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Multiple Functions of Spectrin: Convergent Effects

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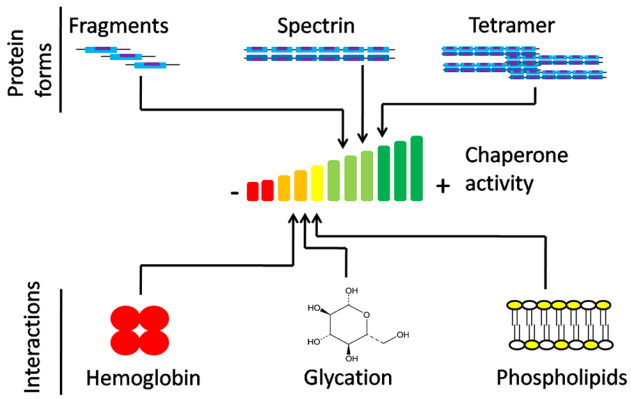
Abstract

Spectrin is a multifunctional, multi-domain protein most well known in the membrane skeleton of mature human erythrocytes. Here we review the literature on the crosstalk of the chaperone activity of spectrin with its other functionalities. We hypothesize that the chaperone activity is derived from the surface exposed hydrophobic patches present in individual "spectrin-repeat" domains and show a competition between the membrane phospholipid binding functionality and chaperone activity of spectrin. Moreover, we show that post-translational modifications such as glycation which shield these surface exposed hydrophobic patches, reduce the chaperone function. On the other hand, oligomerization which is linked to increase of hydrophobicity is seen to increase it. We note that spectrin seems to prefer haemoglobin as its chaperone client, binding with it preferentially over other denatured proteins. Spectrin is also known to interact with unstable haemoglobin variants with a higher affinity than in the case of normal haemoglobin. We propose that chaperone activity of spectrin could be important in the cellular biochemistry of haemoglobin, particularly in the context of diseases.

Graphic Abstract



Keywords Spectrin · Chaperone · Haemoglobin · ANS

Abbreviations

PRODAN	1-[6-(Dimethylamino)-2-naphthalenyl]-1-propanone
ANS	8-Anilino-1-naphthalene sulfonate

Introduction

Spectrin is a cytoskeletal protein which is found in the membrane skeleton of all metazoan cells examined till now. It is a hetero-dimer composed of α and β subunits with molecular masses of 280kDa and 246kDa, respectively. It is most well characterized in mammalian erythrocytes and neuronal cells. Spectrin is a multi-domain protein and is composed mostly of tandemly repeated homologous α-helical motifs called the "spectrin repeat" domains (Goodman et al. 1988).

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In mature human red blood cells (RBCs), the cell membrane is stabilized by a network of spectrin oligomers cross-linked with short actin filaments which are bound to the membrane and make the membrane skeleton (Chakrabarti et al. 2006). Erythrocyte spectrin is encoded by two genes, SPTA1 (UniProt P02549) for α-I spectrin and SPTB (UniProt P11277) for β-I spectrin. Spectrin is present *in-vivo* as a tetramer, which is formed by the "head-to-head" association of two hetero-dimers (Yoshino and Marchesi 1984).

Both spectrin subunits consist mostly of tandem repeats of "spectrin repeat domains", each of which is approximately 106 residues long, with about 30% identity between themselves, and they are aligned in an anti-parallel side to side orientation to give a flexible rod-shaped molecule (Speicher and Marchesi 1984; Yan et al. 1993). The individual spectrin repeat domains are folded into a coiled coil structure which consists of three anti-parallel left handed helices which are connected through a helical linker. The majority, up to 90% of the intact spectrin molecule is made up of these 3-helix repeat (usually 20 in α- and 17 in β-chains) (Pascual et al. 1996; DeSilva et al. 1997).

The available literature on the X-ray structure of "spectrin repeat" motif shows that the 3-helix bundles are held together by hydrophobic interactions between the interior hydrophobic residues and electrostatic interactions between the charged surfaces (Sali et al. 1988). The last helix of one domain forms a continuous helix with the first helix of the second repeat domain. The co-operativity of these spectrin domains is attributed to the presence of linking helical property which is thought to be the source of its ability to expand and contract with mechanical stress.

Non-erythroid spectrin or brain spectrin, also called fodrin is found in neurons and is very similar in structure but has less described function than erythroid spectrin. The two spectrin isotypes are very different in terms of their structure and stability (Bennet et al. 1982; Patra et al. 2015a, b). Brain spectrin is encoded by the genes SPTAN1 (UniProt Q13813) for α-II spectrin which has many isoforms and SPTBN1 (UniProt Q01082), SPTBN2 (UniProt O15020), SPTBN4 (UniProt Q9H254) and SPTBN5 (UniProt Q9NRC6) for β-II-V spectrin which have their respective isoforms.

Non-erythroid spectrin exists as a tetramer *in-vivo* and this tetramer is held together about 15 times stronger than the erythroid spectrin tetramer (Li and Fung 2009). The tetramerization site or the self-associating domain of erythroid spectrin is shown to be different from that of non-erythroid spectrin (Park et al. 2003; Mehboob et al. 2010). Moreover, non-erythroid spectrin is more rigid and thermally stable than erythroid spectrin and interacts more strongly with anionic lipid membranes.

Available literature shows that spectrin is capable of oligomerization by sequential addition of dimers; this process is more or less 'indefinite'; i.e. the association process appears to be unlimited and spectrin can form n-order oligomers. Oligomerization involves opening up the lateral interactions between the helices of each individual 3-helix bundle and the formation of a new 3-helix bundle which has two helices from one peptide chain and one from the other (Henniker and Ralston 1996). Spectrin can also form oligomers by another mechanism where the spectrins are cross-linked by protein 4.1 and actin (Beaven et al. 1985). Electron microscopic, non-denaturing gel electrophoresis, and sedimentation equilibrium studies have been used to report oligomers of a higher order than the tetramer (Morrow and Marchesi 1981; Liu et al. 1984).

Evidence from spectroscopic and physical studies show that spectrin is a highly dynamic protein with multiple types of internal segmental motions which are the source of its unique mobility and flexibility necessary for its function.

The Functions and Properties of Spectrin

Classical knowledge on spectrin function points to the activity of spectrin as a mechanical component in the maintenance of integrity and elasticity of the membrane skeleton (Bennett et al. 1982; Bennett 1990). Recently more literature has become available to show that spectrin has functionality as a component of signalling platforms as well (Fletcher et al. 2015). The binding of spectrin to phospholipids is also well characterized (Grzybek et al. 2006) and our group has demonstrated the chaperone like properties of spectrin and its interactions with heme proteins (Basu and Chakrabarti 2015; Bose and Chakrabarti 2019a, b).

As such available literature suggests that spectrin is a multi-faceted protein with a host of functions associated with it. Here we have listed some of the functions and properties of spectrin:

- Spectrin can act as a chaperone (Bhattacharyya et al. 2004; Basu and Chakrabarti 2015; Bose and Chakrabarti 2019a, b).
- Spectrin dimer is the major functional component responsible for maintaining cell membrane elasticity and strength in erythrocytes (Kirkpatrick 1976; Goodman and Shiffer 1983; Bennett 1985; Mitra et al. 2015a, b; Patra et al. 2015a, b).
- Spectrin interacts with membrane phospholipids directly (Ray and Chakrabarti 2003, An et al. 2004, Ray and Chakrabarti 2004, Chakrabarti and Patra 2015, Mitra et al. 2015a, b, Giri et al. 2017, Sarkar et al. 2018, Sarkar et al. 2019).
- Spectrin can specifically bind to hydrophobic ligands (Chakrabarti 1996; Majee et al. 1999; Haque et al. 2000; Mondal and Chakrabarti 2002; Patra, Mitra et al. 2014; Patra et al. 2015a, b).

- Spectrin can bind hemoglobin and other heme containing proteins (Chakrabarti et al. 2001, Datta et al. 2003, Datta et al. 2006, Das et al. 2015, Mishra et al. 2017).

Thus, it becomes important to address this multifunctional nature of spectrin. Do these functions all arise from distinct features in the protein? Or are they a consequence of some singular deeper character? Moreover, how do these functions fit in with the general role of the protein? Is there some convergent effect?

In the present review, we discuss the multi-functionality of spectrin and explore how these separate functions may be related to each other, specifically as regards to its chaperone function.

Multifunctional chaperones are commonly found where their chaperone function is dependent on or modulated by their other functionalities. For example, in case of PDI, its chaperone activity is dependent on its disulfide isomerase enzyme activity (Puig and Gilbert 1994; Puig et al. 1994; Noiva 1999). Similarly B23 is a multifunctional chaperone, that has protein interactive roles which depends on its chaperone activity (Lindstrom 2011).

In this review we relate how the structure of spectrin, its ability to interact with various partners and its chaperone activity are interlinked.

Chaperone Activity of Spectrin

Our group has previously reported extensively on the chaperone activity of spectrin. We have shown that spectrin is able to prevent aggregation of ADH and insulin (Bose et al. 2017). It is also able to help in refolding of enzyme α-glucosidase (Bose and Chakrabarti 2019a, b). Moreover it also interacts with denatured heme proteins such as HRP and α-globin as a chaperone (Chakrabarti et al. 2001; Basu and Chakrabarti 2015). We have also been able to strongly link the chaperone activity of spectrin with its ability to bind hydrophobic ligands such as ANS and PRODAN (Bhattacharyya et al. 2004; Bose and Chakrabarti 2019a, b). In this review, we have tried to connect the many other functions of spectrin with its chaperone activity and elucidate how they may all converge functionally.

Structure, Oligomerization and Chaperone Activity

It is demonstrated that for chaperones, structure is essential to function; perturbing the structure of a chaperone protein, i.e. inducing minor protein unfolding has been shown to increase chaperone activity in some examples (Cremers et al. 2010). There are also examples where chaperones show activity in an oligomeric state (Horwitz 1992).

We have been able to show that for spectrin, oligomerization leads to increased activity (Bose et al. 2017). Interestingly we have also been able to link the oligomerization linked increase in chaperone activity of spectrin to pH. Like earlier studies on other chaperones, spectrin also shows modulation of chaperone potential with pH (Tanford 1968; Bose et al. 2017).

Literature shows that the ionic strength and pH of the solution play an important role in the structure and conformation of spectrin (Cole and Ralston 1992), as seen in detergent extracted red blood cell membrane skeletons, which have been demonstrated to expand or shrink with changing ionic strength (Johnson et al. 1980; Lange et al. 1982). The properties of the protein as a whole are pH dependent, especially the oligomeric status (Ralston 1991).

We have shown that both forms of spectrin, erythroid and non-erythroid, are better chaperones at pH 4.0 than they are at their native pH of 8.0. At pH 4.0 we see that aggregation of BSA is prevented by about 60% while at pH 8.0 the prevention of aggregation is only 30%. Insulin aggregation experiments were also found to show the same trend.

We could show that conformational changes in spectrin lead to higher order oligomerization and consequently higher chaperone potential. Both dimeric spectrin and tetrameric non-erythroid spectrin undergo oligomerization at low pH of around 4.0 and these oligomers are better chaperones than the native proteins. We could show using fluorescence data that at pH 4.0, both forms of spectrin lacked well defined tertiary structure with 5 nm hypsochromic shift of the emission maxima. The mean residue ellipticity at 222 nm also showed a transition at pH 4.0 with appreciable loss of α-helical structure. This was understood to be a further indication of oligomerization as is seen in some chaperones like α-crystallins (Raman and Rao 1994).

Further, dynamic light scattering data indicated that at pH 4.0, the hydrodynamic radius of the protein increased significantly, which was also supported by 90° light scattering measurements, thus confirming the oligomeric nature of spectrin at pH 4.0.

Moreover as discussed earlier, here too we were also able to implicate the hydrophobic residues in spectrin for increased chaperone activity upon oligomerization. Maximum enhancement of ANS fluorescence intensity was observed at pH 4.0, indicating maximal exposure of hydrophobic clusters.

Thus we found that the structural transitions at pH 4.0 enhanced the chaperone property of erythroid and non-erythroid spectrin perhaps by increasing or reorganizing the hydrophobic surfaces. It is worth noting that similar pH-dependent chaperon interactions between BSA and clusterin have been reported in literature (Poon et al. 2002).

Lipids, Macromolecular Crowders, and Chaperone Activity

Most of the studies regarding the chaperone activity of spectrin have been carried out in single component systems (Bhattacharyya et al. 2004; Bose et al. 2017). Keeping the complex nature of spectrin functionality in mind, specially its interaction with membrane phospholipids our group has elucidated the effect of phospholipid interaction, and presence of macromolecular crowders on chaperone activity.

It is known that protein aggregation inside a cell is affected by presence of biological membranes such as in the case of amyloid-β protein and α-synuclein (Aisenbrey et al. 2008). Moreover there are also reports of chaperones interacting directly with membrane lipids (Torok et al. 2001; Arispe et al. 2002; Tsvetkova et al. 2002).

It is especially important to know how the chaperone property of spectrin is influenced by the presence of membranes, as it is a membrane associated protein and is known to bind lipids directly (Ray and Chakrabarti 2004; Sarkar et al. 2018). Spectrin is also involved in a variety of RBC diseases such as hereditary elliptocytosis and spherocytosis (Blanc et al. 2010; Harper et al. 2013). Specifically detachment of spectrin from the cell membrane is known to be the major reason for these diseases.

Macromolecular crowders are present in a high concentration *in-vivo* and influence protein aggregation (Wang et al. 2012; Breydo et al. 2014; Kuznetsova et al. 2014) and the pathway taken (Munishkina et al. 2008). There are reports that indicate that crowders like proteins and polymers, act to destabilize chaperone-client interactions (Zhou 2013). Presence of macromolecular crowders can affect weak protein–protein interactions as crowder-protein attractive interactions are stronger (Rosen et al. 2011; Sarkar et al. 2014). However crowders can also enhance chaperone-client interactions (Kinjo and Takada 2003).

Investigating the chaperone activity of spectrin in the presence of protein crowder BSA and polymer crowder PVP40 reveals that macromolecular crowders BSA and PVP40 have no significant effect on the chaperone activity of spectrin. In the presence of BSA or PVP40, spectrin is able to prevent insulin and ADH aggregation just as well as in their absence. This is also corroborated by enzyme refolding studies (Bose and Chakrabarti 2019a, b).

It was seen that phospholipids competed with the chaperone client proteins for spectrin binding. The greatest decrease of chaperone activity was noted in the case of SUVs containing DMPE as a large part of spectrin became bound to these membranes (Ray and Chakrabarti 2004) (Mitra et al. 2015a, b; Giri et al. 2017). Thus, as the surface of spectrin was blocked only ~ 8% protections from aggregation were observed.

Similarly large decrease of chaperone activity was seen in case of phospholipid membranes with cholesterol and phosphatidylserine (PS). This was explained by the fact that spectrin has stronger affinity and larger surface coverage for these membranes (An et al. 2004, Mitra et al. 2015a, b). As in the case of macromolecular crowders enzyme refolding in the presence of SUVs showed the same overall trends.

Using AFM imaging we showed that insulin aggregates had a fibrilar nature which was reduced to a finer dispersion in the presence of spectrin. Insulin aggregation in the presence of phospholipid membranes showed the similar fibrilar type of aggregates but looked more clumped and clustered together. Addition of spectrin caused the aggregates to become more finely dispersed; however the dispersion of aggregates was not as great as in the presence of SUVs as in their absence.

This generalized decrease of chaperone potential of spectrin in the presence of phospholipid bi-layers may have important implications in disease states like hereditary elliptocytosis and spherocytosis (Tse and Lux 1999; Zhang et al. 2013) where spectrin is known to be detached from the cell membrane (Goodman and Shiffer 1983; Mohandas and Chasis 1993; Mohandas and Evans 1994). In these cases an increase in chaperone activity could be present which could help modulate cellular stress under such diseased conditions.

Post-translational Modifications and Chaperone Activity

It is known that post-translational modifications affect the functions of molecular chaperones, such as in case of HSP90 (Mollapour and Neckers 2012) and α-crystallins (Cherian and Abraham 1995). Of them glycation in general increases with protein age and generally decreases the chaperone function (Kumar et al. 2007). Spectrin cannot be easily glycated *in-vivo* as the RBC has mechanisms to prevent spectrin glycation (Manno et al. 2010) which involve the membrane association of spectrin. This is absent in disease states that cause membrane detachment of spectrin.

Spectrin is also known to be phosphorylated at the C-terminus of the β-subunit (Harris and Lux 1980; Tang and Speicher 2004). There can be a maximum of 6 phosphorylations, 5 being on serine residues and 1 being on threonine (Tang and Speicher 2004). Phosphorylation effects spectrin interaction with other proteins such as actin (Pinder et al. 1977). There are chaperones which are modulated by phosphorylation (Szebeni et al. 2003; Aquilina et al. 2004). We have reported the effect of hyper- and hypo-phosphorylation on chaperone activity of spectrin.

Glycation was found to decrease the chaperone function of spectrin. Interestingly it is known that the domains that get glycated are those that bind PS (Manno et al. 2010); our

data showed that SUVs with PS in their compositions similarly decrease chaperone potential on spectrin binding. Thus we hypothesized that both glycation and PS binding may act in similar ways—possibly by binding to and occluding spectrin surface (Bose and Chakrabarti 2019a, b).

While phosphorylation affected the interactions of spectrin (Pinder et al. 1977), it was seen that it did not affect chaperone activity. Considering the hypothesis that spectrin binds to its chaperone clients via its extended rod like surface, the limited phosphorylation at the C-terminal of its β subunit is expected to not alter its chaperone activity.

Heme Protein Interactions and Chaperone Activity

Spectrin has been shown to interact with heme proteins such as HRP and free globin chains and act as a chaperone for them (Chakrabarti et al. 2001; Basu and Chakrabarti 2015). In proteomic studies it has been reported that spectrin is a part of the interactome of hemoglobin (Basu and Chakrabarti 2015). Various reports from our group have also shown that spectrin interacts differentially with various hemoglobin isoforms (Datta et al. 2003). However the biochemical significance of these interactions is not fully understood.

We have recently shown that interaction with hemoglobin decreases the chaperone activity of spectrin (Bose and Chakrabarti 2019a, b). We hypothesize that hemoglobin competes with clients for spectrin binding. Hemoglobin has a high stoichiometry for spectrin binding of about 100:1, and thereby covers spectrin surface as suggested in our 'bead on a string' model (Datta et al. 2003; Mishra et al. 2017). The combined observations that spectrin can act as a chaperone for free globin chains and also shows reduced chaperone activity towards other proteins in the presence of hemoglobin lends support to our hypothesis that the major client of spectrin chaperone function is hemoglobin. This can have implications in disease states like β-thalassemia (Datta et al. 2003; Basu and Chakrabarti 2015). Interestingly, spectrin shares structural homology with known erythrocyte resident α-globin chaperone—α-hemoglobin stabilizing protein (AHSP). These two proteins share a common three helix bundle motif (Yan et al. 1993; Kihm et al. 2002; Yu et al. 2007). Structural homology of spectrin with AHSP, along with its higher affinity for structurally aberrant hemoglobin variants and unfolded heme proteins also highlights the chaperone function of spectrin (Chakrabarti et al. 2001; Basu and Chakrabarti 2015).

Interestingly spectrin is also known to play a part in the redox biology of hemoglobin. We have demonstrated the oxidative cross-linking of hemoglobin variants with spectrin (Chakrabarti et al. 2008; Datta et al. 2006). Moreover, spectrin interacts with the greatest affinity to the most unstable hemoglobin isoforms—HbE (Chakrabarti et al. 2008; Datta et al. 2006). It is known that under oxidative stress hemoglobin gets attached to the membrane and it is seen that the pathology of hemoglobin diseases is manifested by the membrane attachment of hemoglobin. This results in lipid peroxidative damage and protein oxidation; for example as seen in sickle cell disease and thalassemia (Kuross et al. 1988; Scott et al. 1993). These membrane alterations are also visible on storage of blood (Kriebardis et al. 2007). As such it is important to understand the interactions of spectrin with hemoglobin isoforms and other heme containing proteins.

We have used a fluorescence quenching technique and a novel, label free second harmonic scattering technique to quantify the interaction of spectrin to hemoglobin isoforms and other heme containing proteins such as cytochrome-c (Mishra et al. 2017). The apparent K_d values were estimated from fluorescence quenching measurements; for cytochrome-c a K_d of 0.9 μM was seen and a K_d of 27.5 μM for adult hemoglobin was observed (unpublished data).

Moreover we have seen that the peroxidase activity of hemoglobin isoforms and cytochrome-c, as determined using the ABTS assay increased in the presence of spectrin (unpublished data), (Chakrabarti and Basak 1996; Haque et al. 1999; Chakrabarti et al. 2001). We are thus able to assign biochemical significance for spectrin heme protein interactions. This observation is supported by our previous work, which shows that in conditions like hereditary spherocytosis, redox-regulators, membrane bound globin chains and cytoskeletal protein damage increase; which indicates involvement of spectrin in redox pathways (Saha et al. 2011).

Localization and Molecular Origin of Chaperone Potential

Since spectrin is composed mostly of "spectrin repeat" domains, we wanted to determine if the chaperone property of spectrin is localized in one of these domains or is a general property of these repeat domains.

These domains are homologous (Speicher and Marchesi 1984), especially they have conserved tryptophans (MacDonald et al. 1994), within themselves. But they also have distinct properties (Djinovic-Carugo et al. 2002).

We had previously hypothesised that this property resides in the self-association domain of the hetero-dimer (Chakrabarti et al. 2001; Bhattacharyya et al. 2004; Basu and Chakrabarti 2015; Bose et al. 2017). Literature indicates that all chaperones have some features in common, namely they possess charged and/or hydrophobic patches which are solvent exposed (Bose and Chakrabarti 2017). Using the knowledge of the presence of hydrophobic patches on spectrin as well as our hypothesis linking hydrophobic patches to chaperone activity (Bhattacharyya et al. 2004; Sarkar et al.

2019), we concluded that the most likely domains on spectrin to harbour chaperone activity would also have hydrophobic patches.

Thus we used nine domains of spectrin, five from α and four from β spectrin to investigate their chaperone activity as versus the native protein. We also checked for the hydrophobic nature of these domains (Bose and Chakrabarti 2019a, b).

We found that spectrin fragments as well as the reconstituted self-association domain could all inhibit protein aggregation, and it was dose dependent. For all the cases the individual domains showed less extent of protection from aggregation than intact spectrin. Based on the molar concentrations of the fragments used, we could hypothesize that the chaperone property of spectrin is the sum total of the activities of its domains. This was corroborated by enzyme refolding studies (Bose and Chakrabarti 2019a, b).

Interestingly, using ANS fluorescence we were able to confirm that all the spectrin domains possessed a similar level of surface exposed hydrophobic patches. The binding parameters of ANS to the spectrin fragments were similarly evaluated and K_d values varied for spectrin and all fragments varied from 40–60 μM with binding stoichiometry of 1 ANS per fragment. However, for intact spectrin the binding stoichiometry was 5. However, only the reconstituted "self-association" domain was able to bind PRODAN, a specific hydrophobic probe for spectrin (Chakrabarti 1996; Bhattacharyya et al. 2004; Bose and Chakrabarti 2019a, b). The apparent binding dissociation constants (K_d) of the reconstituted "self-association" domain was determined to be 13.4 ± 0.4 μM compared to 2.4 ± 0.1 μM for intact spectrin (Chakrabarti 1996; Bhattacharyya et al. 2004; Bose and Chakrabarti 2019a, b).

We could conclude that the molecular site of PRODAN binding in native dimeric erythroid spectrin is located in the self-association domain. Further, the chaperone activity of spectrin is derived from the presence of many surface exposed hydrophobic patches distributed evenly across its many domains (Bose and Chakrabarti 2019a, b).

Linking Functions Together

We see that spectrin chaperone activity is influenced by its other functionality. The major points are summarized as follows:

(1) Chaperone activity of intact spectrin is linked to its ability to bind hydrophobic ligands, implying involvement of hydrophobic patches in spectrin surface.
(2) Chaperone activity enhances with increasing extent of oligomerization and surface hydrophobicity.
(3) Interaction with phospholipid membranes reduce chaperone activity in a way which indicates that chaperone activity is a consequence of client interaction with the extended surface of the rod-like spectrin molecule.
(4) Post-translational modifications such as glycation, which cause large scale alteration of spectrin surface, reduce chaperone activity. Interestingly, parts of spectrin which get glycated are implied to interact with membrane phospholipids.
(5) It is demonstrated that haemoglobin is a spectrin chaperone client. Indeed it is able to displace other denatured proteins and preferentially bind spectrin. There is also some evidence of involvement of spectrin in redox biology of haemoglobin.
(6) Chaperone activity is distributed evenly across the domains of spectrin, and these domains all show surface exposed hydrophobic patches.

Thus we can logically hypothesize that the chaperone activity of spectrin is derived from the presence of surface exposed hydrophobic patches, which seem to be evenly distributed across the surface of the extended rod-like molecule. These patches are also implied to bind membrane phospholipids, so there is a competition between phospholipid binding and chaperone activity. This is important in scenarios where spectrin is known to detach from the membrane surface in-vivo such as in disease states such as hereditary spherocytosis and elliptocytosis.

Moreover processes such as glycation, which is linked to protein aging, can also shield these surface patches and reduce chaperone activity. This implies that as the protein ages, it becomes worse at chaperone function.

Interestingly, spectrin is present as oligomers linked via protein 4.1 in the membrane; this indicates that in-vivo spectrin chaperone activity should be greater than that of the dimer.

Most importantly, spectrin seems to prefer haemoglobin as its chaperone client. Combined with the knowledge that spectrin is able to take part in the redox biology of haemoglobin and also is known to interact more strongly with unstable haemoglobin variants, it can be hypothesized that spectrin chaperone activity is important in the cellular biochemistry of haemoglobin.

The picture thus arises of spectrin chaperone function and its interaction and interdependence on its other functionalities is presented in a diagram, shown in Fig. 1.

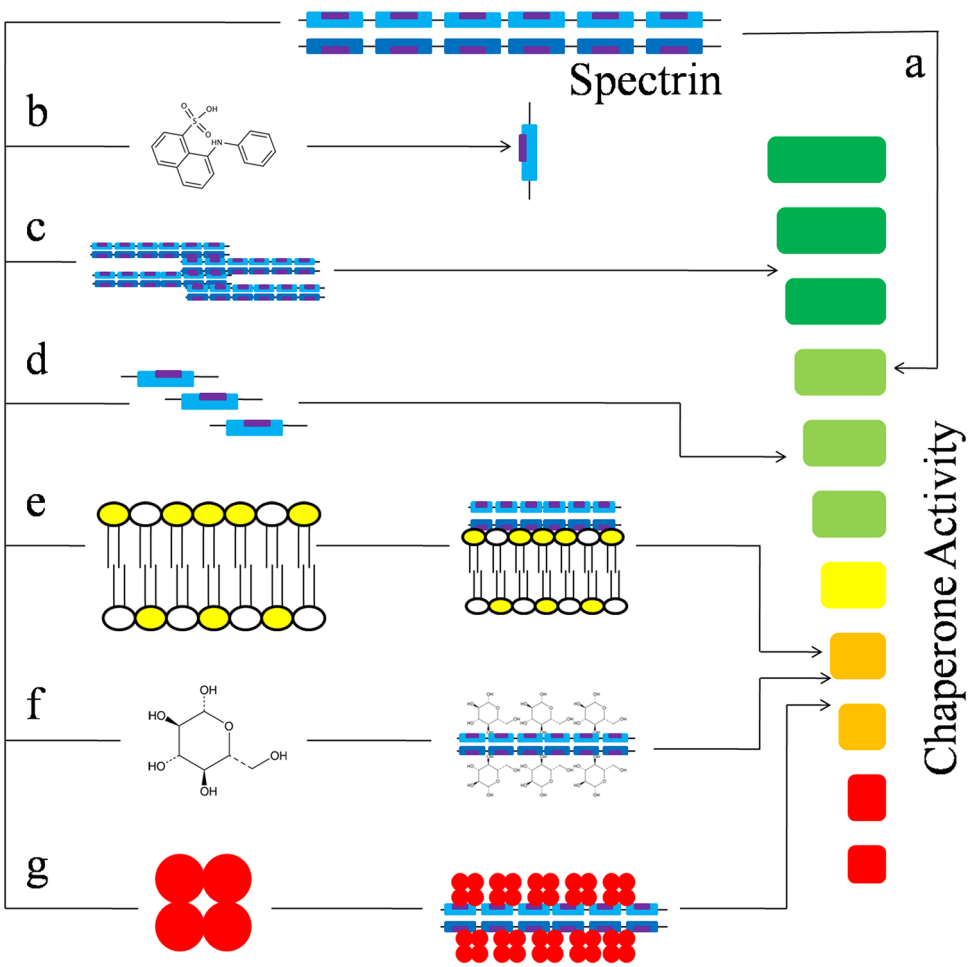

Fig. 1 Figure shows a schematic representation of the effect of the chaperone activity of spectrin interacting with its other functionalities. Generally it is seen that there is a competition between the different functionalities of spectrin and its chaperone potential. Spectrin interactions with phospholipids or hemoglobin, and glycation reduce chaperone activity. Spectrin is shown as a hetero-dimer composed of "spectrin repeat" domains, each of which have surface exposed hydrophobic patches indicated in purple. Path "a" represents the chaperone activity of intact dimeric spectrin. Path "b" shows the interaction of hydrophobic ligand ANS with the hydrophobic patch of the "spectrin repeat" domain. Path "c" shows the chaperone activity of oligomerized spectrin which is higher than that of native dimeric spectrin. Path "d" shows the chaperone activity of "spectrin repeat" domains which is lower than that of native dimeric spectrin. Path "e" shows chaperone activity of spectrin in the presence of membrane phospholipids. Since spectrin surface is blocked, the chaperone activity is lower. Path "f" shows the glycation of spectrin. Here too the surface of spectrin is blocked lowering the chaperone activity. Path "g" shows interaction of spectrin with hemoglobin. Since hemoglobin also blocks spectrin surface, the chaperone activity is reduced

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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