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Effect of stilbenedisulfonate binding on the state of association of the membrane-spanning domain of band 3 from bovine erythrocyte membrane

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The membrane-spanning domain of bovine band 3, the anion transport protein of erythrocyte membrane, was purified in the presence of nonaethyleneglycol lauryl ether ($C_{12}E_9$) and the effect of a covalent attachment of 4,4′-diisothiocyanostilbene-2,2′-disulfonate (DIDS), a potent transport inhibitor, on the state of association of the domain isolated (the 58 kDa fragment) was studied via gel filtration, gel electrophoresis and sedimentation velocity experiments. It was indicated that the DIDS-unlabeled fragment in $C_{12}E_9$ solution forms heterogeneous aggregates which are larger in size than the dimer. This contrasted with the behavior that bovine band 3 is present as dimers or tetramers in the same medium (Nakashima and Makino (1980) J. Biochem. 88, 933–947). When DIDS was covalently attached, the fragment was present as a single molecular species which was indicated to be a dimer by molecular weight determination. The secondary structure of the fragment was not affected by DIDS. The change in the state of association caused by the DIDS-binding was also found in the presence of sucrose monolaurate (SE12), which was a more potent detergent for extraction of the 58 kDa fragment from membranes than $C_{12}E_9$. However, the complex with SE12 was extremely unstable.

Introduction

Band 3 is a major intrinsic erythrocyte membrane protein which functions as an anion transporter [1]. This protein exists as a mixture of dimers and tetramers in membranes and isolated states, and consists of two distinct domains; a cytoplasmic domain and a membrane-spanning domain [1–3]. The former contains the amino-terminus of band 3 and supplies binding sites for several membrane's cytoskeletal and cytoplasmic proteins [4–7]. The latter, the carboxyl-terminal portion of band 3, is involved in anion transport [8,9]. These domains can be isolated following mild proteinase digestion at the internal membrane surface.

Studies with anion transport inhibitory probes, including stilbenedisulfonates, have significantly contributed to the understanding of the structure and function of band 3 [10–17]. For instance, Macara et al. [15] suggested a conformational alteration in transmembrane segment(s) which

Abbreviations: $C_{12}E_9$, nonaethyleneglycol lauryl ether; SE12, sucrose monolaurate; SDS, sodium dodecyl sulfate; DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonate; BADS, 4-benzamide-4′-aminostilbene-2,2′-disulfonate.

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causes exposure of an inhibitor, eosin maleimide, bound from the extracellular surface to the cytoplasmic side of the membrane. Salhany et al. [17] demonstrated that a chymotryptic 17 kDa subdomain of the membrane-spanning domain is allosterically affected by dinitrostilbenedisulfonate bound to a chymotryptic 35 kDa subdomain, indicating the existence of an allosteric interaction between two integral subdomains. In addition to such conformational changes of transmembrane segments in the band 3 monomer, there is some indication [18] that the DIDS-binding to band 3 changes the hemoglobin binding to the cytoplasmic domain from a noncooperative state to negative cooperative state. Moreover, some, though not all, stilbenedisulfonate derivatives, bind to band 3 in a negative cooperative manner under certain conditions [19,20]. These observations suggest the possibility that conformational changes which occur upon binding of stilbenedisulfonates are accompanied by a change in subunit-subunit interaction of band 3 oligomers.

The present experiment was undertaken to investigate the effect of DIDS-attachment to the membrane-spanning domain on the state of association of band 3. Band 3 oligomers are formed by interactions within both the membrane-spanning domain and the cytoplasmic domain between promoters [3]. The dimeric association between the cytoplasmic domains is extremely tight [21,22]. In order to evaluate subunit-subunit interaction within the membrane-spanning domain alone, we examined the state of association of the membrane-spanning domain of bovine band 3 isolated (the 58 kDa fragment) in nonaethyleneglycol lauryl ether ($C_{12}E_9$) solution, in which detailed studies have been carried out on the structure of bovine band 3 [23]. In addition, preliminary studies are also performed in the presence of sucrose monolaurate (SE12). The present results show that the DIDS-binding to the membrane-spanning domain changes the state of association of the 58 kDa fragment isolated from associated towards dissociated forms.

Materials and Methods

Materials. α-Chymotrypsin was purchased from Miles, and diisopropylfluorophosphate and Sepharose 4B were obtained from Fluka and Pharmacia, respectively. Detergents used were as follows: Sodium dodecyl sulfate (SDS) from Nakarai Chemicals, $C_{12}E_9$ from Nikko Chemicals and SE12 supplied from Mitsubishi Chemicals. DIDS and other reagents were purchased from Wako Chemicals. Aminobutyl-Sepharose 4B was prepared according to the previously described procedure [23].

Preparation of membrane-spanning domain of bovine band 3. Unsealed ghosts from intact cells and DIDS-treated cells were prepared according to the procedures described previously [24]. After the ghosts were washed with 0.2 M NaCl, 10 mM Tris-HCl (pH 8.0), they were hydrolyzed with 50 μg/ml of chymotrypsin for 15 h at room temperature in the buffer. The proteinase was inhibited by 0.1 mM diisopropylfluorophosphate. Chymotrypsin-treated ghosts were washed several times with 0.15 M NaCl, 10 mM Tris-HCl (pH 8.0) by centrifugation (15000 rpm for 30 min). The packed membranes (approx. 10 ml) were mixed with 25 ml of 0.35% $C_{12}E_9$, 0.15 M NaCl, 10 mM Tris-HCl (pH 8.0) (final 0.25% $C_{12}E_9$). The suspension was gently stirred on ice for 15 min to remove proteins loosely bound to membranes and then tightly packed by centrifugation. The membrane residues recovered were further incubated with 25 ml of 0.7% SE12, 0.15 M NaCl, 10 mM Tris-HCl (pH 8.0) on ice for 30 min (final 0.5% SE12). After centrifugation at 40000 rpm for 30 min, $C_{12}E_9$ was added to the supernatant (final 1%). (See Results and Discussion on instability of 58 kDa fragment in SE12 solution). The supernatant was dialyzed against 10 mM Tris-HCl (pH 8.0), and then applied to an aminobutyl-Sepharose 4B column (1.6 × 15 cm). The column was washed sufficiently with 10 mM Tris-HCl (pH 8.0) containing 0.1% $C_{12}E_9$, and proteins were eluted with 200 ml of a NaCl linear gradient (the final concentration of NaCl was 0.2 M) in the above buffer containing 0.1% $C_{12}E_9$.

Samples used for experiments in SE12 solution were prepared as follows: The 58 kDa fragment purified in $C_{12}E_9$ solution was again adsorbed to an aminobutyl-Sepharose 4B column (0.6 × 7 cm), then washed with a 10-fold column volume of 10 mM Tris-HCl (pH 8.0) containing 1% SE12, to change the detergent from $C_{12}E_9$ to sE12. Then

the column was further washed with a 30-fold column volume of the above buffer containing 0.1% SE12, and the protein was eluted with 0.1 M NaCl, 10 mM Tris-HCl (pH 8.0) containing 0.1% SE12. This eluate was immediately used for experiments.

Analytical procedures. Concentration of the 58 kDa fragment was determined according to the modified Lowry method [23], using bovine serum albumin as a standard.

Polyacrylamide gel electrophoresis in the presence of 0.1% SDS, $C_{12}E_9$ or SE12 was performed as described previously [23]. For electrophoresis in the presence of nonionic detergents, electrophoresis buffer (0.01 M Tris-0.08 M glycine (pH 8.3)) was circulated by using a peristatic pump from one electrode chamber to the other through a 2-l buffer reservoir to avoid a change of pH during electrophoresis.

High performance gel chromatography was carried out at room temperature using a Shodex WS-803 column (0.8 × 60 cm), which had been equilibrated with 0.1 M Na_2HPO_4-NaH_2PO_4 buffer (pH 7.1) containing 0.1% $C_{12}E_9$ or 0.1% SE12. The column was calibrated using standard proteins with known Stokes radii.

Sedimentation velocity measurements were made with a Hitachi UCA-01 analytical ultracentrifuge equipped with schlieren optics. The runs were done with a protein concentration of approx. 1 mg/ml at 20 °C in 10 mM Tris-HCl (pH 8.0).

The molecular weight of protein moiety in a protein-detergent complex (M) was determined according to the following equation [23].

$$M = (6\pi N\eta R_s s^0_{20,w})/(1-\phi'\rho) \quad (1)$$

Here, N is the Avogadro's number, R_s is the Stokes radius of a protein-detergent complex and ϕ' is the effective specific volume per gram of protein. η and ρ are the viscosity and the density of water at 20 °C, respectively. The factor $(1-\phi'\rho)$ in Eqn. 1 can be expressed in terms of the contribution of bound detergent (δ_D g/g of protein) as follows:

$$1-\phi'\rho = (1-\bar{v}_P\rho) + \delta_D(1-\bar{v}_D\rho) \quad (2)$$

where $\bar{v}_P$ is the partial specific volume of protein, and $\bar{v}_D$ is the partial specific volume of bound detergent. A value of $\bar{v}_P = 0.750$ cm^3/g was calculated on the basis of the amino acid composition of 58 kDa fragment (The compositional data are not shown.) The values of $\bar{v}_D$ were taken from literature as 0.953 for $C_{12}E_9$ [23] and 0.798 for SE12 [25], respectively. The purified fragment was concentrated in aminobutyl-Sepharose 4B column chromatography by stepwise elution, and binding number of detergent was calculated from the excess amount of detergent which was co-eluted with the 58 kDa fragment, as described previously [23]. The Stokes radii of 58 kDa fragment-detergent complexes were determined by high performance gel exclusion chromatography.

Circular dichroic spectra were recorded on a JASCO J-40 spectropolarimeter. Mean residue ellipticity was calculated using the mean residue weight of 58 kDa fragment, 112, based on the amino acid composition. A cell of 1 mm light path was used.

Binding of 4-benzamide-4′-aminostilbene-2,2′-disulfonate (BADS) to the 58 kDa fragment was measured in 28.5 mM sodium citrate (pH 7.5) containing 0.1% $C_{12}E_9$, as described previously [26].

Results

Isolation of membrane-spanning domain of bovine band 3 in nonionic detergent solution

Chymotrypsin attacked only the internal membrane surface of bovine unsealed ghosts at ionic strength higher than 0.1, to produce the membrane-spanning 58 kDa fragment [24], as shown in Fig. 1, lane 2. In a medium of low ionic strength, the enzyme had easy access to the exosurface region at a distance of 17 kDa from the amino-terminal end of 58 kDa fragment [24], resulting in the generation of a 17 kDa and a 41 kDa fragment (Fig. 1, lane 3). When DIDS-labeled ghosts were used, the DIDS fluorescence was visualized at the 58 kDa and/or the 17 kDa band on an SDS gel. The 58 kDa fragment was solubilized from membranes more selectively and effectively by 0.5% SE12 than by 1% $C_{12}E_9$ (data not shown). The 58 kDa fragment was purified from the 0.5% SE12 extract by using an aminobutyl-Sepharose 4B column in 0.1% $C_{12}E_9$. Results of

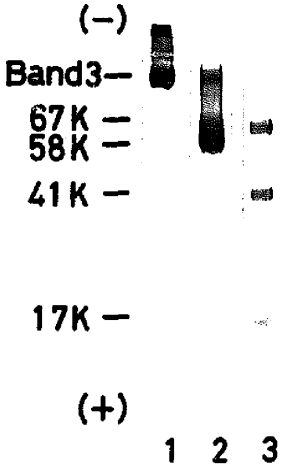

Fig. 1. 0.1% SDS-8% polyacrylamide gel electrophoresis of alkali-stripped unsealed ghosts (lane 1) and alkali-stripped unsealed ghosts which had been digested with α-chymotrypsin in 10 mM Tris-HCl (pH 8.0) containing 0.2 M NaCl (lane 2) or 50 mM NaCl (lane 3). Ghosts were digested for 15 h at room temperature with 50 μg/ml of chymotrypsin. Intact and chymotrypsin-treated ghosts were treated with 0.1 M NaOH and then the membrane residues were electrophoresed on slab gels (0.2×14×14 cm) using an electrophoresis buffer of 0.1 M Na_2HPO_4-NaH_2PO_4 (pH 7.1) containing 0.1% SDS. Gels were stained with Coomassie brilliant blue R. A 67 kDa band is a component which was not cleaved at a bond between a 17 kDa integral subfragment and a 50 kDa cytoplasmic fragment [24].

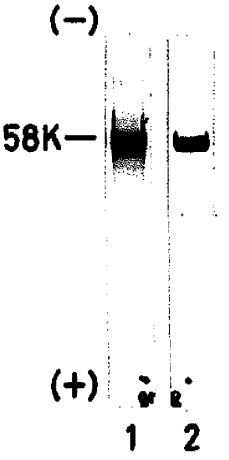

Fig. 2. SDS-polyacrylamide gel electrophoresis patterns of the intact 58 kDa fragment (lane 1) and the DIDS-labeled 58 kDa fragment (lane 2). Samples (approx. 50 μg) which were purified by an aminobutyl-Sepharose 4B column were run on 0.1% SDS-8% polyacrylamide slab gels (0.1×8×8 cm), using 0.1 M Na_2HPO_4-NaH_2PO_4 (pH 7.1) containing 0.1% SDS as a running buffer. Gels were stained with Coomassie brilliant blue R.

SDS-gel electrophoresis for the intact and the DIDS-labeled fragment isolated are shown in Fig. 2, lanes 1 and 2.

Conformation and state of association of DIDS-labeled and intact 58-kDa fragment in $C_{12}E_9$ solution

Circular dichroic spectra in the far-ultraviolet region of the 58 kDa fragment in $C_{12}E_9$ solution showed the presence of an α-helical structure as the predominant secondary structure, whose content was estimated as approx. 50%. The DIDS-attachment did not cause any detectable change in the circular dichroic profile (Fig. 3), as in the case of human band 3 [27]. On the contrary, the hydrodynamic characteristics of the membrane-spanning domain were strongly affected by the DIDS attachment. A typical example is seen in gel filtration experiment shown in Fig. 4A. The results show that the intact 58 kDa fragment exists as a mixture of several molecular species, while the attachment of DIDS onto the fragment eventually produces a single molecular species with a Stokes radius of 75 ± 3 Å (Fig. 5). The same conclusion was drawn from the results of gel electrophoretic

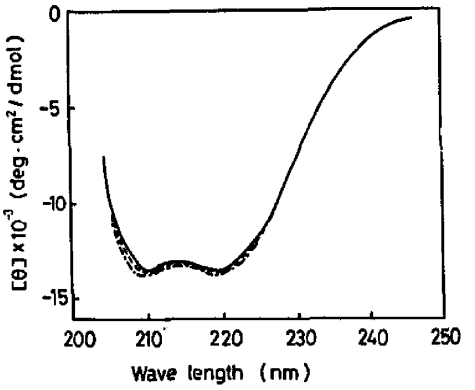

Fig. 3. Circular dichroic spectra in the far-ultraviolet region of the 58 kDa fragment in 10 mM Tris-HCl, 50 mM NaCl buffer (pH 8.0) containing 0.1% $C_{12}E_9$ or 0.1% SE12. ———, the intact fragment in 0.1% $C_{12}E_9$; ------, the DIDS-labeled fragment in 0.1% $C_{12}E_9$; — — —, the DIDS-labeled fragment in 0.1% SE12.

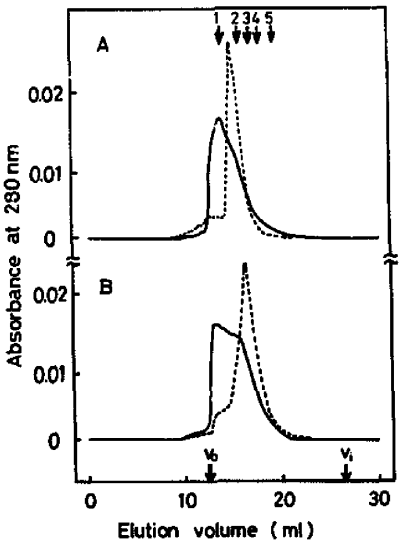

Fig. 4. Elution profiles of the intact 58-kDa fragment (———) and the DIDS-labeled 58 kDa fragment (- - - - - -) on a Shodex WS-303F column in the presence of 0.1% $C_{12}E_9$ or 0.1% SE12. (A) The fragments purified in the presence of 0.1% $C_{12}E_9$ (50 μl each of approx. 1 mg/ml protein solution) were applied to the column which had been equilibrated with 0.1 M Na_2HPO_4-NaH_2PO_4 buffer (pH 7.1) containing 0.1% $C_{12}E_9$ and eluted with the same medium. (B) Similarly, after the detergent $C_{12}E_9$ in the sample solution was displaced by SE12 using aminobutyl-Sepharose 4B, the fragments (50 μl each of approx. 1 mg/ml protein solution) were applied to the column equilibrated with the above buffer containing 0.1% SE12, and eluted with the same buffer. V_0 and V_i indicate the void and internal volume, respectively. Arrows indicate the elution positions of standard proteins: 1, thyroglobulin; 2, ferritin; 3, catalase; 4, bovine serum albumin; 5, hemoglobin. For their molecular weights and Stokes radii, see the figure caption of Fig. 5.

experiments in the presence of $C_{12}E_9$, as shown in Fig. 6. In the gels, the DIDS-labeled fragment migrated as a single band. On the other hand, the unlabeled fragment showed a very diffuse band. This is in marked contrast to the result for whole band 3 showing distinct bands corresponding to the dimer and the tetramer in this gel system [23]. Consistent with the above observations, the DIDS-labeled fragment sedimented as a single component in ultracentrifugation, with a sedimentation coefficient of 4.4 ± 0.2 S.

The molecular weight of a protein moiety was determined for the DIDS-labeled 58 kDa fragment-$C_{12}E_9$ complex which was present as a single molecular species, according to the procedure described under Materials and Methods. Molecular parameters used for the calculation and the molecular weight obtained are summarized in Table I. The result apparently demonstrates that, when the membrane-spanning domain covalently bonded DIDS, the fragment isolated exists as a dimer in $C_{12}E_9$ solution.

The dissociation constant for binding of BADS, a reversible anion transport inhibitor, to the 58 kDa fragment was 115 ± 10 μM in 0.1% $C_{12}E_9$ solution. This value was ten times as high as that observed for whole band 3 under the identical conditions [26].

Studies in SE12 solution

It has been reported [28] that different results may often be obtained when different detergents are used to solubilize the same protein. In the present case, we have observed that SE12 displays a potent ability to extract the membrane-spanning

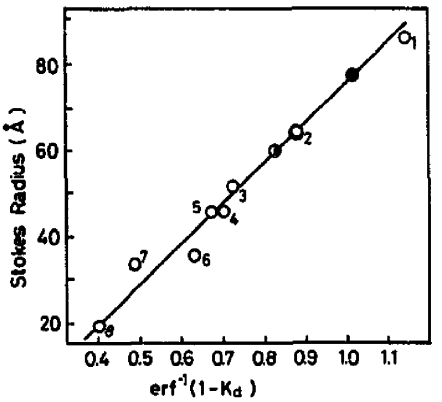

Fig. 5. Determination of the Stokes radii of the DIDS-labeled 58 kDa fragment-detergent complexes by gel filtration described in Fig. 5. ●, the complex with $C_{12}E_9$; ○, the complex with SE12. Each experimental point represents an average of 3 or 4 determinations. K_d is a distribution coefficient. The column was calibrated in detergent-free buffer using standard proteins. The Stokes radii of standard proteins were taken from literature [23]. Standard proteins: 1, thyroglobulin (669000; 85 Å); 2, ferritin (44000; 64 Å); 3, catalase (232000; 52 Å); 4, aldolase (158000; 45 Å); 5, lactate dehydrogenase (140000; 45 Å); 6, bovine serum albumin (67000; 35 Å); 7, hemoglobin (64000; 33 Å); 8, myoglobin (17000; 19 Å). Numbers in parentheses are molecular weights and Stokes radii.

TABLE I

MOLECULAR PARAMETERS AND POLYPEPTIDE CHAIN MOLECULAR WEIGHT OF THE 58 kDa FRAGMENT-DETERGENT COMPLEX

Values are an average of three or four determinations.

Fragment–detergent complex	Stokes radius of complex (Å)	Amount of bound detergent (g/g protein)	Sedimentation coefficient of complex $s_{20,w}$ (S)	Polypeptide chain molecular weight ($\times 10^{-3}$)
Complex with $C_{12}E_9$	75 ± 3	1.0 ± 0.1	4.4 ± 0.2	126 ± 13
Complex with SE12	58 ± 3	2.6 ± 0.4	8.1 ± 0.3	70 ± 14

domain of bovine band 3 from membranes. This motivated us to characterize the 58 kDa fragment in SE12 solution. However, the fragment precipitated during storage, usually within 2 days, even in a solution of SE12 concentration of more than 1%. Therefore, experiments in SE12 solution were performed immediately after the detergent was changed from $C_{12}E_9$ to SE12.

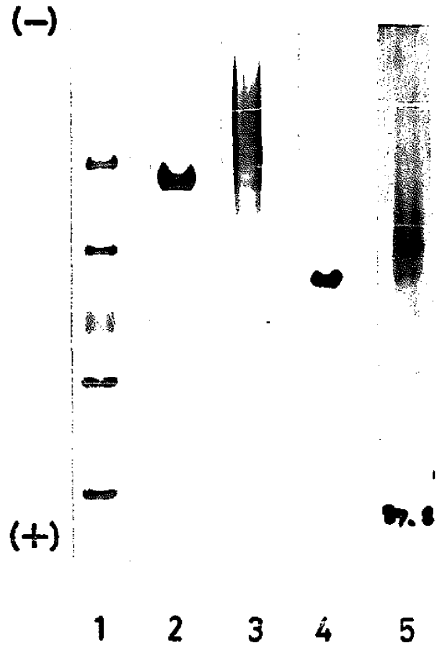

Fig. 6. Polyacrylamide gradient gel electrophoresis in the presence of 0.1% $C_{12}E_9$ or 0.1% SE12. Lane 1, standard proteins in 0.1% $C_{12}E_9$: thyroglobulin, ferritin, catalase, lactate dehydrogenase and bovine serum albumin, from top to bottom. For their molecular weights and Stokes radii, see the legend of Fig. 5. Lane 2, the DIDS-labeled 58 kDa fragment in 0.1% $C_{12}E_9$. Lane 3, the intact 58 kDa fragment in 0.1% $C_{12}E_9$. Lane 4, the DIDS-labeled 58 kDa fragment in 0.1% SE12. Lane 5, the intact 58 kDa fragment in 0.1% SE12. Mobilities of standard proteins in 0.1% SE12 were the same as those in 0.1% $C_{12}E_9$. Electrophoresis was done in 4–15% gradient slab gels (0.2 × 8 × 14 cm) for 20 h at 100 volts at 4°C in 10 mM Tris-80 mM glycine buffer (pH 8.3) in the presence of 0.1% $C_{12}E_9$ or 0.1% SE12. Samples of 40–50 μg each were loaded onto the gel.

Under the conditions used here, the DIDS-labeled 58 kDa fragment-SE12 complex existed as a single molecular species, as seen in gel filtration and gel electrophoresis experiments (Figs. 4B and 6), and sedimented as an 8.1 S component. The fragment in SE12 solution exhibited a circular dichroic profile similar to that seen in $C_{12}E_9$ solution (Fig. 3). DIDS-attachment also appeared to affect the gross conformation of 58 kDa fragment (Fig. 4B), as in the case of $C_{12}E_9$ solution. However, the result of gel filtration experiment apparently indicated that the complex of DIDS-labeled fragment with SE12 possesses a smaller molecular size than that with $C_{12}E_9$, despite the fact that the amount of detergent bound to the fragment is extrodinary large in the former (2.6 ± 0.4 g/g) as compared to that of the latter (1.0 ± 0.1 g/g). The result of molecular weight estimation suggests that the state of association of the fragment in SE12 solution differs markedly from that in $C_{12}E_9$ solution (Table I).

Discussion

The membrane-spanning domain of bovine band 3 isolated was stable in $C_{12}E_9$ solution, but not in SE12 solution. Hence, the 58 kDa fragment was characterized with greater confidence in the former rather than in the latter. We have found in $C_{12}E_9$ solution that (1) the DIDS-unlabeled fragment exists as heterogeneous aggregates which can not be identified as distinct molecular species in

gel electrophoresis, and (2) the attachment of DIDS affects the state of association of the membrane-spanning domain isolated. In contrast to the present results, Reithmeier showed [29] that the membrane-spanning domain of human band 3 exists in the dimer form in Triton X-100 solution, by measurements of R_s, $s_{20,w}$ and the amount of bound Triton X-100, where the latter two were determined by zone centrifugation in a sucrose gradient. Jay [30] reported that the structure of membrane-spanning domain of band 3 may be conserved among species, and the detergents, $C_{12}E_9$ and Triton X-100, have nearly identical characteristics in their physico-chemical properties [23]. On the other hand, there is evidence [31,32] that the presence of sucrose can disturb the state of association and the preferential hydration of a protein. Thus, the discrepancy observed between the membrane-spanning domain of human and bovine origin is probably related to differences in experimental conditions.

Bovine band 3 is present as dimers or tetramers in Triton X-100 and $C_{12}E_9$ solutions [23]. Nevertheless, the state of association of the DIDS-unlabeled 58 kDa fragment in $C_{12}E_9$ solution was apparently distinct from that of whole band 3. Appell and Low [33] have indicated that, in the cytoplasmic and the membrane-spanning domains of band 3, the structural properties of one domain depend in no way on the structural integrity of the complementary domain. In this view, the present result suggests that, at least in isolated bovine band 3, the cytoplasmic domain acts to prevent further association of band 3 dimers and tetramers, probably by an electrostatic fashion based on its highly acidic nature [7]. The reduced affinity for BADS-binding of the 58 kDa fragment observed here can be reasonably interpreted as a result of a masking of the binding site by the associations between the 58 kDa fragments.

The location of the DIDS-reactive site is Lys-558 or Lys-561 (murine band 3 sequence) which might protrude into membrane region inaccessible to the nonspecific proteinase, papain [34,35]. As shown in the present experiment, the binding of DIDS to such a cleft of the band 3 molecule causes a disturbance of the oligomer structure of the membrane-spanning domain isolated, in addition to an increase in thermal stability of the α-helical structure as reported by Oikawa et al. [27]. It has been indicated that the DIDS-reactive site on each protomer of the protein has room enough to accomodate BADS, which has a larger molecular size than DIDS [3.36]. However, in the DIDS-binding process, there is a loss of one protonated lysine-side chain and a gain of two sulfonic acids per the monomer of band 3. An elaborate model for the arrangement of the transmembrane region of murine band 3 described by Jay and Cantley shows [37] that 5–7 positively charged residues and 1–3 negatively charged residues surround the location proposed as the DIDS-attachment site. Thus, it appears that the attachment of DIDS induces a significant change in the ionic environment around the DIDS-reactive site. The accompanying change in electrostatic interaction, rather than the structural perturbation by steric hindrance, might be of importance in shifting the subunit dissociation equilibrium in the membrane-spanning domain of band 3 towards dissociated forms. In any event, it is probable that a conformational disturbance as observed here is closely related to the appearance of negative cooperativity found in interactions of band 3 with some stilbenesulfonates and hemoglobin as noted in the introduction.

It is relevant to again emphasize that ambiguities might be inherent in experimental values obtained here in SE12 solution, because of the instability of the 58 kDa fragment in this medium. However, the results obtained in SE12 solution led also to the indication that the DIDS-attachment disturbs the intermolecular interactions between the 58 kDa fragments. The molecular weight value estimated for the protein moiety of the complex of DIDS-labeled fragment with SE12 was close to that for a monomer rather than a dimer. When allowance is made for the monomeric state of the DIDS-labeled fragment in complex with SE12, a remarkably high binding number of SE12 can be accounted for as the binding of SE12 to a hydrophobic surface responsible for the monomer-monomer contact in the dimer. Although the reason for the instability of the 58 kDa fragment observed in SE12 solution is not clear at present, one possible explanation is that dimerization between the membrane-spanning domains is requisite for stable conformation of band 3.

References

1 Steck, T.L. (1978) J. Supramol. Struct. 8, 311–324.
2 Nigg, E. and Cherry, R.J. (1979) Biochemistry 18, 3457–3465.
3 Jennings, M.L. (1984) J. Membr. Biol. 80, 105–117.
4 Steck, T.L., Koziarz, J.J., Singh, M.K., Reddy, G. and Kohler, H. (1978) Biochemistry 17, 1216–1222.
5 Hargreaves, W.R., Giedd, K.N., Verkleij, A. and Branton, D. (1980) J. Biol. Chem. 255, 11965–11972.
6 Tsai, I.-H., Murthy, S.N.P. and Steck, T.L. (1982) J. Biol. Chem. 257, 1438–1442.
7 Moriyama, R. and Makino, S. (1987) Arch. Biochem. Biophys. 256, 606–617.
8 Grinstein, S., Ship, S. and Rothstein, A. (1978) Biochim. Biophys. Acta 507, 294–304.
9 Dupre, A.M. and Rothstein, A. (1981) Biochim. Biophys. Acta 646, 471–478.
10 Cabantchik, Z.I. and Rothstein, A. (1974) J. Membr. Biol. 15, 207–226.
11 Passow, H. and Zaki, L. (1978) in Molecular Specialization and Symmetry in Membrane Function (Solomon, A.K. and Karnovosky, M., eds.), pp. 229–250, Harvard University Press, Cambridge, MA.
12 Jennings, M.L. and Passow, H. (1979) Biochim. Biophys. Acta 554, 498–519.
13 Frohlich, O. (1982) J. Membr. Biol. 65, 111–123.
14 Verkman, A.S., Dix, J.A. and Solomon, A.K. (1983) J. Gen. Physiol. 81, 421–449.
15 Macara, I.G., Kuo, S. and Cantley, L.C. (1983) J. Biol. Chem. 1785–1792.
16 Boodhoo, A. and Reithmeier, R.A.F. (1984) J. Biol. Chem. 259, 785–790.
17 Salhany, J.M., Rauenbuechler, P.B. and Sloan, R.L. (1987) J. Biol. Chem. 262, 15965–15973.
18 Salhany, J.M., Cordes, K.A. and Gaines, E.D. (1980) Biochemistry 19, 1447–1454.
19 Dix, J.A., Verkman, A.S., Solomon, A.K. and Cantley, L.C. (1979) Nature 282, 520–522.
20 Macara, I.G. and Cantley, L.C. (1981) Biochemistry 20, 5095–5105.
21 Appell, K.C. and Low, P.S. (1981) J. Biol. Chem. 256, 11104–11111.
22 Moriyama, R., Kitahara, T., Sasaki, T. and Makino, S. (1985) Arch. Biochem. Biophys. 243, 228–237.
23 Nakashima, H. and Makino, S. (1980) J. Biochem. 88, 933–947.
24 Makino, S., Moriyama, R., Kitahara, T. and Koga, S. (1984) J. Biochem. 95, 1019–1029.
25 Makino, S., Ogimoto, S. and Koga, S. (1983) Agric. Biol. Chem. 47, 319–326.
26 Moriyama, R. and Makino, S. (1985) Biochim. Biophys. Acta 832, 135–141.
27 Oikawa, K., Lieberman, D.M. and Reithmeier, R.A.F. (1985) Biochemistry 24, 2843–2848.
28 Tanford, C. and Reynolds, J.A. (1976) Biochim. Biophys. Acta 457, 133–170.
29 Reithmeier, R.A.F. (1979) J. Biol. Chem. 254, 3054–3060.
30 Jay, D.G. (1983) J. Biol. Chem. 258, 9431–9436.
31 Simons, K., Helenius, A. and Garoff, H. (1973) J. Mol. Biol. 80, 119–133.
32 Timasheff, S.N., Lee, J.C., Pitt, E.P. and Tweedy, N. (1976) J. Colloid Interface Sci. 55, 658–663.
33 Appell, K.C. and Low, P.S. (1982) Biochemistry 21, 2151–2157.
34 Kopito, R.R. and Lodish, H.F. (1985) Nature 316, 234–238.
35 Falke, J.J., Kanes, K.J. and Chan, S.I. (1985) J. Biol. Chem. 260, 13294–13303.
36 Lieberman, D.M. and Reithmeier, R.A.F. (1983) Biochemistry 22, 4028–4033.
37 Jay, D. and Cantley, L. (1986) Annu. Rev. Biochem. 55, 511–538.