Journal of Supramolecular Structure 8:361–373 (1978)
Normal and Abnormal Red Cell Membranes 83–95

Physical-Chemical Studies of Spectrin

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In recent years considerable progress has been made in the understanding of the structure and function of the red blood cell membrane. The protein spectrin, of high molecular weight and propensity for self-association, appears to play a major role, in concert with actin, in maintaining the shape and integrity of the membrane. A study of the physical-chemical properties of spectrin, and its size, shape, self-association pattern, and its interaction with other components, leads to a plausible model for the way this protein performs its biological role. The evidence from the structure and interactions of spectrin suggests a structure which is relatively symmetrical yet highly expanded, and which allows extensive, two-dimensional network formation with actin. In these respects, the structure of spectrin is quite different from that of myosin, to which it has often been likened.

Key words: spectrin, actin, hydrodynamic properties, structure of spectrin

When Marchesi and his colleagues first isolated spectrin back in 1969 [1, 2], they recognised from the way the red cells fragmented after extraction of the protein that spectrin probably plays an important role in maintaining the integrity and shape of the cell. Sedimentation velocity studies on the isolated spectrin showed the presence of multiple boundaries [2, 3] and suggested that spectrin was capable of self-association. A study of the physical-chemical properties of spectrin, and its size, shape, self-association behaviour, and its interaction with other components of the membrane, is a prerequisite for an understanding of the functional role of spectrin.

Before an unambiguous analysis of the properties of spectrin could be undertaken, the isolation of a pure preparation was essential. In early studies with this protein, spectrin was isolated from other components in the water-soluble extract by the use of gel filtration on Sephadex G-200. Although this allows the removal of many of the contaminating proteins, chiefly hemoglobin, it is now clear that not all other proteins are removed by this means. Traces (up to 5%) of other components, chiefly component 5, are eluted with the spectrin in the void volume fraction from the Sephadex G-200 columns [4–6]. Furthermore, the spectrin prepared this way is itself heterogeneous, as was indicated in Marchesi's sedimentation analysis [2].

Received for publication March 5, 1978; accepted April 24, 1978.

0091-7419/78/0803-0361$02.60

Rechromatography of the void volume fraction from the G-200 columns on 4% agarose gels, a medium capable of resolving globular proteins up to 15×10^6 daltons, revealed a series of components occupying elution positions from the void volume through almost the entire fractionation range of the column [4]. Figure 1 shows the elution profile for a sample of the water-soluble proteins prepared at low temperature. The peaks labelled "tet" and "di" correspond to a tetramer of 960,000 daltons and a dimer of 480,000 daltons, respectively [4]. These oligomers do not appear to interconvert on prolonged storage in moderate salt concentrations and at temperatures below 30°C. In other words, the elution profile represents discrete, nonequilibrating species, rather than a reversible interaction pattern.

The void volume peak, V_0 (Fig 1), has been shown to contain small amounts of components 4.5 and 5, in addition to spectrin [7]. We observe a constant stoichiometry for the components of this fraction, one molecule of component 4.5 and two molecules of component 5 for each spectrin tetramer, over a large number of different preparations. This ratio is the same as that for the whole membrane, or for the pellet after Triton X-100 extraction. The constant stoichiometry, and our inability to separate the spectrin from the other components by physical means, leads us to believe that the void volume fraction is a well-defined complex, rather than a series of separate, large homotropic polymers of the individual components. Since this material also elutes in the void volume from 2% agarose columns (G.B. Ralston and J.C. Dunbar, unpublished results), it would appear to be of very large size and very highly agregated.

A small peak near 190 ml (Fig 1) comprises a number of nonspectrin proteins, chiefly components from the 3 region, and components 5 and 7. The elution position of these proteins suggests that they all exist in oligomeric forms, with molecular weights near one million.

The presence of these multiple components in the void volume fraction from Sephadex G-200 columns, and thus in the preparations of "spectrin," may in large part explain the variability in the physical properties of the protein isolated in this way

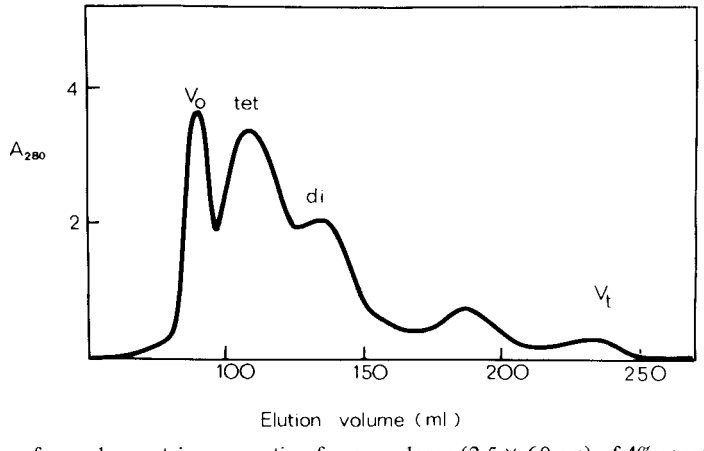

Fig 1. Elution of a crude spectrin preparation from a column (2.5 × 60 cm) of 4% agarose gel beads. The water-soluble proteins, prepared from human erythrocyte ghosts at 4°C, were eluted from the column with 0.1 M NaCl, 0.01 M sodium phosphate, 5 mM EDTA, and 5 mM mercaptoethanol (pH 7.5). V_0) the void volume peak; tet) the spectrin tetramer; di) the spectrin dimer; V_t) the total volume peak.

(compare Refs 2 and 3). In particular, the size of the very highly aggregated complex, and its variable proportion in different preparations [4] would be expected to influence markedly the properties of the preparation. In addition, further complications can arise from a temperature-dependent dissociation of the tetramer to the dimer and monomer [8], so that preparations made at 37°C will not be directly comparable with those made at low temperature.

In spite of the probable variation in the composition of the spectrin preparations from different laboratories, a number of consistent points emerge. Major components of low-temperature preparations are of approximately 9 S and 12 S [2, 4, 6, 8], corresponding to the dimer and tetramer, respectively [4], while 37°C preparations seem to comprise mainly the 9 S component, together with another of from 4 to 7 S, presumably the monomer [4, 6, 8].

THE EFFECTS OF SALT

A number of early reports claimed that spectrin is aggregated in moderate concentrations of salt: Clarke [3] reported that sodium chloride concentrations above 10 mM caused aggregation. These conclusions have generally been based on the increase in sedimentation coefficient that is seen when the salt concentration is raised from very low levels, and they are questionable on two counts. First, the primary charge effect would be expected to operate at very low salt concentrations, and this effect may be large. Second, the preparations used in some of the earlier studies were almost certainly heterogeneous, such that a single peak in the sedimentation pattern at low salt concentrations may not represent only a single component. The appearance of several, faster boundaries on addition of salt may thus arise from both the swamping of the charge effect and the improved resolution of the several species already present.

The above interpretation has been confirmed in studies with isolated oligomers of spectrin. The change in sedimentation coefficient of both the tetramer and the dimer, on increasing the salt concentration, follows very closely the relationship derived by Pedersen [9] for the primary charge effect: The sedimentation coefficient decreases linearly with the inverse of the solution conductance. In addition, the effect is linearly dependent on the protein concentration, again as required by the primary charge effect. The dependence of sedimentation coefficient on the protein concentration, in both low and high salt concentrations (Fig 2), is quite different from that to be expected from aggregation.

Support for this interpretation has come from gel filtration experiments, which have shown that as the salt concentration is raised from very low values up towards 0.1 M, both the tetramer and dimer elute at increasingly greater elution volumes. This behaviour is quite the reverse of aggregation, and again is better explained in terms of the operation of charge exclusion phenomena in the gel matrix [10]. The tetramer and dimer components in total water-soluble protein extracts behave identically with the isolated oligomers, eliminating the possibility of changes having taken place in the proteins during isolation [10].

Although salt does not appear to induce the aggregation of spectrin, there is increasing evidence for salt-dependent conformation changes in spectrin. The limiting sedimentation coefficient for spectrin in low-ionic-strength solution is not identical with that in the presence of 0.1 M NaCl (G.B. Ralston, unpublished results). This effect is

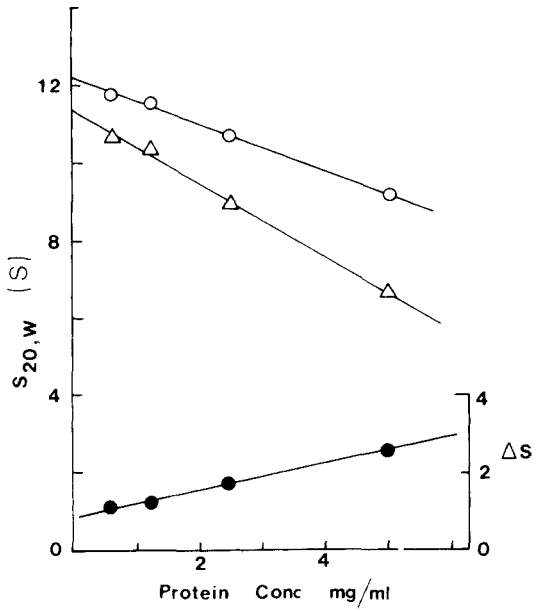

Fig 2. Effect of salt and protein concentration on the sedimentation coefficient of the spectrin tetramer. ○—○) 0.1 M NaCl, 0.01 M sodium phosphate, pH 7.5; △—△) 5 mM sodium phosphate, pH 7.5; •—•) the difference in sedimentation coefficient, Δs, (right-hand ordinate) as a function of protein concentration.

demonstrated for the tetramer in Figure 2. Similar effects have been seen with the isolated dimer (J.C. Dunbar and G.B. Ralston, unpublished results). Bennett and Branton [11], in density gradient sedimentation velocity studies, find a lower sedimentation coefficient for the spectrin dimer in 0.3 mM phosphate buffer (7S) than after the addition of 20 mM KCl (8 S). These data are supported by the results of analytical ultracentrifugation studies by Shotton and Branton (quoted in Ref 11).

THE TETRAMER OF SPECTRIN

The tetramer appears to be the largest homopolymeric aggregate of spectrin to be isolated from the red cell membrane. The tetramer, purified by gel filtration on 4% agarose gels, appears to be homogeneous by the criteria of gel filtration, sedimentation velocity, acrylamide gradient electrophoresis, and sedimentation equilibrium [12]. Electrophoresis in dodecyl sulphate yields only the two spectrin components of 220,000 and 250,000 daltons, respectively, in equal amounts (Fig 3).

This purified tetramer has a molecular weight of 960,000 determined both by sedimentation equilibrium and sedimentation/diffusion [4, 12].

The sedimentation coefficient of 12.2 S for this material (Table I) is a great deal lower than the value of approximately 35 S expected for a compact globular protein of this molecular weight, and it corresponds to a frictional ratio of 2.8. Similarly, the elution of the tetramer from agarose columns is considerably earlier than expected.

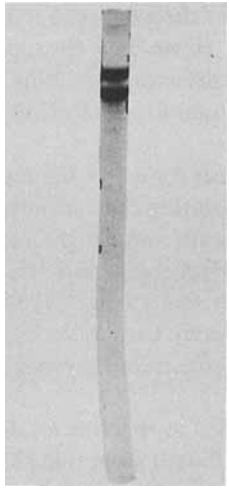

Fig 3. Electrophoresis of the purified tetramer of spectrin. Electrophoresis was performed in 5.6% acrylamide gels containing 1% SDS, essentially by the method of Fairbanks, Steck, and Wallach [23].

TABLE I. Hydrodynamic Properties of Spectrin*

Property	Tetramer (a)	Dimer (b)	Monomer (c)
MW	960,000 [4, 12]	480,000 [4] 430,000 [6]	225,000 [6]
$s^{\circ}_{20,w}$	12.2 S [12] 12.6 S [6]	9.7 S [6] 9.3 S	4.4 S [6] ~ 7 S [4]
$D^{\circ}_{20,w}$ ($cm^2 sec^{-1}$)	1.1×10^{-7} [12]	1.75×10^{-7}	–
f/f_0	2.8	2.1 [6] 2.3	2.9 [6]
$K_S/[\eta]$	1.33 [12]	1.15	–
a/b (0.5 g/g hydration)	–	5 [6]	13 [6]
a/b (from $K_S/[\eta]$)	4	7	–

*Values not referenced are unpublished results of J.C. Dunbar and G.B. Ralston

The anomalous hydrodynamic properties have traditionally been explained by proposing a long rod shape for spectrin [3]. A model of this nature has also appeared attractive in the light of supposed similarities between myosin and spectrin [13] and of possible contractile roles for spectrin [14].

However, because asymmetry and expansion can both give rise to an increase in the frictional ratio, it is notoriously difficult to determine both the size and shape of a molecule from hydrodynamic properties alone. If a shape is known, the dimensions of this shape can be determined from the frictional coefficient. Usually, a compact ellipsoid of rotation is chosen to represent the shape of the molecule, partly because of the daunting complexity of the calculation for other shapes. Moderate values of the frictional ratio are taken to indicate a globular particle with a degree of "hydration" or departure from

truly spherical. Very large values of the axial ratio in this treatment generally are taken to indicate a long, rod-like molecule. However, if the molecule is not compact but is highly expanded, this treatment is not satisfactory, resulting in an anomalous, high value of the axial ratio. By these criteria, for example, a spherically symmetrical random coil appears to have a high axial ratio.

There is some theoretical basis for use of the ratio $K_S/[\eta]$, the ratio of the concentration-dependence of the sedimentation coefficient to the intrinsic viscosity, as a measure of symmetry of the molecule. Creeth and Knight [15] have tested this relationship empirically and found that for the spherical particles, ranging from denatured proteins in guanidine hydrochloride to latex spheres, $K_S/[\eta]$ was close to 1.6, while for rod-like particles the value of $K_S/[\eta]$ decreased with increasing axial ratio, approaching zero for very long rods. For a number of proteins of known shape, $K_S/[\eta]$ appeared to be a function of axial ratio [15].

When this treatment is applied to spectrin, a value of 1.33 (Table I) was found for the tetramer [12], a value very different from that of 0.2 for myosin; it indicates a shape no more asymmetric than serum albumin or glutamate dehydrogenase, with a probable axial ratio no more than about 4, estimated by use of the empirical relationship quoted by Creeth and Knight.

It is therefore unlikely that the spectrin tetramer can be represented adequately by the model of a long rod. The unusual hydrodynamic properties may then be due more to a highly expanded but basically symmetrical structure than a grossly elongated one [12]. On the basis of a symmetrical model, hydrodynamic data from viscosity, diffusion, and gel filtration all suggest a particle radius of about 200 Å [12].

THE DIMER

A number of laboratories have used the more rapid method of preparing spectrin by means of extraction at low ionic strength at 37°C. Although this method removes most of the spectrin within 15 min rather than many hours, the spectrin so isolated is in a dimer form, of molecular weight about 480,000 [6, 8].

The dimer produced in this way appears to be very similar to that isolated from low-temperature preparations, but sufficient study has not yet been made to determine whether the two forms are identical. Gratzer and Beaven [6] measured the sedimentation coefficient of the dimer of human spectrin as 9.7 S, and in our laboratory (J.C. Dunbar, unpublished results) a value of 9.3 S has been obtained, both for low-temperature and 37°C preparations. Clarke [3] reported a limiting sedimentation coefficient for her spectrin preparations in the range of 8–10 S, and although this preparation may have been contaminated with higher aggregates of spectrin, it would appear that the dimer was a prominent component.

Gratzer and Beaven [6] also reported the presence of monomeric spectrin in their 37° preparations. This species appeared to have a sedimentation coefficient of 4.4 S, and its proportion increased with time of storage and on freezing and thawing the ghosts. The monomer and dimer were stable aggregates, and like the tetramer and dimer system from low temperature preparations they did not appear to be in rapid equilibrium [6].

The sedimentation coefficient of the dimer corresponds to a frictional ratio of 2.1 [6]. Allowing for 0.5 g/g hydration Gratzer and Beaven [6] calculate an axial ratio of about 5 for the dimer, and about 13 for the monomer (Table I). However, they admit

that the prolate ellipsoid is not a very realistic model and suggest that a flexible coil may be a better approximation. Certainly, the physical properties of the dimer are again quite different from those of myosin.

Recently we have subjected the dimer of spectrin to the Creeth-Knight analysis for symmetry [15]. With the dimer from 37°C preparations, the value of 1.15 for $K_s/[\eta]$ indicates a more asymmetric structure for the dimer than was seen for the tetramer, but still not a grossly asymmetric one. A value of 7.2 can be estimated for an axial ratio of the dimer, if a prolate ellipsoid is a valid model, using the empirical equation given by Creeth and Knight [15]. This value is in reasonable agreement with that of 5 estimated by Gratzer and Beaven [6] (Table I).

Results somewhat different from these have been obtained by Schechter et al [16] with spectrin in the detergent, deoxycholate. These workers have found that in this solvent, the dimer and monomer of spectrin have very similar sedimentation coefficients: between 5.5 and 6 S. These data are consistent with dimerisation as an end-to-end association of monomers, since the frictional coefficients of rod-like molecules of constant diameter increase almost linearly with the length of the rod. Although this interpretation is not in dispute, it is very unlikely that the protein in deoxycholate is similar in overall conformation to that of the protein in the absence of detergents. In fact, Schechter et al [16] report that the aqueous extract was heterogeneous, but with an average sedimentation coefficient near 8 S. Addition of deoxycholate resulted in dissociation and a decrease in sedimentation coefficient to about 6 S. Furthermore, addition of deoxycholate caused a decrease in the mean residue ellipticity of about 15%. Although Schechter et al [16] suggest that this is not a major conformational change, it is nevertheless a significant one that may be expected to alter the hydrodynamic properties of the protein. The changes, both in optical properties and in the sedimentation coefficient, indicate that the conformation of spectrin in the presence of deoxycholate is not the same as that in its absence.

SECONDARY STRUCTURE

The chiroptical properties of spectrin have been examined in a number of different studies, but again there has been a marked variation in the results from different laboratories, as indicated by the data of Table II. Most experiments have been done with the void volume peak from Sephadex G-200 chromatography, a preparation already shown to be heterogeneous. The results reported by Brandts et al [17] were made on the unfractionated water-soluble extract. It is not possible unequivocally to determine which of the oligomers predominated in the preparations used for the data of Table II.

Although methods such as sedimentation velocity, in which a partial separation of components occurs, can still give reasonable estimates of the properties of noninteracting components in a mixture, the optical properties of components in such a mixture can not easily be extracted. It is likely that the data of Table II underestimate the helical content of spectrin. Schechter et al [16] report that a crude extract of spectrin in deoxycholate had a mean residue ellipticity of $-19{,}000$ deg · cm^2 · dmole^{-1}, while after purification the same sample in the same solvent showed an increase in the magnitude of the trough to about $-23{,}000$ deg · cm^2 · dmole^{-1}, a change of approximately 20%. Since the presence of deoxycholate appeared to reduce the magnitude of the circular dichroism (CD) trough [16], it is likely that the magnitude of this trough is even greater for aqueous solutions.

Measurements on the purified tetramer in our laboratory [12] were originally made on rather dilute solutions in the visible region, where the optical rotation is very small. These studies indicated about 45% α-helix. We have since repeated these measurements on very carefully prepared samples, over a range of protein concentrations. These results (Table III) indicate an α-helix content close to 60% for both the tetramer and the dimer, based on the Moffitt-Yang b_0 parameter. The fit of the data to the Moffitt-Yang plots is very good, with correlation coefficients in the region of 0.9995. The specific rotation at the mercury 578 line is very low, about −6°. Within the limits of accuracy of these measurements, we are unable to detect a difference in the optical rotatory dispersion of the tetramer and dimer.

However, studies on the purified proteins in the far UV circular dichroism spectrum indicate an even greater apparent helix content, and significant differences between the tetramer and dimer (Fig 4). The molar ellipticity of the tetramer (−28,600 deg • cm^2 • $dmole^{-1}$) is quite a bit greater in magnitude than any of the values listed in Table II.

To some extent, this difference presumably reflects the greater purity of the sample in the latter case, but there may well be other factors involved, such as differences between the tetramer and dimer, temperature and solvent effects on the proteins, and perhaps conformational changes.

TABLE II. Chiroptical Properties of Spectrin

a. Circular dichroism	$-[\Theta]_{222} \times 10^{-4}$ deg • cm^2 • $dmole^{-1}$	% helix[a]
Pinder et al [5]	1.9	54
Gratzer and Beaven [6]	1.6–1.8	45–50
Schechter et al [16]	2.2	60
Vincentelli et al [18]	–	61
Brandts et al [17]	2.1	63
b. Optical rotatory dispersion	$-[m']_{233} \times 10^{-3}$ deg • cm^2 • $dmole^{-1}$	% helix[b]
Reynolds and Trayer [27]	3	25
Brandon [19]	5.95	48

[a]Based on $[\Theta]^H_{222} = -39{,}000$ [21].
[b]Based on $[m']^H_{233} = -14{,}600$ for polylysine [21].

TABLE III. Chiroptical Properties of Spectrin Oligomers*

	Dimer (a)	Tetramer (b)
$[\alpha]_{578}$	−6 ± 2°	−6 ± 2°
b_0	−382°	−385°
% helix (b_0)	60 ± 5	60 ± 5
$[m']_{233} \times 10^{-3}$	−9.62°	–
% helix (ORD)	66	–
$[\Theta]_{222} \times 10^{-3}$	−26.7°	−28.6°
% helix (CD)	68	73

*All measurements made in 0.1 M NaCl, 0.01 M sodium phosphate (pH 7.5), 25–27°C.

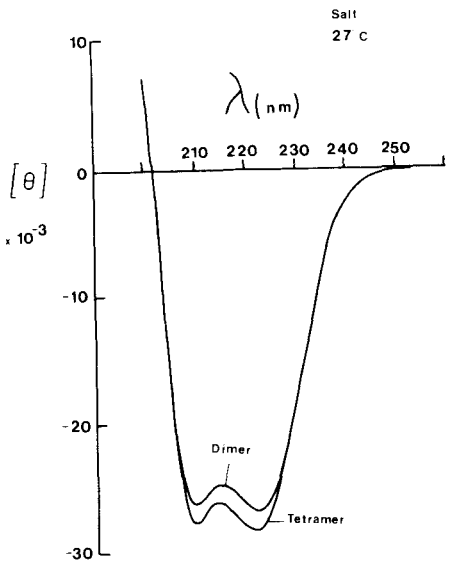

Fig 4. Circular dichroic spectra for the tetramer and dimer of spectrin in 0.1 M NaCl, 0.01 M sodium phosphate, pH 7.5. Spectra were recorded in a Cary 60.01 polarimeter, with cells of 1 mm pathlength. Protein concentrations: tetramer, 0.42 mg/ml; dimer, 0.37 mg/ml.

Chen, Yang, and Martinez [20] have developed a method for the calculation of helical and β-structure content in proteins from the circular dichroism spectrum. This method is based on the average properties of structured regions in a number of different proteins, whose structures are known from crystallographic studies. Structure distributions determined in this way are thought to be more meaningful for globular proteins than those determined from the use of long helical models such as polyamino acids. However, when the apparent helical content is as high as it appears to be in spectrin, there are good grounds for retaining the use of polyamino acids as models. This procedure has been adopted in the present case, with the use of the summarized values for polyamino acids listed by Jirgensons [21], and yields an α-helical content of 73% for the tetramer and 68% for the dimer, both in 0.1 M NaCl at 27°C.

The CD spectrum of spectrin, in an undefined oligomeric state, has been analysed in detail by Vincentelli, Devogel, and Leonis [18] with the use of the method of Chen, Yang, and Martinez [20]. Their analyses suggest 61% helix and 20% β-structure. The helical content is lost rapidly and almost completely on heating to 100°C, but the β-structure appears to be surprisingly stable. Brandts et al [17] also find a residual structure, resistant to heating. However, both of these studies were performed on unfractionated mixtures, so the conclusions must be tempered with caution.

CONFORMATION CHANGES

The chiroptical properties of spectrin are markedly dependent on salt concentration and temperature. In the presence of 0.1 M NaCl and at temperatures between 15° and 40°C, the optical rotating dispersion (ORD) and CD spectra of spectrin are relatively

constant, the magnitude of the rotation or ellipticity decreasing by only a small amount with increasing temperature, as shown for the specific rotation in Figure 5. At higher temperatures, thermal denaturation appears to occur with a midpoint near 50°C. This transition has been observed calorimetrically and by circular dichroism by Brandts et al [17] using unfractionated spectrin. At even higher temperatures, there appears to be a further structure change, centered near 60°C (Fig 5), but this change does not appear to correspond to changes in enthalpy. Aggregation phenomena, as well as further unfolding, may contribute to this high temperature transition.

In very low salt concentration, the temperature-dependent changes are more interesting. At 20°C, the lower salt concentration seems to result in a small conformational change, reflected in about a 5% decrease in the magnitude of the ellipticity. This optical change seems to correspond to the change in sedimentation coefficient as the salt concentration is lowered, and may reflect a slight expansion of the molecule, promoted by the increased electrostatic free energy. As the temperature is raised, however, further, more substantial changes take place. Instead of the slight decrease in laevorotation with increasing temperature seen in 0.1 M NaCl, laevorotation in low salt concentrations steadily increases (Fig 5). By 37°C, the optical rotation has already undergone a substantial change.

These temperature-dependent changes in low salt concentrations parallel the dissociation from the tetramer to the dimer. Possibly, the change in optical rotation reflects a change in conformation that is required to enable the dissociation step. The presence of salt prevents this change, stabilizing the tetramer conformation, and thus inhibiting dissociation.

What is not clear at present is why the dissociation is apparently irreversible. Gratzer and Beaven [6] have also noted that dissociation of the dimer to monomers is not a reversible process: Reversible unfolding in guanidine hydrochloride regenerates only dimers

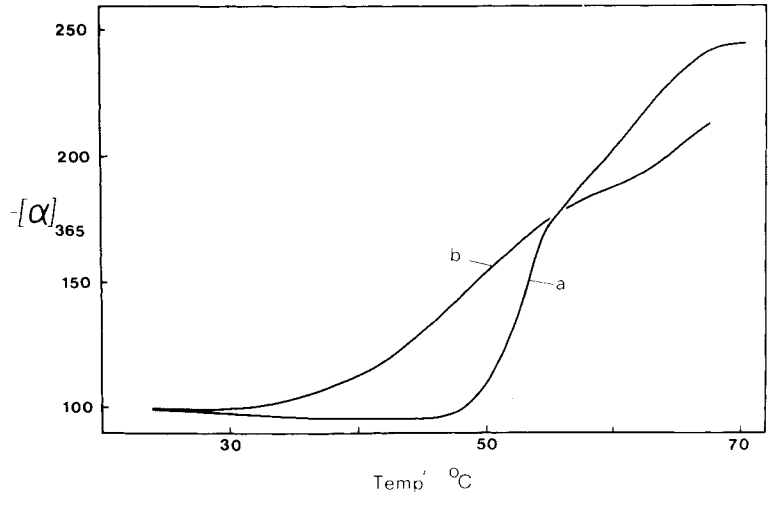

Fig 5. Temperature dependence of specific rotation for the spectrin tetramer. Measurements were made on a Perkin-Elmer polarimeter, model 241, with cells of pathlength of 10 cm. a) Tetramer in 0.1 M NaCl, 0.01 M sodium phosphate, pH 7.5; b) Tetramer in 5 mM sodium phosphate, pH 7.5. The temperature plotted is that of the circulating water bath and has not been corrected for the temperature differential between the interior of the cell and the bath.

from dimers, and only monomers from the monomers. It is possible that irreversible changes in covalent bonds, either peptide bonds, or phosphoryl groups, may accompany the dissociation process, but evidence is lacking at present.

A MODEL FOR SPECTRIN

On the basis of the hydrodynamic properties, a number of models for the structure of spectrin can be eliminated. The compact globular structure is eliminated by the high frictional ratio, and the long-rod model is inconsistent with the value of $K_s/[\eta]$. Any model that is proposed must also be consistent with increasing symmetry as the monomer units associate to form dimers and tetramers. A side-by-side association of rods with axial ratios of about 13 may satisfy the data, but a structure like this is not consistent with other requirements.

The tetramer is the largest known homotropic association state of spectrin. Higher association states seem to comprise actin in the ratio of one actin molecule for each two chains of spectrin. This stoichiometry suggests a continuous two-dimensional network of spectrin and actin, in which each actin molecule links two adjacent spectrin monomers. The spectrin monomers are envisaged as being organized into tetramer units. In order to satisfy the spectrin-actin stoichiometry, and to allow two-dimensional continuity, a cross structure is proposed, as shown in Figure 6. A structure like this has two-dimensional symmetry and would be expected to behave quite differently than a rod. The dimer and monomer dissociation states, however, would show increasing asymmetry. The radius of the cross would be expected to be near 200 Å, the Stokes radius of the tetramer. Given, then, the area of a single tetramer "cell" as 1.6×10^5 Å^2 and the number of copies of the

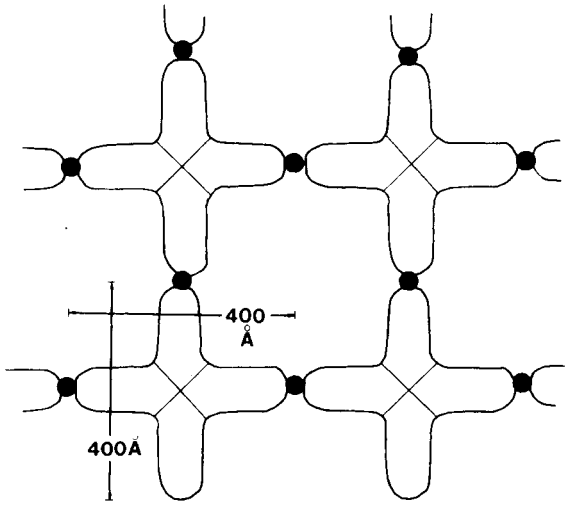

Fig 6. A model for the spectrin-actin network. The spectrin tetramer is envisaged as being composed of monomers arranged such that one actin molecule can bridge two adjacent monomer "arms." The "unit cell" of the repeating network is approximately 400 Å × 400 Å, based on the Stokes radius of the tetramer.

tetramer per erthyrocyte as approximately 8.5×10^4 [23], the total area that the spectrin network can cover is 1.36×10^{10} Å^2, in excellent agreement with estimates of the surface area of the ghost [24].

This model has certain points of similarity with that proposed by Kirkpatrick [28], but differs in the important respects of the shape of spectrin (allowing better membrane coverage), and the interaction with actin (allowing two-dimensional network formation).

As a further check on the validity of the model, it is possible to calculate the sedimentation coefficient of a structure that can be approximated by an assemblage of spherical subunits [25]. The cross structure can be approximated by an arrangement of nine spherical subunits, each of mass 100,000 daltons, and of radius 60 Å. The diameter of this cross will be 600 Å, and the total mass 900,000 daltons. The dimer can be approximated by two adjacent arms, and the monomer by a single linear arm. For these structures, the sedimentation coefficents are shown in Figure 7, together with the experimental values. Although the dimensions and arrangements of this structure are rather arbitrary and crude, nevertheless such a structure is reasonably consistent with the experimental data.

The proposed structure for the dimer is also consistent with the "C" structures seen in negative contrast preparations in the electron microscope [22] and with the three-domain structures, approximately 500 Å long, seen in high-resolution low-angle shadowing experiments (D. Branton and D. Shotton, personal communication).

Very recently, Hainfeld and Steck [26] have shown scanning electron micrographs of a "sub-membrane reticulum," appearing as a continuous web over the cytoplasmic face of the membrane. The size and arrangement of the components of this reticulum bear a surprising and gratifying resemblance to the sort of structure just proposed for spectrin. This is a very welcome convergence from two completely different experimental approaches.

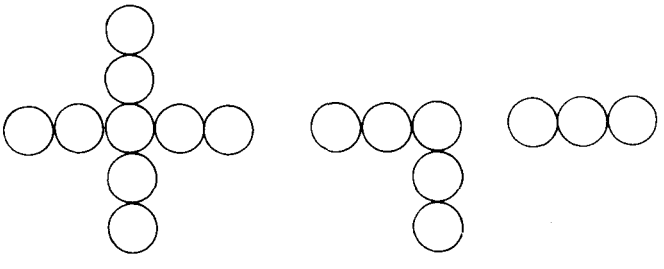

Fig 7. Model representations of the spectrin oligomers. The tetramer, dimer, and monomer were represented by arrangements of spherical subunits, for the purposes of calculation of approximate sedimentation coefficients. Each spherical subunit has a radius of 60 Å and a mass of 100,000 daltons. For each oligomer, the experimental and calculated sedimentation coefficients are compared.

ACKNOWLEDGMENTS

The original research described in this review was supported by grants from the Australian Research Grants Committee and the University of Sydney Consolidated Medical Research Fund.

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