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The concentration dependence of the activity coefficient of the human spectrin heterodimer. A quantitative test of the Adams–Fujita approximation

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Abstract

The heterodimer and tetramer states of human spectrin, in equilibrium at 30°C, have been resolved by means of sedimentation velocity experiments at low temperature. This has allowed quantification of the concentrations of each oligomer at equilibrium, as a function of the total concentration of spectrin. In separate sedimentation equilibrium experiments, the thermodynamic activity of the heterodimer has been estimated as a function of the total spectrin concentration through the use of the Omega function. Combination of the concentration and thermodynamic activity of the spectrin heterodimer allowed estimation of the activity coefficient of the heterodimer as a function of total spectrin concentration. Over the accessible concentration range of 0–16 g/L, the logarithm of the activity coefficient of the heterodimer was linear in the total concentration, and the apparent equilibrium constant for tetramer formation was only weakly dependent on concentration, implying that a single virial coefficient is sufficient to describe the nonideality of this system over this concentration range.

Key words: Self-association; Excluded volume; Activity coefficient; Second virial coefficient; Oligomers; Sedimentation equilibrium; Ultracentrifuge

1. Introduction

The durability and deformability of the erythrocyte is attributed to its membrane cytoskeleton which consists of a two-dimensional protein network covering the cytoplasmic surface of the erythrocyte membrane [1]. The major cytoskeletal protein is spectrin which consists of two non-identical polypeptide chains: α(280 kDa) [2] and β(246 kDa) [3] which associate laterally by coiling around one another to form a double-stranded heterodimer. This molecule has the appearance of a long flexible rod in the electron microscope, approximately 100 nm in contour length, in which the two component chains can be seen loosely twisted about each other [4]. The spectrin heterodimer can undergo a reversible self-association reaction [5,6] to yield a tetramer of contour length ≈ 200 nm [4]. It is now well established that spectrin can self-associate beyond the tetramer to produce a series of higher oligomers: hexamers, octamers and so on [7–12]. Although

Abbreviations: SDS, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane.

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the tetramer appears to be the predominant species in vivo [6], the higher oligomers of spectrin represent a significant fraction of the spectrin on the membrane [12].

While the existence of the higher oligomers has been demonstrated by means of both sedimentation equilibrium [8,10,11] and gel electrophoresis [7,9], some discrepancies have been noted in the values of the equilibrium constants estimated from these two approaches. In particular, the equilibrium constant for addition of a further heterodimer unit to a pre-existing oligomer to form oligomers larger than the tetramer (K_{iso}) was estimated to be $\approx 10^6$ L/mol from sedimentation equilibrium studies [10,11], but significantly less than this from gel electrophoresis [8,9].

Measurements at sedimentation equilibrium allow analysis of self-association reactions at chemical equilibrium without perturbation of the reaction. However, such analysis is complicated by the non-ideality arising from the finite volume that macromolecules occupy in solution, and the charges they carry. In self-association reactions, particularly those in which many species coexist, rigorous assessment of the nonideality is a very complex problem, requiring detailed knowledge of the geometry and charges of the different species.

Adams and Fujita [13] proposed that (at least over a limited concentration range) a first-order approximation to the effects of nonideality in self-associating systems could be made by considering that the logarithm of the activity coefficient of each species could be considered to be linearly dependent on the total protein concentration, c,

$$\ln \gamma_i = B_i M_i c, \tag{1}$$

where γ_i is the activity coefficient, M_i the molar mass, and B_i the second virial coefficient of the ith species pertaining at a total solute concentration c. With the further assumption that the same value of the second virial coefficient B (in units of L mol g^{-2}) applies to all oligomers, i.e.

$$B_1 = B_2 = B_3 = \ldots = B, \tag{2}$$

it follows that $\gamma_i = \gamma_1^i$ at any fixed concentration, c, so that the thermodynamic equilibrium constants become equal to the apparent constants (based on concentrations rather than activities). In addition, the approximation allowed the true weight average molecular weight to be estimated from the apparent value, given an estimate of the global second virial coefficient, B,

$$M_{w,app} = \frac{M_w}{1 + BM_w c}, \tag{3}$$

$$\frac{1}{M_{w,app}} = \frac{1}{M_w} + Bc, \tag{4}$$

where $M_{w,app}$ is the *apparent* weight average molecular weight, obtained from the local slope of plots of ln c versus r^2, M_w is the true weight average molecular weight, and c is the concentration of the protein in the centrifuge cell at the radial position at which $M_{w,app}$ was calculated.

The Adams–Fujita approximation is frequently employed in the analysis of self-association, but it is recognised that the approximation will not be valid at high protein concentrations [14]. Furthermore, in the case of a protein with the unusual geometry of spectrin, the validity of the approximation, and the estimation of the concentration range over which it may be assumed, remains to be tested.

The present study takes advantage of the high activation energy of the spectrin association reaction to 'freeze' the reaction by rapid chilling [6], to allow separate quantification of the dimer and tetramer over a range of spectrin concentrations. The dimer concentration is compared with the dimer activity, obtained from sedimentation equilibrium [15], to allow an estimation of the activity coefficient of the dimer. The linear dependence of this quantity on the spectrin concentration provides an explicit test of the Adams–Fujita approximation for this protein over the concentration range 0–16 g/L.

2. Experimental

2.1. Preparation of spectrin

Freshly drawn blood from donors was obtained from the Red Cross Transfusion Service, Sydney,

as packed cells in an anti-coagulant, preservative solution (0.33% citric acid, monohydrate; 2.6% sodium citrate, dihydrate; 0.25% sodium dihydrogen phosphate, dihydrate; 4.64% dextrose; 0.027% adenine). The cells were stored at 4°C in this medium and were used within 48 h of collection.

Packed erythrocytes were washed twice by centrifugation (10 min, $1400 \times g$) in six volumes of 5 mM phosphate buffer, pH 7.5, containing 0.95% NaCl at 2–4°C. The washed cells were then hemolysed in six volumes of cold 5 mM phosphate buffer, pH 8.0, and the membranes were collected by centrifugation at $16000 \times g$ for 17 min. This procedure was repeated six times, or until the membranes were a pale cream colour. Care was taken to remove the buffy coat during the initial washing of the cells, and the leucocyte pellet during the hemolysis in order to minimise proteolysis.

The hemolysed membranes were diluted 1:1 with cold distilled water to lower the ionic strength of the suspension in order to facilitate dissociation. The membranes were then centrifuged at $16000 \times g$ and the resulting ghost pellet was resuspended in an equal volume of distilled water. This suspension was incubated at 37°C at low ionic strength for 1 h to solubilize the spectrin, and the suspension was centrifuged at $16000 \times g$ for 30 min at 4°C to sediment the membrane vesicles. The supernatant fraction containing the water-soluble proteins was then recentrifuged twice under the same conditions to remove any residual membrane fragments. Phenylmethylsulfonyl fluoride was included in all incubations to minimise proteolysis.

2.2. Purification of spectrin dimer

The low ionic strength extract was concentrated by dialysis against Aquacide III and the mixture of water-soluble proteins was separated by gel filtration on a Sepharose CL-4B column (60 cm × 2.5 cm) with a buffer comprising 0.01 M sodium phosphate/5 mM EDTA, pH 7.5, containing 0.1 M NaCl, 1 mM dithiothreitol and 0.3 mM sodium azide, at a flow rate of 16.22 mL/h. The dimer fractions were concentrated to approximately 5 mL by dialysis against Aquacide III at 4°C. The concentrated dimer was then dialysed for 15 min against 100 mL of the above buffer, before repeating the gel filtration chromatography on the same Sepharose CL-4B column. Fractions from the centre of the peak were taken and concentrated to a protein concentration of up to 32.2 g/L by centrifugation at $3000 \times g$ through a Centricon microconcentrator (Amicon Corp, USA), followed by overnight dialysis against the appropriate buffer.

The concentration of spectrin solutions was estimated from the absorbance at 280 nm and the absorbance coefficient $A_{1\ g/L,1\ cm} = 1.07$ [16]. Corrections were made for light scattering by subtracting the absorbance at 360 nm. The purity of spectrin samples was examined with the aid of SDS polyacrylamide gel electrophoresis [17]. The purified spectrin was used as soon as possible for experimentation to minimise proteolytic damage that may occur during storage.

2.3. Ultracentrifugation

Sedimentation velocity experiments were performed in cells fitted with double-sector aluminum-filled epon centrepieces and quartz windows, in a Beckman Optima XL-A analytical ultracentrifuge. Some measurements were made in a Beckman model E instrument; no significant differences were found between the data obtained from different instruments.

Solutions containing spectrin at a concentration between 0 and 32 g/L were incubated for 16 h at 30°C in a buffer comprising 0.01 M sodium phosphate, pH 7.5, 0.1 M NaCl, 5 mM EDTA and 1 mM dithiothreitol. After incubation, the samples were chilled immediately on ice, and diluted to 1.0 g/L with ice cold buffer. These diluted samples were centrifuged at 42000 rpm and at a temperature of 2–4°C. During acceleration, the rotor was held at a speed of 3000 rev/min while the samples were scanned at 280 and 360 nm for the estimation of the total spectrin concentration. Then the rotor was accelerated to 42000 rpm, and scans were made at 40

min intervals over a period of 2–3 h, until the individual dimer and tetramer boundaries were clearly resolved.

The scans at 280 nm were corrected for baseline deviation from partial masking or window defects by subtracting the scans at 360 nm. The concentration changes across the dimer and tetramer boundaries were measured and corrected for radial dilution,

$$c_0 = c_t \frac{r_t^2}{r_0^2}, \tag{5}$$

where c_0 is the concentration corrected for radial dilution, c_t is the concentration measured at time t across the boundary which is located at radial position r_t, and r_0 is the radial position of the meniscus. Multiple samples were measured from several incubation concentrations in order to assess the variance of the estimates of heterodimer concentration. In one experiment an ill-defined boundary was observed to move more slowly than the dimer boundary, and possibly represented degraded or fragmented spectrin. This experiment was rejected.

The sedimentation equilibrium experiments have already been described [15]. Since the Omega function values were obtained with the use of a molar mass of 480 kg/mol for the spectrin heterodimer, these values were converted to those relevant to 526 kg/mol,

$$\ln \Omega(r)_{526} = \tfrac{526}{480} \ln[\Omega(r)_{480}] + (\tfrac{526}{480} - 1) \ln\left(\frac{c(r_{\mathrm{ref}})}{c(r)}\right). \tag{6}$$

The set of $(\Omega(r), c(r))$ points between 0 and 2 g/L was fitted with the cooperative isodesmic (or 'SEK III') model [15] in order to estimate $a_1(r_{\mathrm{ref}})$, the thermodynamic activity of the spectrin heterodimer at the reference concentration of 1.0 g/L,

$$a_1(r_{\mathrm{ref}}) = c_1(r_{\mathrm{ref}}) \exp[BM_1 c(r_{\mathrm{ref}})]. \tag{7}$$

The value of $a_1(r_{\mathrm{ref}})$ was calculated for each fit from the best-fit values of the equilibrium constants and the second virial coefficient. This procedure is equivalent to extrapolating the Omega function to zero concentration and calculating $a_1(r_{\mathrm{ref}})$ from the extrapolated intercept [18]. The

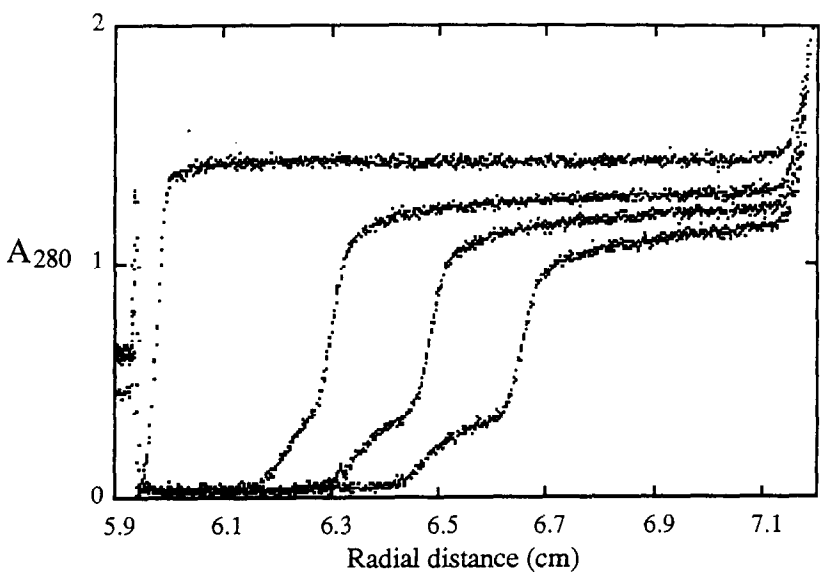

Fig. 1. Sedimentation velocity profiles of spectrin, incubated at 30°C for 24 h at a concentration of 3.18 g/L, then chilled rapidly on ice, diluted to 1.27 g/L with ice-cold buffer, and centrifuged at 42000 rpm. The dimer and tetramer boundaries are clearly resolved.

approach outlined above was preferable to an arbitrary extrapolation to zero concentration, since the SEK III model has already been shown to fit the data well. Furthermore, for the range of data between 0 and 1 g/L, the value of $a_1(r_{ref})$ was not sensitive to the particular model chosen to fit the data; essentially the same estimate of $a_1(r_{ref})$ was returned from application of the SEK III model, a dimer–tetramer–hexamer model, or a dimer–tetramer–hexamer–octamer model.

With a value of $a_1(r_{ref})$ available, the thermodynamic activity of the heterodimer (the protomer of the reaction) at all other concentrations of spectrin could be obtained,

$$a_1(r) = \frac{a_1(r_{ref})c(r)}{\Omega(r)c(r_{ref})}. \quad (8)$$

2.4. Non-linear regression

The non-linear regression program used for fitting concentration and Omega data with the model was based on the Marquardt algorithm [19]. In fitting the c_1 versus c distribution, the value of c_1 at each value of c was estimated for each new set of parameter estimates by solution of the implicit relationship

$$c = c_1\left[1 + y(2-x)/(1-x)^2\right], \quad (9)$$

where $x = K_{iso}c_1/M_1$ and $y = K_{2,4}c_1/M_1$, with the aid of the Newton–Raphson method.

3. Results

The dimer boundary could be clearly resolved after about 90 min sedimentation at 42000 rpm (Fig. 1). Within a given experiment, measurements subsequently made of the dimer concentrations and corrected for radial dilution were generally within 2% of each other. The tetramer boundary was not so clearly resolved until later in the experiment; there was often noticeable overlap with the boundaries of the hexamer and high oligomers. Nevertheless, satisfactory measurement of the tetramer concentration was achieved in most cases, though with less precision. The concentrations of the dimer and tetramer boundaries are plotted against the total spectrin concentration in Fig. 2. These data reveal that at

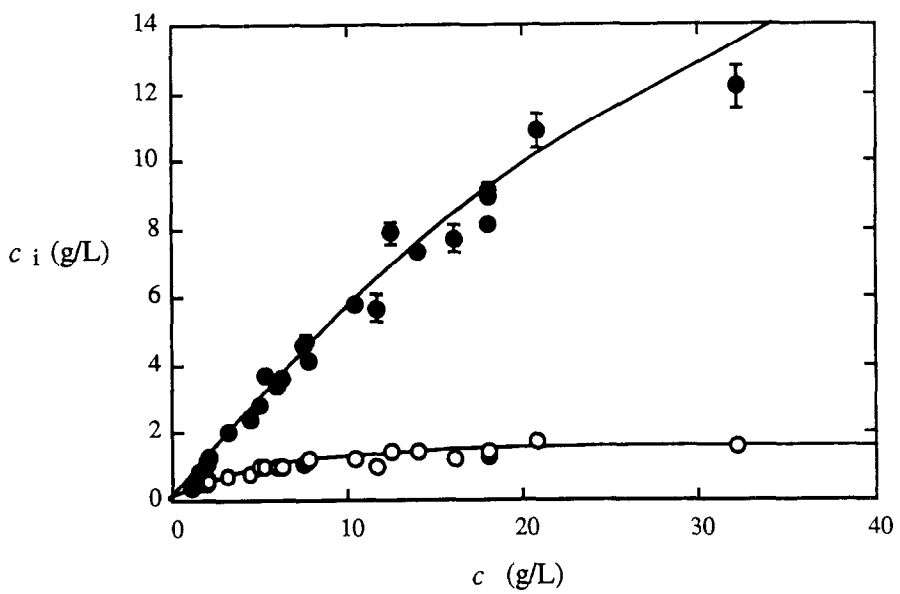

Fig. 2. Dependence on total spectrin concentration of the concentrations of (○) the heterodimer and (●) the tetramer. The error bars indicate the standard error of the mean of three or four estimates of the oligomer concentrations from the same sample measured at 40 min intervals.

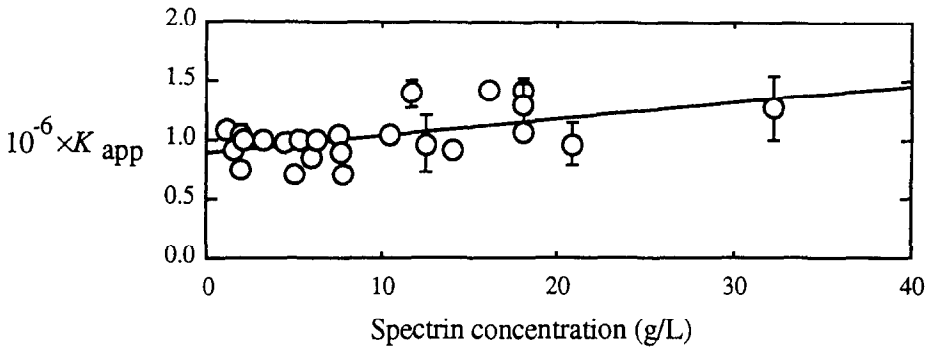

Fig. 3. Dependence of the apparent equilibrium constant for the association of spectrin heterodimers to form the tetramer on the total concentration of spectrin. The concentrations of heterodimer and tetramer at equilibrium at 30°C were determined by means of sedimentation velocity at 2–4°C after rapid chilling of the reaction mixtures. The slope of this plot is significantly different from zero ($P < 0.01$).

high concentrations, the proportion of oligomers larger than the tetramer was appreciable. At a total of 10 g/L spectrin, the dimer contributed ≈ 1 g/L and the tetramer ≈ 5 g/L; larger oligomers comprised the remaining 40%.

The apparent equilibrium constant for the dimer–tetramer step, $K_{2,4}^{app}$, could be calculated from the data of Fig. 2,

$$K_{2,4}^{app} = K_{2,4} \frac{(\gamma_{di})^2}{\gamma_{tet}} = C_{tet}/C_{di}^2 = M_1 c_{tet}/2c_{di}^2, \quad (10)$$

where $K_{2,4}$ represents the thermodynamic equilibrium constant, C represents molar concentrations, c the concentrations in the g/L scale, and M_1 the molar mass of the heterodimer. $K_{2,4}^{app}$ showed a very gradual increase with the total concentration of spectrin as shown in Fig. 3. Although the slope of this plot was shown by application of the t-test to be significantly different from zero, ($P < 0.01$), the increase in $K_{2,4}^{app}$ with concentration is very modest.

The distribution of c_1 as a function of total spectrin concentration was fitted with the SEK

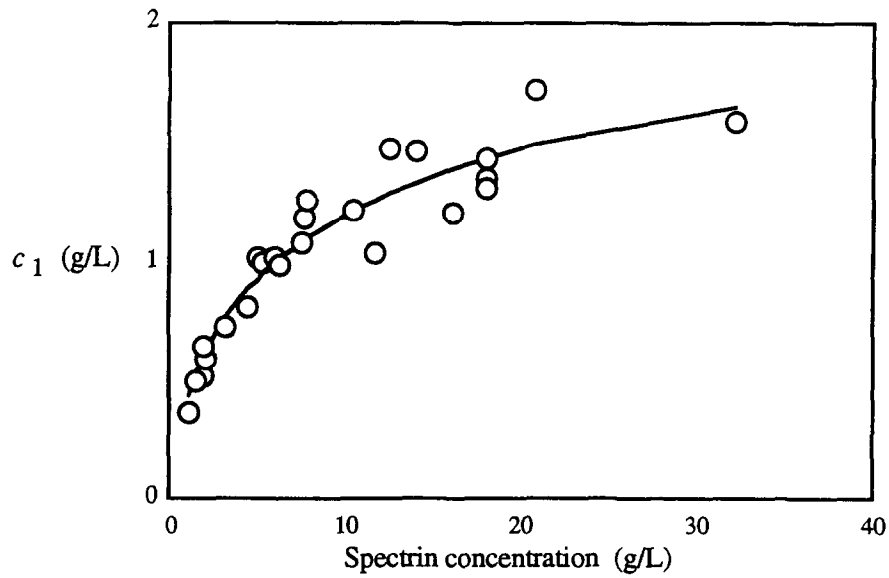

Fig. 4. Fitting of the concentration of the spectrin heterodimer (c_1) versus total spectrin concentration (c) to a cooperative isodesmic model. The runs test indicated that the residuals were randomly distributed around zero ($P > 0.05$).

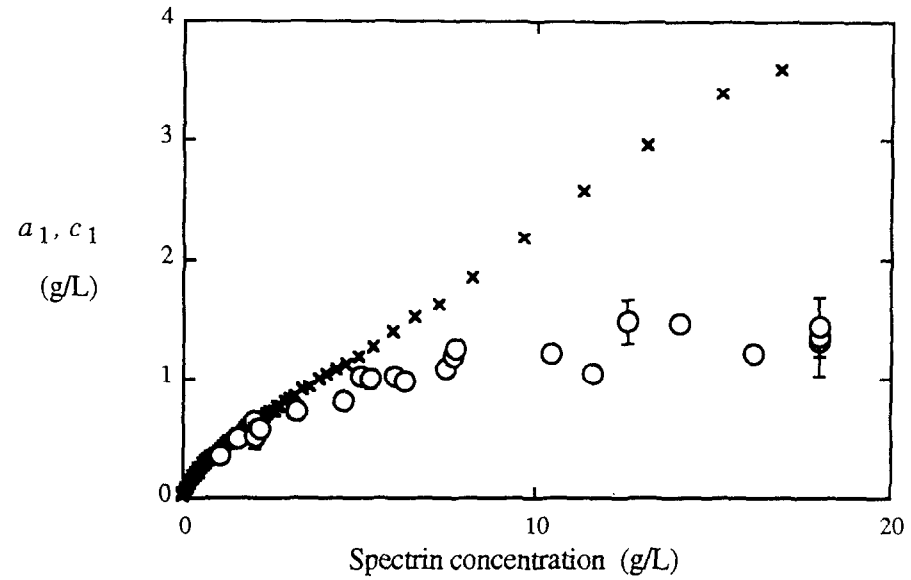

Fig. 5. Dependence on total spectrin concentration of the thermodynamic activity of the heterodimer (×) and the concentration of the heterodimer (○).

III model (Fig. 4). The fit returned values for $K_{2,4}$ of 0.8×10^6 L/mol and for K_{iso} of 0.2×10^6 L/mol. The goodness of fit was acceptable by the criterion of the runs test ($P > 0.05$).

From the previously published Omega function data [15] the thermodynamic activity of the protomer was calculated and is plotted against total spectrin concentration in Fig. 5. With a

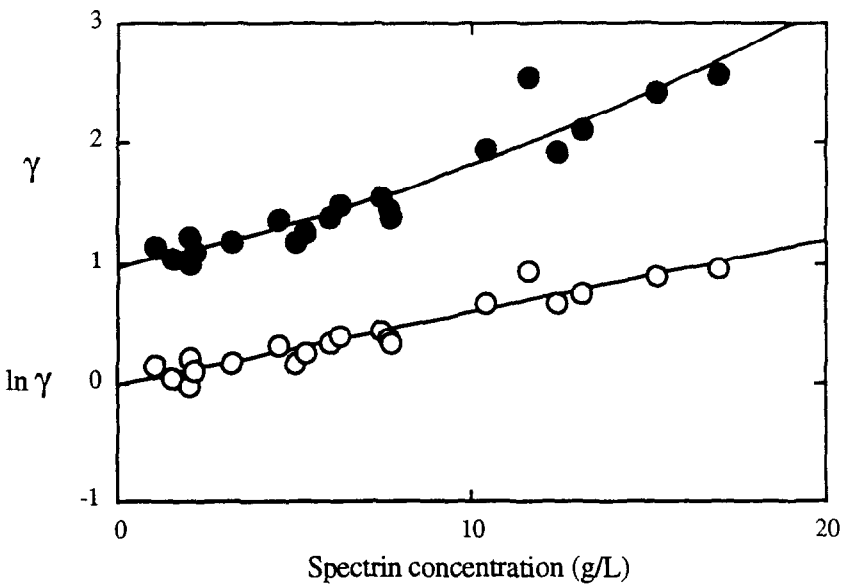

Fig. 6. Dependence of the activity coefficient of the spectrin heterodimer and its natural logarithm on total spectrin concentration. (●) Activity coefficient. An exponential fit ($r^2 = 0.915$) was significantly better than a linear fit ($r^2 = 0.894$); (○) the logarithm of the activity coefficient. The slope of the straight line of best fit gives an estimate of the quantity BM_1, from which B is estimated to be 1.2×10^{-7} L mol g^{-2}.

protomer molar mass of 480 kg/mol, the calculated activity distribution fell below that of the heterodimer concentration at total spectrin concentrations below 3 g/L; i.e. below 3 g/L total spectrin, the activity coefficient would be less than 1.0. However, with the value of 526 kg/mol, obtained from the cDNA sequences [2,3], the activity distribution merged with the concentration distribution (Fig. 5), so that the activity coefficient of the heterodimer approached 1.0 in the limit of infinite dilution.

Values of the thermodynamic activity of the heterodimer corresponding to the measured heterodimer concentrations were linearly interpolated from the activity data of Fig. 5. From the a_1 and c_1 data, the activity coefficient was calculated, and is plotted against total spectrin concentration in Fig. 6. A plot of γ_1 versus c showed detectable curvature, and was fitted well with a single exponential. A plot of ln γ_1 versus c was more closely linear, as shown by the lower curve in Fig. 6. Fitting the ln γ_1 versus c data with a quadratic returned a coefficient of the second order term of 10^{-5}, verifying the absence of significant curvature in this plot. The slope of the linear plot, equal to BM_1, allowed calculation of the second virial coefficient, B, as 1.2×10^{-7} L mol g^{-2}.

4. Discussion

While earlier work with spectrin [8,10,11] was based on a molar mass for the heterodimer of 480 kg/mol, estimated from SDS gel electrophoresis, recent sequence studies [2,3] suggest that a molar mass of 526 kg/mol is more appropriate. This revised estimate of the molar mass of spectrin is supported by the present study. The use of the lower value of 480 kg/mol results in a limiting activity coefficient for the heterodimer that is less than 1.0, while with the use of the revised value of 526 kg/mol, the activity coefficient of the heterodimer approaches 1.0 at infinite dilution (Fig. 6).

The unusual and variable shapes of the spectrin oligomers preclude modelling of the nonideality through simple geometric shapes [21]. However, with spectrin we are in the fortunate position of being able to determine independently the thermodynamic activity of the heterodimer from the Omega distribution [15,18,20] at equilibrium at 30°C, and the heterodimer concentration at equilibrium from the sedimentation velocity experiments, thanks to the very large enthalpy of activation of the association reaction, which allows the distribution of oligomers to be kinetically 'trapped' on chilling to 2–4°C [6]. Comparison of the concentration and thermodynamic activity of the spectrin heterodimer allows the explicit determination of the activity coefficient of the spectrin heterodimer as a function of total spectrin concentration and thus allows a quantitative test of the Adams–Fujita approximation in this case.

The linear relationship between ln γ_1 and total spectrin concentration implies that, at least over the accessible concentration range, a single virial coefficient is adequate for the description of the nonideal behaviour of the spectrin heterodimer, in spite of the considerable variation in solution composition over the measured concentration range. Furthermore, the relative insensitivity of $K_{2,4}^{app}$ to total spectrin concentration also implies that, as a first approximation, ln $\gamma_{tet} \approx 2$ ln γ_{di}. It has not yet proved possible to extend this analysis to higher oligomers of spectrin.

The use of the Adams–Fujita approximation appears then to be vindicated for this protein under these conditions, at least with respect to the Omega analysis, for which ln γ_1 can be formulated as a linear function of total spectrin concentration (Eq. (1) and Refs. [10,11,15]). It must be stressed, however, that this linear empirical dependence is likely to be the fortuitous result of the cancellation of several factors, and should not be taken as a general validation of the approximation. Firstly, there is no escape from the fact that the logarithm of the activity coefficient for the heterodimer must include higher order terms in concentration as the total protein concentration is increased. The estimated second virial coefficient implies an excluded volume of 63 mL/g of spectrin; at 20 g/L, the volume occupancy would be such that three-particle and higher-order interactions are unavoidable.

However, the addition of heterodimers to higher oligomers of spectrin appears to be made in a manner reminiscent of spokes attached to a central hub [12]. In this manner, the second virial coefficient for self-interaction of spectrin oligomers (in units of L mol g^{-2}) would be expected to decrease with increasing oligomer size. Indeed, even for the dimer and tetramer, admittedly rough calculations of the second virial coefficient of the dimer and tetramer [10] suggest a slight decrease in the value of B for the tetramer compared with that of the dimer.

Wills et al. [21] have calculated the activity coefficients of the monomer and oligomeric species of lysozyme as a function of total lysozyme concentration for a variety of self-association models and assuming spherical geometry for all species. The results indicated that the Adams–Fujita approximation was not valid for the lysozyme system beyond a limited concentration range. It has been pointed out [22] that for end-to-end association of long rod-like particles, or the self-association of highly charged molecules, the Adams–Fujita approximation is theoretically reasonable. However, spectrin oligomers cannot be adequately represented either as compact spherical particles, nor as end-to-end associations of rod-like molecules.

The value of the second virial coefficient for the spectrin dimer, 1.2×10^{-7} L mol g^{-2}, is in reasonable agreement with that obtained from fitting the Omega function or molecular weight distributions over the same concentration range (1.24×10^{-7} L mol g^{-2} [15]). With a value of the second virial coefficient of the spectrin heterodimer available experimentally, this quantity need no longer be used as an ill-defined additional parameter to be determined, allowing a more precise estimation of the remaining parameters of the reaction.

The experimental values of the second virial coefficient and $K_{2,4}$ were used to fit the SEK III model to the previously published Omega data over the concentration range 0–20 g/L [15]. In this case, only the equilibrium constant K_{iso} and the precise reference concentration were parameters whose values were to be estimated. This exercise returned a value of 0.2×10^{6} M^{-1} for K_{iso}, both for adequate description of the data over the whole concentration range, as well as in piecewise sections between 0–2, 2–6, and 4–16 g/L.

The availability of the *concentration* of the spectrin heterodimer as a function of total spectrin concentration further allows an assessment of the self-association reactions of this protein in the absence of nonideality. Fitting of this relationship with the cooperative isodesmic model (Eq. (9)) returned a value of the equilibrium constants $K_{2,4} = (0.8 \pm 0.08) \times 10^{6}$ M^{-1} and $K_{iso} = (0.2 \pm 0.02) \times 10^{6}$ M^{-1}, in gratifying agreement both with previously published values (1.18×10^{6} and 0.14×10^{6} M^{-1}, respectively [18]), based on a molar mass of 526 kg/mol, and with the values obtained above.

It is acknowledged that the cooperative isodesmic fit is not unique, and that other models may give an equally good fit within the precision of the measurements (e.g. dimer–tetramer–hexamer–octamer). Nevertheless, any realistic model for the self-association of spectrin must take into account the fact that the association pattern is apparently indefinite, with species up to the dodecamer clearly resolved [7,9]. Furthermore, the cooperative isodesmic model is also consistent with a plausible mechanism of association whereby the satisfied valencies within the heterodimer must be broken before association can proceed [10,11].

The self-association of spectrin has been studied in a number of different laboratories and with several different methods. While estimates of the values of successive equilibrium constants have not shown overwhelming agreement, nevertheless there has been general agreement that the mode of association is indefinite and proceeds via the addition of heterodimers to a preexisting oligomer. There has also been general agreement that the equilibrium constants for addition of dimers to oligomers larger than the tetramer are smaller than that for the formation of tetramers from two dimers.

Part of the discrepancy between estimates of equilibrium constants from different laboratories has arisen from the use of an inappropriate value of the molar mass of the heterodimer. While

estimates of the molar equilibrium constant from gel electrophoresis studies will only be in error proportional the error in the protomer molar mass, the error from sedimentation equilibrium increases non-linearly with the error in molar mass. The use of a molar mass of 526 kg/mol for the heterodimer, instead of 480 kg/mol, considerably lowers the estimates of the equilibrium constants, particularly K_{iso} [15]. To a large extent, the error involved in using the lower molecular weight is partially offset through increases in the estimated second virial coefficient; these parameters are highly correlated. However, it is unwise to use B as a fitting parameter whose value can be varied to mop up all sorts of deficiencies in the data elsewhere.

In the present study, we have available a value of the second virial coefficient (or at least, a measure of d ln γ/dc) that has been determined independently of the fitting process. We have also verified that 526 kg/mol is a more appropriate molar mass for spectrin. With these values, we now find that the self-association of spectrin is best predicted with a value of $\approx 1.0 \times 10^6$ for $K_{2,4}$, a value of 0.2×10^6 for K_{iso}, and a value of 1.2×10^{-7} L mol g^{-2} for B. These values now agree well (Table 1) with those obtained from fitting sedimentation equilibrium data with $M_1 =$ 526 kg/mol [15], and also agree satisfactorily with those determined from gel electrophoresis [9] and sedimentation velocity [6].

While the present study has shown that the Adams–Fujita approximation is reasonable for spectrin self-association over the concentration range studied, it must be stressed that this finding is not likely to be of general applicability. Association of compact spherical particles has been shown not to be described well by this approximation [14]. The applicability of the approximation in the case of spectrin may be due to the unusual geometry the spectrin oligomers.

Table 1
Published estimates of the equilibrium constants for spectrin self-association near pH 7.5 and 30°C

Method	$10^{-6} \times K_{2,4}$ (L/mol)	$10^{-6} \times K_{iso}$ (L/mol)	M_1 (kg/mol)	Ref.
sedimentation velocity	1.5	–	480	[6]
gel electrophoresis	0.4 [a]	< 0.4 [a]	480	[7]
	1.4	0.7	480	[23]
	1.1	0.05–0.1	480	[9]
sedimentation equilibrium	2.0	1.0	480	[10]
	2.0	0.28	480	[15]
	1.2	0.14	526	[15]
	1.0	0.4	480	[11]
	1.4	0.2	526	[24]
	1.0	0.2	526	this study
HPLC	1.2	–	526	[25]

[a] These values were estimated indirectly.

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