\alpha_{IIb}\beta_3$ complex.

RESULTS

Stokes Radius and Molecular Weight of Octyl Glucoside Micelles. Dynamic light scattering measurements were made on solutions of buffered octyl glucoside at a detergent concentration of 0.03 M, which is in excess of its critical micellar concentration (0.02–0.025 M) (Lasch et al., 1983; Chattopadhay & London, 1984; Brito & Vaz, 1986), in order to determine the Stokes radius (R_s) of the octyl glucoside micelles under conditions used here for isolation of the $\alpha_{IIb}\beta_3$ complex. Data obtained at each of four scattering angles (45°, 60°, 90°, 120°) were analyzed by second-order cumulants analysis (Koppel, 1972) to determine the decay constant of the first-order autocorrelation function, which contains the z-average diffusion coefficient and an angular-dependent term: $\Gamma = -D_t(4\pi n/\lambda_0)^2 \sin^2(\theta/2)$. Figure 2 shows a plot of Γ vs $\sin^2(\theta/2)$; linear regression (with the intercept constrained to zero) yielded a value of $D_t = (1.03 \pm 0.05) \times 10^{-6}$ cm^2 s^{-1} for octyl glucoside micelles at 25 °C. After correction for the viscosity of the buffered octyl glucoside solution, we obtained $D_t^{(20,w)} = 9.24 \times 10^{-7}$ cm^2 s^{-1} and $R_s = 2.31 \pm 0.11$ nm for the octyl glucoside micelle. This value agrees with $R_s = 2.65 \pm 0.2$ nm reported by Aerts et al. (1990) based on dynamic light scattering measurements of octyl glucoside at 0.025 M in 0.1 M sodium phosphate.

Figure 2 also shows data obtained from these same solutions by light scattering intensity measurements, performed as a function of angle. As expected for particles of this size, the reciprocal scattering intensity function, $Kc/R(\theta)$, was independent of angle (over the range 60–120°) so these data were averaged to obtain a molecular weight of $(6.0 \pm 0.2) \times 10^3$ for the octyl glucoside micelle. We note that this value is somewhat lower than the micelle molecular weight of 8000 reported by Rosevear et al. (1980), based on size exclusion

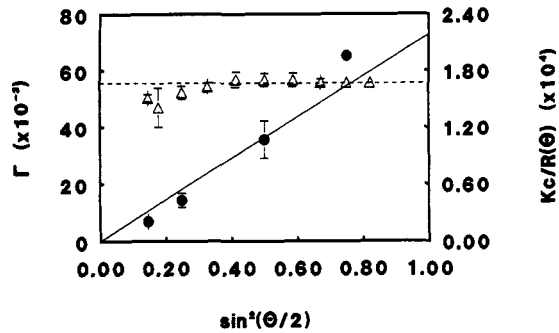

FIGURE 2: Angular dependence of dynamic light scattering and classical light scattering intensity data obtained with octyl glucoside. Left ordinate (solid circles): Exponential decay constant Γ, obtained from fitting the autocorrelation functions at each angle with a second-order cumulants analysis. The solid line resulted from a linear regression, constrained to have zero intercept and yields $D_t^{(20,w)} = 9.24 \times 10^{-7}$ cm^2 s^{-1}, $R_s = 2.31$ nm. Right ordinate (open triangles): Reciprocal scattering function $Kc/R(\theta)$, calculated from the intensity measurements at each angle. The dashed line is the average of data obtained from 60° to 120° and yields $M_w = 6.0 \times 10^3$. Dynamic light scattering and light scattering intensity data were collected from solutions of 0.03 M octyl glucoside, 0.13 M NaCl, 0.01 M HEPES, 0.002 M $CaCl_2$, and 3×10^{-7} M trypsin inhibitor, pH 7.2, as described in the text.

chromatography. However, additional light scattering intensity and dynamic light scattering data (not shown) indicated the extent of aggregation of octyl glucoside increased steeply at higher detergent concentrations, reaching $M_w = 1.9 \times 10^4$, $R_s = 2.8$ nm at 0.06 M and $M_w = 2.7 \times 10^4$, $R_s = 3.6$ nm at 0.09 M octyl glucoside.

Size Determinations for Particles Suspended in Octyl Glucoside. The preceding results indicate that, in order to accurately determine the hydrodynamic size of a particle suspended in octyl glucoside using dynamic light scattering, it will be necessary to explicitly account for the contribution of detergent micelles to the intensity autocorrelation function. The theoretical basis for our approach is outlined under Experimental Procedures, *Dynamic Light Scattering: Data Analysis*. Here we describe experimental tests of the validity of this method.

Polystyrene spheres of nominal radius 10 ± 0.75 nm were diluted 30–1000-fold in either water or buffered octyl glucoside, and right-angle dynamic light scattering measurements were performed. The data were fit by second-order cumulants analysis, initially without correction for the contribution of the octyl glucoside micelles to the intensity autocorrelation functon (Koppel, 1972). As shown in Figure 3 (open circles) for beads suspended in water, the apparent R_s of 9.15 ± 0.20 nm was independent of particle concentrtation. Analyzing the same data without correction for higher order terms yielded $R_s = 10.0 \pm 0.5$ nm, in agreement with the value cited by the manufacturer. In contrast, measurements performed in octyl glucoside showed a striking dependence on bead concentration, in that R_s ranged from an apparent value of 5.1 nm at 4.5×10^{12} particles/mL to 8.1 nm at a 1.5×10^{14} particles/mL (Figure 3, open squares). Next, dynamic light scattering measurements were performed with buffered octyl glucoside using the same instrumental parameters, and the appropriate intensity-weighted first-order correlation function was subtracted from the corresponding function determined for each mixture of beads plus detergent, as outlined under Experimental Procedures (eqs 1 and 2). As shown in Figure 4, the reduced first-order correlation function experimentally determined for polystyrene beads (6×10^{12} mL^{-1}) in octyl glucoside (squares) contains terms due to both

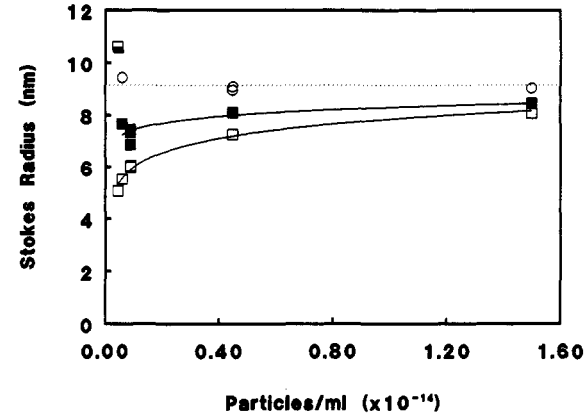

FIGURE 3: Apparent Stokes radius of polystyrene spheres as a function of particle concentration. Open circles (dashed line): Particles suspended in water; size measurements from the right angle autocorrelation functions determined by cumulants analysis yields an average $R_s = 9.15$ nm. Open squares: Particles suspended in buffered octyl glucoside; size measurements by cumulants analysis without correction for the contributions of octyl glucoside. Solid squares: Particles suspended in buffered octyl glucoside; size measurements according to eqs 1 and 2 with correction for intensity-weighted contribution of the octyl glucoside micelles to the autocorrelation function. Note the overestimate of R_s obtained at the lowest particle concentration (half-filled square).

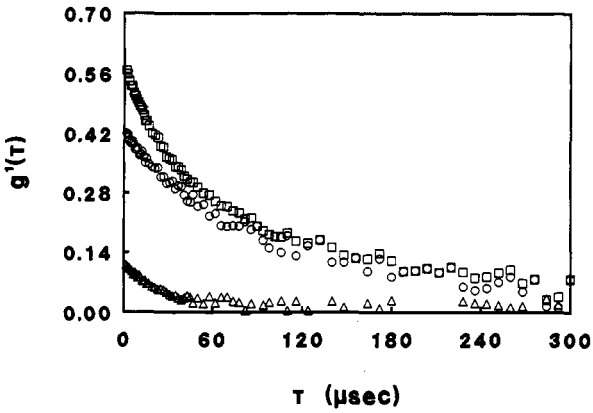

FIGURE 4: Reduced first-order autocorrelation functions for octyl glucoside and polystyrene spheres suspended in octyl glucoside. Triangles: $g_\tau^{(1)}$ determined from the experimentally determined intensity autocorrelation function for buffered octyl glucoside, without corrections. Squares: $g_\tau^{(1)}$ for polystyrene spheres suspended at 6×10^{12} particles/mL in octyl glucoside. Circles: Corrected $g_\tau^{(1)}$ for polystyrene beads in octyl glucoside, calculated with eq 1, with subtraction of the intensity-weighted (0.23) contribution of the detergent micelles to the reduced first-order correlation function of the bead/detergent suspension. The first 64 channels of the BI-2030 AT correlator were divided into four blocks, each of 16 channels, using sample times of 1, 2, 4, and 8 μs; the last eight channels were delayed by 8.2 μs and used to define the baseline. Data were collected for 20 min, with a detector aperture to 0.4 mm; signal/noise ratios of 0.20–0.25 were obtained.

detergent and beads; analysis of these data without correction for these terms yielded an apparent $R_s = 5.5$ nm. In contrast, the correlation function for octyl glucoside micelles (triangles) exhibits a more rapid decay, characterized by $R_s = 2.2$ nm. Following correction for the detergent contribution (fractional intensity 0.23) using eq 1, these data (circles) were analyzed by eq 2, which yielded $R_s = 7.7$ nm.

As illustrated in Figure 3 (solid squares), application of this procedure to the complete data set removed most of the curvature in a plot of R_s vs particle concentration. The ratio of the corrected Stokes radius in octyl glucoside to that determined in water ranged from 0.80, at a 6–9 × 10^{12}

particles/mL dilution, to 0.92 at 1.5×10^{14} particles/mL. The improved agreement between the corrected data and those obtained in the absence of detergent verifies the utility of these algorithms for obtaining more accurate estimates of diffusion coefficients of particles suspended in solutions of detergent micelles. However, these experiments also revealed a limitation of the correction procedures, for at the lowest bead concentration examined (4.5×10^{12} particles/mL; fractional intensity due to octyl glucoside = 0.27) application of the algorithms significantly overestimated R_s (half-filled square).

Classical and Dynamic Light Scattering of Fibrinogen. Light scattering measurements were carried out with fibrinogen following gel filtration chromatography in buffer with and without 0.03 M octyl glucoside to assess the effects of the detergent micelles on molecular weight and translational diffusion coefficient measurements of a well-characterized soluble protein. In the absence of detergent, $R_s = 10.1 \pm 0.4$ nm and $M_w = 2.77 \times 10^5$ resulted from right-angle dynamic light scattering and intensity measurements, respectively, carried out at 0.31 mg/mL fibrinogen. This value of R_s agrees with that of 10.5 nm determined by Palmer et al. (1979) with the same techniques. The lower molecular weight that we have obtained, in contrast to the accepted value of 3.38×10^5 (Henschen et al., 1983), could be due to partial proteolysis of the samples, as evidenced by SDS–PAGE, which showed heterogeneity in the fibrinogen α-chains (data not shown). With a sample of fibrinogen chromatographed in buffered octyl glucoside and concentrated to 0.29 mg/mL, $M_w = 2.99 \times 10^5$ and an apparent $R_s = 5.70 \pm 0.15$ nm were obtained. After correction for the detergent micelle contribution to the correlation function (fractional intensity 0.26), $R_s = 8.46 \pm 0.32$ was obtained. These results indicate that application of the correction procedures results in a substantial improvement in our ability to obtain reliable dynamic light scattering data from a macromolecule in a solution of detergent micelles.

Light Scattering Intensity Measurements of $\alpha_{IIb}\beta_3$ in Octyl Glucoside. Light scattering intensity measurements were also performed on solutions of $\alpha_{IIb}\beta_3$ in octyl glucoside in order to determine the molecular weight of the detergent-solubilized macromolecule. Consistent with the predictions of Rayleigh–Gans theory (Johnson & Gabriel, 1981) for particles of this size (16-nm diameter for $\alpha_{IIb}\beta_3$ is less than 5% of the wavelength of the scattered light), the scattering intensity was independent of angle, over the range 45–120° (Figure 5), so that data from both right angle and angular-dependent intensity measurements were analyzed to obtain M_w. The results are illustrated in Figure 6a, which shows $Kc/R(\theta)$ vs the $\alpha_{IIb}\beta_3$ concentration. We note that over the concentration range of 0.15–0.82 mg/mL, M appeared to exhibit significant concentration dependence. Analysis of these data by linear regression and extrapolation to $c = 0$ (Johnson & Gabriel, 1981) yielded a molecular weight of $(1.94 \pm 0.25) \times 10^5$ for the $\alpha_{IIb}\beta_3$ complex. This value agrees, within 15%, with the molecular weight of 2.28×10^5 calculated from the amino acid and carbohydrate compositions of the α_{IIb} and β_3 polypeptides (Eirin et al., 1986) and within 8% of the value of 2.10×10^5 determined by Rivas et al. (1991) by low-angle light scattering. This agreement is reasonable, especially when the limitations imposed by our use of a published value for the refractive index increment for $\alpha_{IIb}\beta_3$ in Triton X-100 (Rivas et al., 1991) are considered (see Experimental Procedures).

Moreover, if the value at the highest concentration tested is not considered, the concentration dependence is no longer apparent, and averaging the $Kc/R(\theta)$ values for the six lowest

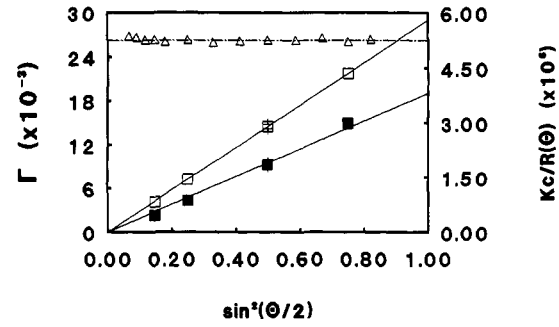

FIGURE 5: Angular dependence of dynamic light scattering and light scattering intensity data obtained with $a_{IIb}\beta_3$ in octyl glucoside. Open squares: Exponential decay constant Γ at each angle, with $\alpha_{IIb}\beta_3$ at 0.43 mg/mL. Solid line (upper) determined by linear regression, with constrained zero intercept, yields $R_s = 5.8$ nm. Filled squares: Exponential decay constant Γ obtained from eqs 1 and 2 with correction for the intensity-weighted contribution of octyl glucoside to the reduced first order correlation functions at each angle. Solid line (lower) determined by linear regression, with constrained zero intercept, yields $R_s = 8.8$ nm. Open triangles: Reduced scattering intensity data from the same solutions; averaging these data over the angular range 60 – 120° yielded a molecular weight of 1.9×10^5 for $\alpha_{IIb}\beta_3$ at 0.43 mg/mL.

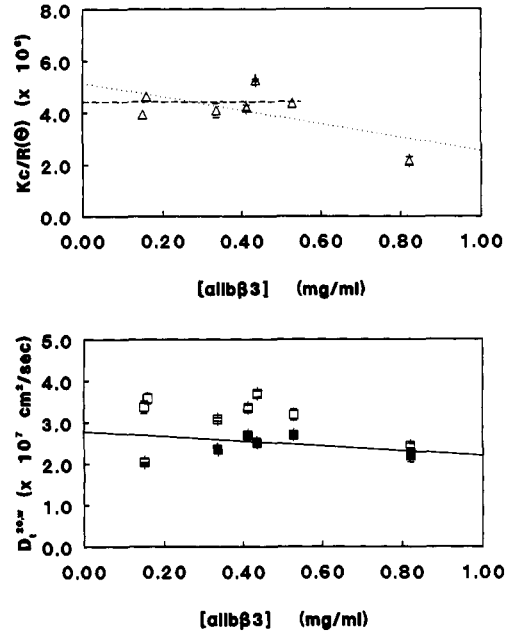

FIGURE 6: (a, top) Concentration dependence of the reduced scattering intensity of $\alpha_{IIb}\beta_3$ in octyl glucoside. Open triangles: $Kc/R(\theta)$ determined at each protein concentration (in buffered octyl glucoside) from light scattering intensity measurements. Dotted line: Linear regression, extrapolated to zero protein concentration, yields $M_w = (1.94 \pm 0.25) \times 10^5$ for $\alpha_{IIb}\beta_3$. Dashed line: Averaging values at the six lowest protein concentrations yields $M_w = (2.26 \pm 0.22) \times 10^5$ for $\alpha_{IIb}\beta_3$. (b, bottom) Translational diffusion coefficient of $\alpha_{IIb}\beta_3$ in octyl glucoside as a function of protein concentration. Open symbols: $D_t^{(20,w)}$ measurements for $\alpha_{IIb}\beta_3$ determined by cumulant analysis without correction for the contributions of octyl glucoside. Closed symbols: $D_t^{(20,w)}$ measurements for $\alpha_{IIb}\beta_3$ from eqs 1 and 2 with correction for the intensity-weighted contribution of octyl glucoside micelles to the autocorrelation functions. Solid line: Linear regression, extrapolated to zero protein concentration to obtain $D_t^{o(20,w)} = (2.78 \pm 0.31) \times 10^{-7}$ cm² s⁻¹, corresponding to $R_s = 7.67 \pm 0.85$ nm for $\alpha_{IIb}\beta_3$. As the correction algorithms underestimate D_t at low sample intensities (see text and Figure 3), the data point represented by the half-filled square was not used in the regression analysis.

protein concentrations results in $M_w = (2.26 \pm 0.22) \times 10^5$ (Table I), in excellent agreement with the 2.28×10^5 value calculated from primary structure data (Eirin et al., 1986).

Table I: Hydrodynamic Parameters for Octyl Glucoside Micelles and the $\alpha_{IIb}\beta_3$/OG Complex

macromolecule	M_w	$D_t^{(20,w)}$ ($cm^2\ s^{-1}$)	R_s (nm)
OG micelle	$(6.0 \pm 0.2) \times 10^3$	$(9.2 \pm 0.4) \times 10^{-7}$	2.3 ± 0.1
$\alpha_{IIb}\beta_3$/OG	$(2.3 \pm 0.2) \times 10^5$ [a]	$(2.8 \pm 0.3) \times 10^{-7}$	7.7 ± 0.8

[a] Polypeptide moiety only.

Thus, the higher M_w measured at 0.8 mg/mL may result from the onset of concentration-dependent self-association of $\alpha_{IIb}\beta_3$, a phenomenon already suggested by other investigators (Carrell et al., 1985; Rivas et al., 1991).

Dynamic Light Scattering of $\alpha_{IIb}\beta_3$ in Octyl Glucoside. Dynamic light scattering measurements were performed on the purified platelet integrin $\alpha_{IIb}\beta_3$, which had been isolated by gel filtration chromatography in buffered octyl glucoside. Figure 5 also shows the angular dependence of dynamic light scattering data obtained in experiments with an $\alpha_{IIb}\beta_3$ concentration of 0.43 mg/mL (open squares), without correction for the octyl glucoside contributions. Fitting the data obtained at each angle by cumulants analysis (Koppel, 1972) and plotting the resultant exponential decay term, Γ vs $\sin^2(\theta/2)$, gives the expected the linear dependence (Johnson & Gabriel, 1981; Pecora, 1983). The slope of a best-fit line passing through the origin yields an apparent diffusion coefficient of 4.13×10^{-7} $cm^2\ s^{-1}$ at 25 °C, and a R_s = 5.8 nm. The effects of correction for the contribution of octyl glucoside micelles to the intensity autocorrelation function (using eqs 1 and 2) can be seen in Figure 5 as the filled squares. When the decay due to the faster-moving detergent micelles is removed from each angular-dependent correlation function, the value of the apparent diffusion coefficient drops to 2.71×10^{-7} $cm^2\ s^{-1}$, at 25.0 °C, and R_s increases to 8.81 ± 0.19 nm.

The original and corrected $D_t^{(20,w)}$ values resulting from application of these procedures to samples of $\alpha_{IIb}\beta_3$ at five protein concentrations in the range 0.3–0.9 mg/mL are shown in Figure 6b. Due to the tendency of the correction algorithms to underestimate D_t at low sample concentrations, the data point obtained at 0.15 mg/mL $\alpha_{IIb}\beta_3$ (half-filled square) was not used in this analysis. The corrected data (filled squares) exhibit a modest concentration dependence, and extrapolation to zero protein concentration (Johnson & Gabriel, 1981) yields $D_t^{o(20,w)} = (2.78 \pm 0.31) \times 10^{-7}$ $cm^2\ s^{-1}$, corresponding to R_s = 7.67 ± 0.85 nm for the $\alpha_{IIb}\beta_3$ complex, isolated in octyl glucoside (Table I).

DISCUSSION

In this study, we have employed dynamic and classical light scattering measurements to characterize the solution conformation of the prototypical integrin receptor, $\alpha_{IIb}\beta_3$. In the process, we have also measured the molecular weight and translational diffusion coefficient of octyl glucoside micelles and developed a method, based on the theory of dynamic light scattering, to resolve the intensity autocorrelation function of particles suspended in detergent from the signal due to micelles alone. The validity of these algorithms has been demonstrated by determining the Stokes radius of spherical particles of known size, as well as R_s for the asymmetric macromolecule fibrinogen, in the presence and absence of octyl glucoside micelles.

This report is the first direct determination of the translational diffusion coefficient of $\alpha_{IIb}\beta_3$ by a physically rigorous technique, i.e., dynamic light scattering. A translational diffusion coefficient of $(2.78 \pm 0.31) \times 10^{-7}$ $cm^2\ s^{-1}$, corresponding to a Stokes radius of 7.67 ± 0.85 nm, was obtained for $\alpha_{IIb}\beta_3$ isolated in octyl glucoside. Classical light scattering intensity measurements yielded $M_w = (2.26 \pm 0.22) \times 10^5$ for the polypeptide moiety of the $\alpha_{IIb}\beta_3$ complex, a value that agrees with its amino acid and carbohydrate composition (Eirin et al., 1986). Both M_w and D_t have been obtained from data corrected for protein concentration-dependent effects to minimize the contributions of $\alpha_{IIb}\beta_3$ self-association (Carrell et al., 1985; Rivas et al., 1991).

It is important to stress that while the data reported here have been corrected for the contributions of octyl glucoside micelles to the intensity autocorrelation function, the resultant R_s is that for a detergent/protein complex. An estimate of the hydrated volume of the detergent-solubilized $\alpha_{IIb}\beta_3$ complex, V_h = 548 nm^3, may be obtained from the polypeptide molecular weight (228 000; Eirin et al., 1986) utilizing values of partial specific volume (0.730 cm^3/g) and theoretical water of hydration (0.392 g H_2O/g protein) calculated from the amino acid and carbohydrate compositions of the α and β subunits (Rocco et al., 1987). The contribution of bound detergent (0.38 g of Triton X-100/g of protein; Rivas et al., 1991) was estimated using the partial specific volume of octyl glucoside, 0.86 cm^3/g, reported by Aerts et al. (1990). A spherical particle of this size would exhibit $D_t^{(20,w)} = 4.2 \times 10^{-7}$ $cm^2\ s^{-1}$ (R_{calc} = 5.1 nm). In contrast, the experimentally determined translational diffusion coefficient for the $\alpha_{IIb}\beta_3$/octyl glucoside complex is ~30% smaller, indicative of significant deviation from spherical geometry (f/f_{calc} = 1.5).

We note that Jennings and Phillips (1982) obtained R_s = 7.1 nm from size exclusion chromatography and Rivas et al. (1991) calculated R_s = 7.4 nm from their experimentally determined molecular weight and sedimentation velocity values for the $\alpha_{IIb}\beta_3$ complex. While the $R_s = 7.7 \pm 0.8$ nm obtained by dynamic light scattering is 4–8% higher than those values, concentration-dependent self-association of the $\alpha_{IIb}\beta_3$/OG complex may contribute to this difference. Furthermore, in both previous reports the $\alpha_{IIb}\beta_3$ complex was isolated in Triton X-100. Triton X-100 forms micelles with a molecular weight of 90 000 (Robson & Dennis, 1977) and Stokes radius = 4.7 ± 0.1 nm (Hantgan, unpublished results). In contrast to octyl glucoside (M_w = 6000, R_s = 2.3 nm), Triton X-100 micelles are likely to make a major contribution to the hydrodynamic properties of the 228 000-Da $\alpha_{IIb}\beta_3$ complex.

In summary, our results confirm the asymmetry of the $\alpha_{IIb}\beta_3$ integrin shape previously suggested by others, on the basis of either electron microscopic observations (Carrell et al., 1985; Rivas et al., 1991; Weisel et al., 1992) or less direct hydrodynamic techniques (Jennings & Phillips, 1982; Rivas et al., 1991). Moreover, we believe that the ability to directly monitor both the hydrodynamic properties and aggregation status of detergent-solubilized membrane-spanning proteins with the light scattering techniques and correction algorithms described here will prove very useful in studying their solution conformation and interactions.

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