Biochimica et Biophysica Acta, 1151 (1993) 21–27

BBAMEM 76040

The state of association of Band 3 of the human erythrocyte membrane: evidence of a hexamer

Pierre Wong

Institut de Recherches Cliniques de Montréal and Département de Biochimie, Université de Montréal, C.P. 6128, Montréal, Québec (Canada)

(Received 30 October 1992)
(Revised manuscript received 2 March 1993)

Key words: Erythrocyte membrane; Band 3; Glycoprotein; Membrane; Association state; (Human)

Band 3 of the human erythrocyte membrane was isolated from ^{32}P-labelled erythrocytes in nonaethyleneglycol n-dodecyl ether ($C_{12}E_9$), Triton X-100, and Brij 58 solutions, and its states of association were studied by sucrose gradient sedimentation and by Sepharose 4B column chromatography. The sedimentation and elution profiles indicated that Band 3 exists under two stable forms in each detergent solution, a slow form and a fast form. The fraction of the fast form in a Brij 58 solution was 2–3-times higher than those of the fast forms in $C_{12}E_9$ and Triton X-100 solutions. Moreover, depending of the conditions of isolation, only the slow form or the fast form was present in a Brij 58 solution. The apparent values of sedimentation coefficients, Stokes radii, and effective masses of the slow and fast forms in $C_{12}E_9$, Triton X-100, and Brij 58 solutions were determined. On the basis of these values, we have concluded that the slow and fast forms of Band 3 were dimer and tetramer in $C_{12}E_9$ and Triton X-100 solutions but were dimer and hexamer in a Brij 58 solution.

Introduction

Band 3 of the human erythrocyte is a 93 kDa transmembrane glycoprotein with its carbohydrate moiety on the extracellular side [1]. It represents 25% of the total erythrocyte membrane protein. It catalyzes a tight one-for-one anion exchange, and it binds the membrane skeleton to the cytoplasmic surface of the lipid bilayer through ankyrin [1,2]. Band 3 also appears involved in the water transport [3]. Its catalysis equilibrates chloride and bicarbonate across the membrane according to the Gibbs-Donnan equilibrium, thus allowing the transport of carbon dioxide from tissues to lungs as plasma bicarbonate. Band 3 can be divided operationally into two domains: a 50 kDa transmembrane domain responsible for the anion exchange, and a 43 kDa water-soluble domain binding ankyrin and other proteins [4].

The state of association of Band 3 has been the subject of several studies and of a number of reviews [4–14]. Widely different conclusions have been reached in the studies, but it is generally believed that Band 3 is either a dimer, a dimer-tetramer equilibrium, or a tetramer in the membrane.

We have undertaken studies on the state of association of Band 3 in order to define it more precisely. Toward this end, its states of association were studied after its isolation in $C_{12}E_9$, Triton X-100 and Brij 58 solutions. The first two nonionic detergents were selected because of different conclusions reached in previous studies on its states of association in solutions of these detergents [5–10], while Brij 58 was chosen since it was not expected to be effective at breaking protein–protein interactions of integral membrane proteins [15–18].

Materials and Methods

Materials

Human blood (10, 50 or 100 ml) was freshly drawn in the presence of heparin. Concanavalin A-Sepharose 4B and Sepharose 4B were obtained from Pharmacia; Brij 58, $C_{12}E_9$, α-methyl-D-mannoside (99% pure),

Correspondence to: P. Wong, Div. Pharmacokinetics (M 719), Royal Victoria Hospital, 687 Pine Ave. West, Montreal, Quebec, Canada, H3A 1A1.

Abbreviations: $C_{12}E_9$, nonaethyleneglycol n-dodecyl ether; $C_{16}E_{20}$ (Brij 58), icosaethyleneglycol n-hexadecyl ether; Con A, concanavalin A; EGTA, ethylene bis(oxyethylenenitrile)tetraacetic acid; PAS, periodic acid Schiff; PMSF, phenylmethanesulfonyl fluoride; SDS, sodium dodecyl sulfate; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; Tris, tris(hydroxymethyl)aminomethane; TX-100, Triton X-100.

phenylmethanesulfonyl fluoride, Triton X-100 and Trizma base were purchased from Sigma; Coomassie blue R-250 and reagents for preparing the polyacrylamide gels were obtained from Bio-Rad Laboratories; ^{32}P-labelled phosphoric acid, carrier free in 0.01 M HCl (5 mCi), was obtained from New England Nuclear; water was distilled with a central distilling apparatus and then deionized with a Barnstead nanopure apparatus; other reagents used were of analytical grade.

^{32}P-labelling of erythrocytes

10 ml of blood were centrifuged at $3000 \times g$ for 10 min at 4°C. After removal of the supernatant and buffy coat, the erythrocytes were washed three times with a 30 mM Tris-HCl, 130 mM KCl, 5.4 mM NaCl, 0.8 mM $MgCl_2$, 1.0 mM EGTA, 5 mM glucose buffer (pH 7.5 at 4°C) and suspended to a hematocrit of 25% [19]. 9 ml of this suspension was added to a 40-ml centrifuge tube containing 9 μmol of dried sodium [^{32}P]phosphate having a specific activity of 0.166 mCi/μmol. The tube was gently shaken at 37°C for 20–22 h, its content was diluted with 25 ml of an isotonic sodium phosphate buffer (pH 7.4 at 4°C) and centrifuged at $12\,100 \times g$ for 30 s at 4°C. The labelled erythrocytes were then washed five times with the diluting buffer.

Isolation of Band 3

Labelled and unlabelled erythrocyte membranes were isolated according to the procedure of Dodge et al. [20] using a 5 mM sodium phosphate, 0.1 mM PMSF buffer (pH 8.0 at 4°C) as the lysis buffer. They were warmed at room temperature and solubilized with 1 vol. of a freshly prepared 10% detergent solution either of $C_{12}E_9$, Triton X-100 or Brij 58 (weight ratio of detergent to protein were 65:1 and 35:1 for labelled and unlabelled erythrocyte membranes, respectively). After diluting with 1 vol. of a 200 mM Tris-HCl buffer (pH 7.5), 0.2 mM each of $CaCl_2$, $MgCl_2$ and $MnCl_2$, the solution was applied at a flow rate of 20–30 ml/h to a 3–5 ml Con A-Sepharose 4B column equilibrated with a 100 mM NaCl, 10 mM Tris-HCl buffer (pH 7.5) containing either 0.2% $C_{12}E_9$, 0.2% Triton X-100 or 0.5% Brij 58. The column was rapidly washed with 20–30 bed volumes of the equilibrium buffer and the fraction of Band 3 specifically retained on the column was eluted with the equilibrium buffer containing 300 mM α-methyl-D-mannoside [21]. The isolated Band 3 was 80–85% pure, as indicated by densitometry following SDS-PAGE and Coomassie blue staining (gel not shown). The same degree of purity was also obtained by a 10-times decrease of NaCl concentration, a pH variation between 6.5 and 8.5, a 2.5-times increase of detergent concentration in the solubilization, or a prior spectrin extraction. Contaminants were residual peripheral membrane proteins spectrin, ankyrin and its degradation products and Band 4.2, and residual integral membrane protein glycophorin A, as detected by a PAS staining of the gel (gel not shown). These contaminants have been previously observed and are not unexpected since they specifically interact with Band 3 [2,22–24]. The isolated Band 3 represented 10% of the total Band 3 assuming that it represents 25% of the total membrane protein [22], and migrated on a SDS-polyacrylamide gel in the presence or absence of β-mercaptoethanol at the same position. No phospholipids were found bound to the isolated Band 3, as measured by a colorimetric phosphate assay [25].

Sepharose 4B chromatography

The chromatography on a Sepharose 4B column was done as previously described [26]. The detergents $C_{12}E_9$ and Triton X-100 and Brij 58 were present at concentrations of 0.2% or 0.5% in the columns. At these concentrations they had no effect on the chromatographic characteristics of the column. Samples of ^{32}P-labelled and unlabelled Band 3 were applied to the column at a cpm/ml and protein concentrations of 1000–70 000 and 0.8–1 mg/ml, respectively. The Band 3-SDS complex sample was prepared by mixing 0.5 ml of an isolated Band 3 sample (0.8 mg/ml) with 0.5 ml of a 6% SDS solution and 10 μl of β-mercaptoethanol. Band 3 concentrations of collected fractions were determined spectrophotometrically at 230 nm or by Cerenkov radioactivity counting. Elution volumes of the detergent micelles were determined as previously described [26].

Sedimentation in a sucrose gradient

Linear gradients of 11 ml of 5–20% sucrose (w/w) buffered at pH 7.5 with a 100 mM NaCl, 10 mM Tris-HCl solution containing either 0.2% $C_{12}E_9$, 0.2% Triton X-100 or 0.5% Brij 58 were prepared in centrifuge tubes for the S.W. 41 rotor. 0.5-ml samples of ^{32}P-labelled Band 3 having 12 000–25 000 cpm/ml were layered on top of the gradients and the tubes were centrifuged at 34 000 rpm for 16 h at 20°C. The solution was then pumped out from the bottom of the centrifuge tube and equal volume fractions were collected in 3-ml polyethylene counting vials. Band 3 concentrations in the fractions were then determined by Cerenkov radioactivity counting. The standards were centrifuged in parallel with the Band 3 samples, but occasionally they were centrifuged with the Band 3 samples. The calibration curve was linear and the detergents had no effect on its position at concentrations used.

Other methods

Protein concentrations were determined by the procedure of Lowry et al. using a sodium carbonate solution containing 3% SDS, and bovine serum albumin as

standard [27]. Phospholipid concentrations were determined by the procedure of Bartlett [25]. Amounts of [^{32}P]phosphate in Band 3 fractions were determined by Cerenkov radioactivity counting with a Beckman LS 7000 model spectrophotometer. Band 3 fractions were counted twice for 2 or 3 min, but those having less than 200 cpm were recounted for at least 10 min. SDS-PAGE was done in glass tubes of 0.6 × 15 cm according to the procedure of Weber and Osborn [28]. Protein samples (30–60 μg) were submitted to electrophoresis at 25–30 volts overnight in 6.5% bisacrylamide gels. The gels were stained with a 0.01% Coomassie blue, 25% isopropanol and 10% acetic acid solution overnight at room temperature, and destained with a 5% methanol, 7.5% acetic acid solution. The gels were stained with the PAS reagent according to the procedure of Zaccharius et al. [29]. Coomassie blue stained protein were quantitated using an Isco gel scanner connected to an Isco UA-5 recorder or by a procedure previously described [30].

Calculations

The effective masses were calculated with this equation:

$$M_p(1 - \phi'\rho) = 6\pi N\eta R_s s$$

where M_p and $(1 - \phi'\rho)$ are the molecular weight and the effective buoyancy of the protein; R_s and s are the protein Stokes radius and sedimentation coefficient; N and η represents the Avogadro's number and solvent viscosity [15].

Results

Band 3 concentrations

Band 3 concentrations in fractions collected after sucrose gradient sedimentation and during Sepharose 4B column chromatography could be determined by Cerenkov radioactivity counting after its isolation from ^{32}P-labelled erythrocytes. However, the ability to determine its concentrations by this method was not due to its phosphorylation by an endogenous protein kinase, but to ^{32}P-labelled nonpolar phospho-organic substances bound noncovalently to Band 3 since almost all the radioactive substances eluted on a SDS-Sepharose 4B column at the same position as that of the SDS micelle having a Stokes radius of 3.5 nm and that Band 3 eluted on a SDS-Sepharose 4B column with a Stokes radius of 10 nm (data not shown).

A feature of the ^{32}P-labelled nonpolar phospho-organic substances is that their binding to Band 3 is not

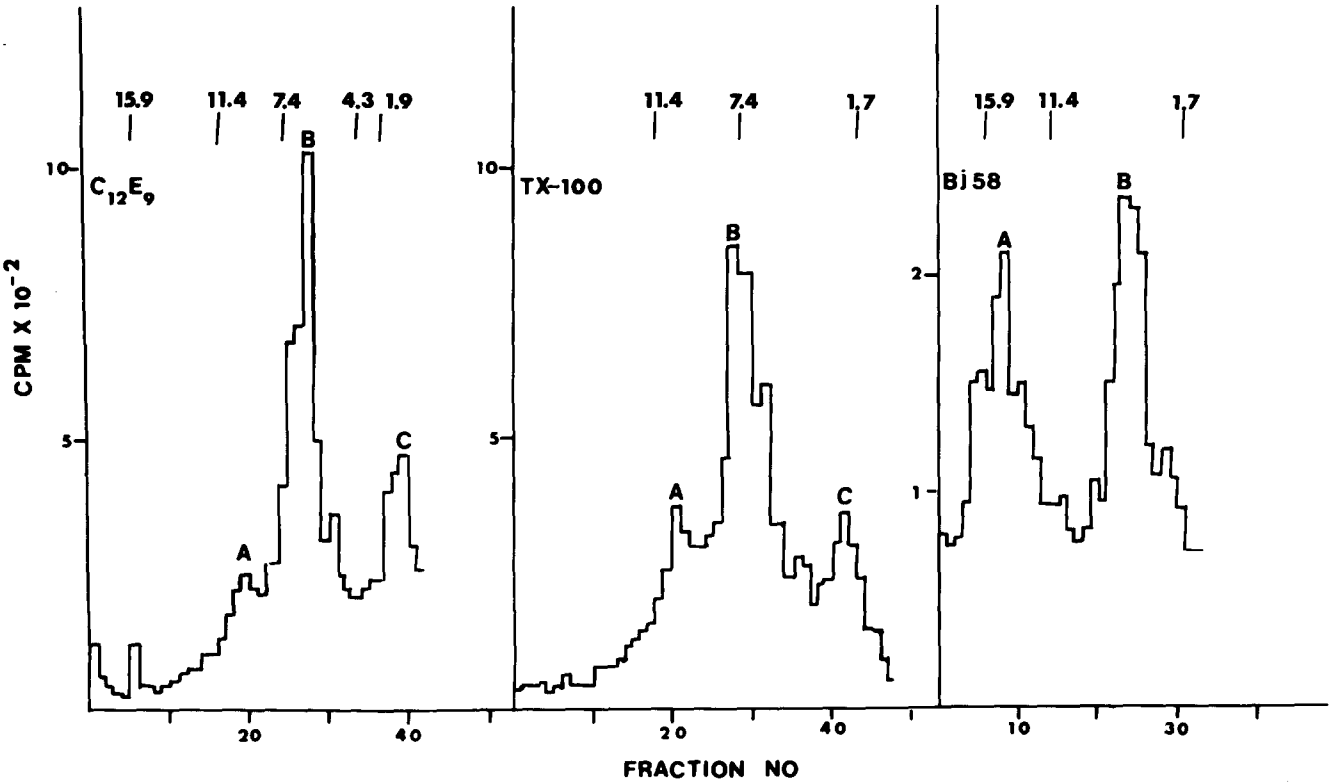

Fig. 1. Sucrose gradient sedimentation profiles of Band 3 isolated in $C_{12}E_9$, Triton X-100 and Brij 58 solutions from ^{32}P-labelled erythrocytes. Samples of Band 3 in $C_{12}E_9$, Triton X-100 and Brij 58 solutions with cpm of 12000, 17000 and 6000 were centrifuged, respectively. The different Band 3 samples were prepared at different times, and larger size fractions were collected in the Brij 58 solution. The positions of sedimentation of the standards for each centrifugation are indicated. The standards were: β-galactosidase (15.9 S), catalase (11.3 S), aldolase (7.4 S), bovine serum albumin (4.3 S), lysozyme (1.9S) and cytochrome *c* (1.7 S). Count recoveries were 70–75%.

tight in the nonionic detergent solutions used, as mainly indicated by the presence of radioactive peaks after sucrose gradient sedimentation and Sepharose 4B column chromatography at sedimentation and elution positions of the detergent micelles. The fraction of ^{32}P-labelled phospho-organic substances unbound to Band 3 in a Brij 58 solution was relatively low, but in $C_{12}E_9$ and Triton X-100 solutions could represent 30–50%. The ^{32}P-labelled phospho-organic substances have not been identified. However, they are likely to be phospholipids since phospholipids extracted from ^{32}P-labelled erythrocyte membranes eluted on a SDS-Sepharose 4B column at the same elution position as that of the SDS micelle. Presumably, they would be phosphatidic acid and phosphatidylinositol derivatives since almost all the ^{32}P-labelled inorganic phosphate incorporated into phospholipids is found in these two groups, which represent 1–3% of the total phospholipids [31].

Band 3 in $C_{12}E_9$, Triton X-100 and Brij 58 solutions

The sucrose gradient sedimentation profiles of Band 3 isolated in $C_{12}E_9$, Triton X-100 and Brij 58 solutions from about 2 ml of ^{32}P-labelled erythrocytes indicated the presence of two forms of Band 3 in each detergent solution, a slow form and a fast form (Peaks A and B): the peaks observed at the tops of the gradients in $C_{12}E_9$ and Triton X-100 solutions (peaks C) were due to ^{32}P-labelled phospho-organic substances in the micelles of these detergents (Fig. 1). The elution profiles of Band 3 in the three detergent solutions were typical,

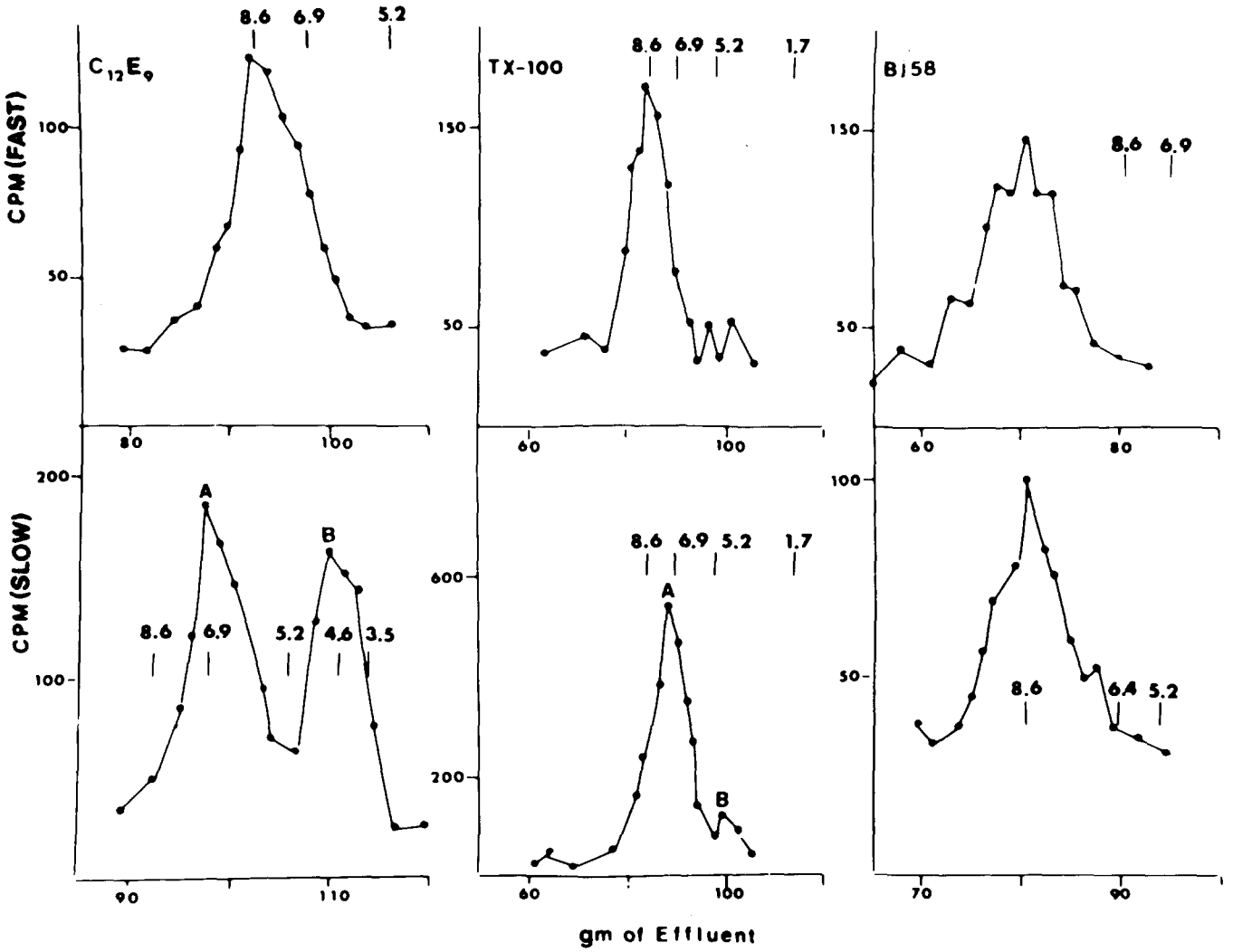

Fig. 2. Elution profiles of the fast and slow forms of Band 3 in $C_{12}E_9$, Triton X-100 and Brij 58 solutions on Sepharose 4B columns after their separation by sucrose gradient sedimentation. The fast and slow forms of Band 3 in the different detergent solutions that were applied on the Sepharose 4B columns were the same as those observed in the sucrose gradient sedimentation profiles shown in Fig. 3. Peaks A and B in some elution profiles of the slow forms of Band 3 are at the positions of elution of the slow form of Band 3 and of the detergent micelle, respectively. The positions of elution of the standards are indicated. The standards were: thyroglobulin (8.6 nm), β-galactosidase (6.9 nm), ferritin (6.4 nm), catalase (5.2 nm), aldolase (4.6 nm), bovine serum albumin (3.5 nm), and cytochrome *c* (1.7 nm), respectively. Different Sepharose 4B columns were used in the different detergent solutions. Count recoveries were 70–85%.

except that two of the four sedimentation profiles of Band 3 obtained in a Brij 58 solution suggested the presence of a third peak near the fast form peak. Peak area measurements indicated that fractions of the slow and fast forms of Band 3 in $C_{12}E_9$ and Triton X-100 solutions were 80–85% and 15–20% while in a Brij 58 solution they were about 55% and 45%.

The following observations further confirmed that Band 3 exists under two stable forms in the different detergent solutions. (1) After sucrose gradient sedimentation, each form eluted on a Sepharose 4B column as a single species with an apparent Stokes radius consistent with its apparent sedimentation coefficient: peak B in some of the elution profiles is due to ^{32}P-labelled phospho-organic substances in the detergent micelle (Fig. 2, Table I). (2) The elution profile on a Sepharose 4B column of Band 3 isolated in a Brij 58 solution from ^{32}P-labelled erythrocyte, showed the presence of two forms of Band 3 whose positions and relative proportions were the same as those observed after sucrose gradient sedimentation (data not shown). (3) SDS-PAGE of Band 3 isolated in a Triton X-100 solution from unlabelled erythrocyte indicated the presence of two forms of Band 3 whose proportions and relative positions were the same as those isolated from labelled erythrocytes (Fig. 3). (4) The sedimentation coefficients and Stokes radii of the two forms of Band 3 in the $C_{12}E_9$ solution were the same as those of the two forms of human and bovine Band 3 previously observed in the same detergent solution, which have been shown to be dimer and tetramer [7,8].

Table I summarizes apparent values of sedimentation coefficients, Stokes radii and calculated effective masses of the slow and fast forms of Band 3 in the different detergent solutions.

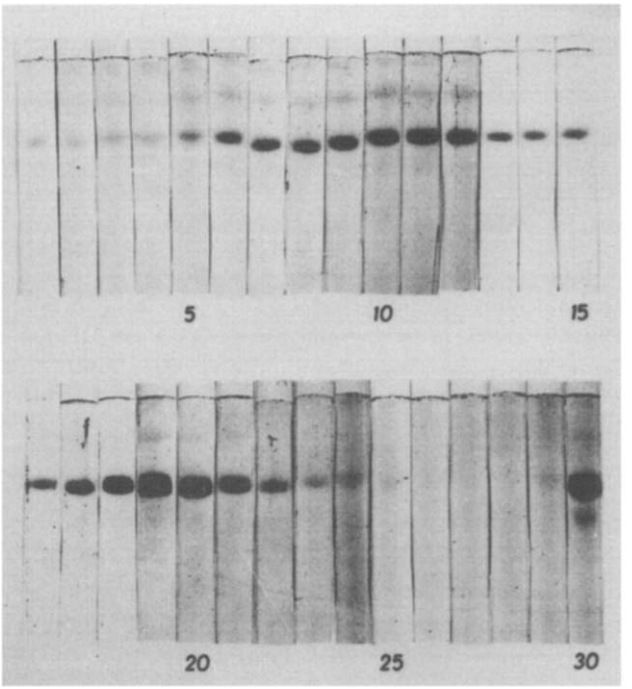

Fig. 3. The sucrose gradient sedimentation profile of Band 3 isolated in a Triton X-100 solution from unlabelled erythrocyte membranes. A 0.5 ml of an isolated Band 3 sample was centrifuged (approx. 1 mg) and fractions of equal volume collected. Aliquotes of 250 μl of each fraction were submitted to SDS-polyacrylamide gel electrophoresis. Gels 1 and 29 represent the bottom and top fractions of the gradient, respectively. Gel 30 is the isolated Band 3 sample centrifuged. The markers catalase (11.3 S) and aldolase (7.4 S) were in maximum amounts in the fractions 9 and 19, respectively. A quantitation of the amounts of Coomassie blue bound to Band 3 indicates that the relative proportion of the two forms of Band 3 is the same as those observed with Band 3 isolated from ^{32}P-labelled erythrocytes.

TABLE I

Apparent values of sedimentation coefficients, Stokes radii and effective masses of the slow and fast forms of Band 3 in different detergent solutions

Average values ± average deviations in n determinations (n given in parentheses). The apparent Stokes radii (nm) of the $C_{12}E_9$, Triton X-100 and Brij 58 micelles were 4.9 ± 0.4 (3), 4.9 ± 0.2 (3), 6.4 ± 0.2 (6), respectively, while their apparent sedimentation coefficients (S) were 0.53, 1.49 and 0.92, respectively. The latter were calculated on the basis of the molecular weights, partial specific volumes and Stokes radii of the detergent micelles [7,15,33].

Detergent	Form	s (S)	R_s (nm)	$M_p(1-\phi'\rho)$ ($\times 10^{-3}$)	Mass ratio
$C_{12}E_9$	slow	6.5 ± 0.4 (4)	7.2 ± 0.2 (3)	53.1	
	fast	9.7 ± 0.2 (4)	8.6 ± 0.1 (2)	94.7	1.78
TX100	slow	7.7 ± 0.4 (4)	7.5 ± 0.2 (4)	65.5	
	fast	10.9 ± 0.4 (4)	8.9 ± 0.1 (2)	110.1	1.68
Brij 58	slow	5.2 ± 0.3 (4)	8.5 ± 0.2 (9)	50.2	
	fast	13.7 ± 0.6 (4)	9.9 ± 0.1 (9)	153.9	3.06

The fractions of the slow and fast forms of Band 3 in a Brij 58 solution depended of the weight ratio of detergent to protein used for membrane solubilization. In one experiment, Band 3 was isolated in a Brij 58 solution after solubilization of ^{32}P-labelled erythrocyte membranes with a 25% $C_{12}E_9$ solution instead of a 10% Brij 58 solution. The sedimentation and elution profiles indicated only the presence of the slow form of Band 3 (data not shown). In another experiment, Band 3 was isolated in a Brij 58 solution in an amount 25–50 times greater from unlabelled erythrocyte membranes solubilized with a weight ratio of detergent to protein of 35:1 instead of 65:1. The elution profiles of two Band 3 samples indicated only the presence of the fast form while that of a third one showed that the fraction of the fast form was about 60% instead of 45% observed when the isolation was done from ^{32}P-labelled erythrocyte membranes (data not shown). These results are not entirely unexpected since an increase of the

weight ratio of detergent to protein would promote the dissociation of Band 3 subunits or prevent their association.

Discussion

The studies of the states of association of Band 3 in $C_{12}E_9$, Triton X-100, and Brij 58 solutions by sucrose gradient sedimentation and by Sepharose 4B column chromatography show that Band 3 in each detergent solution exists in two stable forms. The similarities of the Stokes radii and sedimentation coefficients of the two forms of Band 3 in $C_{12}E_9$ and Triton X-100 solutions with those of human and bovine Band 3 previously isolated in these detergent solutions clearly indicate that they are dimer and tetramer (Table I) [7,8].

Concerning the two forms of Band 3 in the Brij 58 solution, the following pieces of evidence indicate that they are dimer and hexamer. (1) The Band 3 dimers in $C_{12}E_9$ and Triton X-100 solutions were the smallest species observed when Band 3 was isolated at a low concentration from ^{32}P-labelled erythrocyte membranes, indicating a very tight binding between the Band 3 dimer subunits which is unlikely to be broken by the nonionic detergent Brij 58. (2) The size of the fast form of Band 3 in the Brij 58 solution is inconsistent with that of a tetramer since its sedimentation coefficient is higher than that of the corresponding form of Band 3 in Triton X-100 and $C_{12}E_9$ solutions, while the sedimentation coefficient of the slow form of Band 3 in the Brij 58 solution is lower than those of the corresponding forms of Band 3 in Triton X-100 and $C_{12}E_9$ solutions (Table I). (3) It appears unlikely that the Stokes radius of the fast form of Band 3 in the Brij 58 solution has been grossly overestimated since the Stokes radii of the fast forms of Band 3 are both significantly higher than those of slow forms in $C_{12}E_9$ and Triton X-100 solutions (Table I). (4) The ratio of the effective mass of the fast form to that of the slow form of Band 3 in each detergent solution is about two, except that in a Brij 58 solution which is about three. (5) The effective mass of a protein is the product of its molecular weight (M_p) and buoyancy factor ($1 - \phi'\rho$), which depends on the amounts of phospholipids and detergent bound to the protein and their partial specific volumes [15]. It is unlikely that the buoyancy factor differs significantly for the different detergents since the effective masses of the slow forms of Band 3 in different detergent solutions, are about the same (Table I).

While the data of Band 3 in $C_{12}E_9$ and Triton X-100 solutions further support the view that Band 3 is a mixture of stable dimer and tetramer in these detergent solutions [7,8], the data of Band 3 in the Brij 58 solution are potentially of greater interest since they suggest for the first time that Band 3 may exist as a hexamer in the membrane. However, further works would be required to confirm this possibility since relatively little is known of factors affecting Band 3 states of association in the membrane and in detergent solutions. Toward this end the radiochemical method described in this article for monitoring Band 3 in detergent solutions may prove useful for investigating them.

Acknowledgements

This work was initiated in the laboratories of Dr. Allen D. Roses and Dr. Charles Tanford. It was extensively pursued in the laboratory of the late Dr. André Barbeau. This work was partly supported by the grant NSERC OGPIN 002. We thank Dr. Darryl R. McClaslin for advice and Ms. Lorraine Charette for secretarial assistance.

References

1 Cabantchik, Z.I., Knauf, P.A. and Rothstein, A. (1978) Biochim. Biophys. Acta 515, 239–302.
2 Bennett, V. (1985) Annu. Rev. Biochem. 54, 273–304.
3 Yoon, S.C., Toon, M.R. and Solomon, A.K. (1984) Biochim. Biophys. Acta 778, 385–389.
4 Low, P.S. (1985) Biochim. Biophys. Acta 864, 145–167.
5 Clarke, S. (1975) J. Biol. Chem. 250, 5459–5469.
6 Reithmeier, R.A.F. (1979) J. Biol. Chem. 254, 3054–3060.
7 Nakashima, H. and Makino, S. (1980) J. Biochem. (Tokyo) 88, 993–947.
8 Nakashima, H., Nagawa, Y. and Makino, S. (1981) Biochim. Biophys. Acta 643, 509–518.
9 Pappert, G. and Schubert, D. (1983) Biochim. Biophys. Acta 730, 32–47.
10 Schubert, D., Boss, K., Dorst, H.J., Flossdorf, J. and Pappert, G. (1983) FEBS Lett. 163, 81–84.
11 Cuppoletti, J., Goldinger, J., Kang, B., Inho, J., Berenski, C. and Jung, C.Y. (1985) J. Biol. Chem. 260, 15714–15717.
12 Jennings, M.L. (1984) J. Membr. Biol. 80, 105–117.
13 Jay, D. and Cantley, L. (1986) Annu. Rev. Biochem. 55, 511–538.
14 Passow, H. (1986) Rev. Physiol. Biochem. Pharmacol. 103, 61–203.
15 Tanford, C. and Reynolds, J.A. (1976) Biochim. Biophys. Acta 457, 133–170.
16 Helenius, A, McClaslin, D.R., Fries, E. and Tanford, C. (1979) Methods Enzymol. 56, 734–749.
17 Reynolds, J.A. and Karlin, A. (1978) Biochemistry 17, 2035–2038.
18 Schliwa, M., Van Blerkon, J. and Porter, K.R. (1981) Proc. Natl. Acad. Sci. USA 78, 4329–4333.
19 Dzandu, J.K. and Johnson, R.M. (1980) J. Biol. Chem. 255, 6382–6386.
20 Dodge, J.T., Mitchell, C. and Hanahan, D. (1963) Arch. Biochem. Biophys. 101, 119–130.
21 Findlay, J.B.C. (1974) J. Biol. Chem. 249, 4398–4403.
22 Yu, J. and Steck, T.L. (1975) J. Biol. Chem. 250, 9170–9175.
23 Ross, A.H., McConnel, H.M. (1977) Biochem. Biophys. Res. Commun. 74, 1318–1325.
24 Bennet., V. (1982) Biochim. Biophys. Acta 689, 475–484.
25 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468.

26 Wong, P., Barbeau, A. and Roses, A.D. (1985) Anal. Biochem. 146, 191–198.
27 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–27.
28 Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412.
29 Zaccharius, R.M., Zell, T.E., Morrison, J.H. and Woodlock, J.J. (1969) Anal. Biochem. 30, 148–152.
30 Wong, P., Barbeau, A. and Roses, A.D. (1985) Anal. Biochem. 150, 288–293.
32 Ferrell, J.E., Jr. and Huestis, W.H. (1984) J. Cell Biol. 98, 1992–1998.
33 Helenius, A. and Simons, K. (1975) Biochim. Biophys. Acta 415, 29–79.