Molecular Characterization of the Band 3 Protein from Southeast Asian Ovalocytosis*

(Received for publication, November 6, 1992, and in revised form, February 5, 1993)

Vivian E. Sarabia, Joseph R. Casey, and Reinhart A. F. Reithmeier

From the Medical Research Council Group in Membrane Biology, Department of Medicine and Department of Biochemistry, University of Toronto, Toronto, Ontario M5S 1A8, Canada

Southeast Asian ovalocytosis (SAO) is a hereditary form of elliptocytosis resulting in rigid, oval-shaped erythrocytes resistant to invasion by malaria parasites. The molecular defect is due to deletion of codons 400–408, encoding a 9-amino-acid sequence located at the boundary between the cytosolic and the first transmembrane segment in Band 3, the erythrocyte anion transport protein. We have carried out an extensive characterization of Band 3 isolated from SAO erythrocytes which contain about 50% mutant Band 3. A slightly higher proportion of Band 3 in SAO erythrocytes was left associated with the cytoskeleton after extraction of ghost membranes with non-ionic detergents. Size exclusion high performance liquid chromatography analysis showed that SAO Band 3 contained a higher proportion of tetramers relative to dimers (50% tetramer) than normal Band 3 (33% tetramer). The circular dichroism spectrum of Band 3 from SAO erythrocytes was very similar to the spectrum for normal Band 3. Enzymatic deglycosylation and tomato lectin binding showed that SAO Band 3 lacked the polylactosaminyl oligosaccharide found on normal Band 3. SAO Band 3 was unable to bind the anion transport inhibitor 4-benzamido-4'-aminostilbene-2,2'-disulfonate, suggesting a dramatic alteration in the inhibitor binding site. In conclusion, deletion of 9 amino acids from Band 3 on the cytosolic side of the membrane affects the properties (glycosylation and inhibitor binding) of Band 3 on the opposite side of the membrane without dramatic changes in the secondary and quaternary structure of the protein.

Southeast Asian Ovalocytosis (SAO) is a red blood cell condition marked by altered cell morphology, which is found in certain individuals of Southeast Asian descent. Individuals with the SAO phenotype show resistance to invasion by the malarial parasites Plasmodium knowlesi and Plasmodium falciparum (Kidson et al., 1981; Hadley et al., 1983). However, individuals with SAO are asymptomatic and are not adversely affected by the altered red cell morphology (Liu et al., 1990). The mechanism by which the SAO mutation affords resistance to malarial invasion has not been determined. Alteration of cytoskeleton-membrane interaction is implicated since cytoskeletal rearrangements are required for erythrocyte invasion by malarial parasites (Bannister and Dziukiewicz, 1990). In addition to the ovalocytic shape of SAO red cells, red cell ghosts prepared from cells of SAO individuals undergo salt-induced shape changes which control individuals do not (Liu et al., 1990). SAO red cells are very rigid, (Mohandas et al., 1984; Saul et al., 1984; Schofield et al., 1992b) perhaps due to increased interaction of the cytoplasmic domain of Band 3 with the cytoskeleton, since the rotational (Tilley et al., 1991) and lateral (Liu et al., 1990; Mohandas et al., 1992) mobilities of Band 3 are reduced in SAO red cells.

The molecular basis for SAO was recently identified as a 9-amino-acid deletion (residues 400–408) in Band 3 (Jarolim et al., 1991; Tanner et al., 1991; Mohandas et al., 1992; Schofield et al., 1992a). Band 3, the 95-kDa anion-exchange protein of the erythrocyte membrane, has been cloned and sequenced (Kopito and Lodish, 1985; Tanner et al., 1988). The protein is composed of two domains: an NH2-terminal 43-kDa cytoplasmic domain and a 52-kDa membrane domain, predicted to span the bilayer 12–14 times (Kopito and Lodish, 1985). The membrane domain alone is responsible for anion-exchange activity (Grinstein et al., 1979; Lepke et al., 1992). The mutation spans the boundary between the cytoplasmic domain and the predicted first transmembrane segment of Band 3. Heterozygotes for SAO are afforded protection against malarial infection, but the homozygous state is probably lethal since no homozygotes have been found (Jarolim et al., 1991). In all alleles coding for SAO Band 3, an associated mutation (Lys → Glu) known as the "Memphis mutation" (Yannoukakos et al., 1991), was found. The Memphis mutation alters the electrophoretic mobility of Band 3 (Mueller and Morrison, 1977). This allows identification of SAO and control Band 3 by SDS-polyacrylamide gel electrophoresis of proteolytic fragments of Band 3 (Palatnik et al., 1990).

The cytoplasmic domain of Band 3 links the cytoskeleton to the plasma membrane by binding to ankyrin and Band 4.2 protein (Bennett, 1990). Band 3 is associated in the membrane as a mixture of dimers and tetramers, and the tetramers are thought to interact preferentially with the cytoskeleton (Casey and Reithmeier, 1991). The SAO mutation may increase Band 3 binding to the intermediary protein, ankyrin (Liu et al., 1990). Anion-exchange is sensitive to inhibition by stilbene disulfonates such as DIDS, BADS, and 4-acetamido-4'-isothiocyanato-2,2'-disulfonate, which bind to an extracellular site on Band 3 (Cabanthich and Greger, 1992). Marked
alteration of the membrane domain of SAO Band 3 is indicated by the inability to label the protein with several anion-exchange inhibitors (Moriyama et al., 1992; Schofield et al., 1992a). Moreover, the protein is not able to carry out anion exchange (Moriyama et al., 1992; Schofield et al., 1992a). Differential scanning calorimetry of SAO membranes has shown that the membrane domain is largely denatured (Moriyama et al., 1992). In contrast, the cytoplasmic domain retains its normal structural and functional properties (Moriyama et al., 1992).

To understand the consequences of the SAO deletion upon Band 3 structure, we have undertaken a characterization of Band 3 isolated from individuals with SAO.

**EXPERIMENTAL PROCEDURES**

*Materials*—Biotinylated tomato lectin was purchased from Sigma. Triton X-100, chymotrypsin, and glycosidase F mixture, containing both endoglycosidase F and N-glycosidase F, were obtained from Boehringer Mannheim, Germany. Vectastain® was obtained from Vector Laboratories. C12E8 was purchased from Sigma, Nikko Chemical Co., Tokyo. Proteins used as references for calibration were supplied by Pharmacia LKB Biotechnology Inc. The SEC 4000 columns were from Beckman. Blood was collected from control individuals and individuals with SAO into acid-citrate-dextrose as anticoagulant. All other chemicals were reagent grade or better.

**Preparation of Mutant Band 3**—Red cells (25% hematocrit in 0.1 M NaCl, 5 mM sodium phosphate, pH 7.4) were treated at 37°C with 0.5 mg/ml of chymotrypsin, to digest Band 3 at a known exosialic site (Palatnik et al., 1990). Digestion was stopped by addition of phenylmethylsulfonyl fluoride to 1 mM and incubation for 10 min. Band 3 was then prepared from the cells as described below. Upon SDS-polyacrylamide gel electrophoresis, SAO Band 3 differed from normal Band 3 in the migration position of the 60-kDa chromyotryptic fragment (Palatnik et al., 1990). The proportions of the two forms of Band 3 were quantitated by gel scanning of the Coomassie Blue-stained gel, using a Hoefer GS 300 densitometer and Rainin MacIntegrator™ software.

**Measurement of the Fraction of Band 3 Bound to the Cytoskeleton**—The fraction of Band 3 retained by the cytoskeleton was determined in parallel for erythrocytes from both control and SAO individuals. Red cell ghosts (4 mg protein/ml) were extracted with 5 volumes of 0.1% (w/v) C12E8, 5 mM sodium phosphate, pH 8.0, or 1% (v/v) Triton X-100 solution phosphohexose, and incubated on ice for 15 min. The cytoskeleton and any associated Band 3 were pelleted by centrifugation at 35,000 revolutions/minute in a Beckman Ti-75 rotor. The supernatant was removed, and the tubes were each made up to their original volume with 5 mM sodium phosphate, pH 8.0. Aliquots of the sample before centrifugation and of the pellet and supernatant after centrifugation were solubilized with sample buffer (Laemmli, 1970). After electrophoresis, the amount of Band 3 in each lane was quantitated by scanning, as above.

**Isolation of Band 3 and the Membrane Domain**—Band 3 was prepared as previously described (Casey et al., 1989). Briefly, red cells were washed, ghosts were prepared by osmotic hemolysis, and the membranes were stripped of peripheral proteins with ice-cold 2 mM EDTA, pH 12. Membranes were solubilized with 1% C12E8 (w/v), applied to aminomethyl-Sepharose 4B, and eluted with a 0–0.25 M linear NaCl gradient in 0.1% C12E8 (v/v), 5 mM sodium phosphate, pH 8.0. The 52 kDa membrane domain of Band 3 was prepared by trypsin treatment of red cell ghosts, followed by alkali stripping, solubilization in C12E8, and DEAE-Sepharose chromatography (Casey et al., 1989). 

**Deglycosylation of Band 3**—Purified Band 3 (0.5–2 mg protein/ml) in 0.1% C12E8, 100 mM NaCl, 5 mM sodium phosphate, pH 8.0, was treated with N-glycosidase F mixture (2 units/mg protein) at room temperature for 24 h, at room temperature (Casey et al., 1989). Control samples were incubated under identical conditions, including a blank solution of the same composition as the enzyme solution. Cleavage of the carbohydrate chain from Band 3 was assessed by an increase in mobility and sharpening of the protein band in Laemmli gels (Laemmli, 1970). After electrophoresis, the amount of Band 3 in each lane was quantitated by scanning, as above.

**RESULTS**

**Proportion of Mutant Band 3 in Ovalocytes**—Fig. 1 compares the SDS-gel electrophoresis pattern of proteins from normal (lane 1) and SAO (lane 2) ghost membranes prepared from control (panel A) and chymotrypsin-treated cells (panel B). While the mobility of intact Band 3 is indistinguishable in the two samples, the SAO ghosts prepared from chymotrypsin-treated cells contains a doublet at the position of the 60,000-dalton chromyotryptic fragment of Band 3. The lower band comigrates with the normal 60,000 dalton band while the upper band represents the SAO form of Band 3 (Palatnik et al., 1990). The slower mobility of the chromyotryptic fragment

**FIG. 1.** Electrophoretic properties and quantitation of mutant Band 3. A, SDS-polyacrylamide gel electrophoresis of solubilized red cell ghost protein (10 μg) from a control individual (I) and an individual with SAO (2). Arrow indicates the position of Band 3. B, SDS-PAGE of ghosts prepared from red cells that had been treated with chymotrypsin. Lower arrow indicates the 60-kDa fragment from the wild-type form of Band 3, and the upper arrow marks the 68-kDa SAO form.
of SAO Band 3 ($M_r = 63,000$) is due to a point mutation (Memphis Band 3, Lys$^{66} \rightarrow$ Glu) in SAO Band 3 (Mueller and Morrison, 1977) that is present in addition to the 9-amino-acid deletion (Ala$^{404}-$Ala$^{408}$). Quantitation of the proportion of 63,000 and 60,000 fragments by gel scanning showed that SAO Band 3 made up 48% of the Band 3 in ghost membranes. The production of the 63,000-dalton fragment indicates that SAO Band 3 retains the exofacial chymotrypsin sensitive site.

**Glycosylation**—The broad nature of Band 3 in SDS gels is due in part to the heterogeneous nature of the oligosaccharide chain attached to Asn$^{42}$ (Tsuji et al., 1980; Fukuda et al., 1984). Purified samples of normal and SAO Band 3 were treated with N-glycosidase F to remove the oligosaccharide chain. The mobility of Band 3 on SDS gels increased upon deglycosylation, with a considerable sharpening of the protein band for Band 3 isolated from both control and SAO erythrocytes (Fig. 2). Deglycosylated Band 3 from SAO individuals ran as a broader band than Band 3 from normal erythrocytes with some overlap between the leading edge of SAO Band 3 ($\text{panel A, lane 3}$) and the trailing edge ($\text{panel A, lane 4}$) after deglycosylation. The slower migrating deglycosylated protein may represent the SAO form, as is the case for the amino-acid deletion (Ala$^{404}-$Ala$^{408}$), if plunged into a hydrophobic environment, would assume a helical conformation. The deletion of a portion of the first transmembrane segment may also result in a more global conformational change in Band 3 structure due to disruption of helix-helix interactions within the membrane domain. Conformational changes of this type may be detected by circular dichroism spectroscopy. The circular dichroism spectra of Band 3 purified from normal and SAO erythrocytes are presented in Fig. 4. No significant difference in the spectra were detected, suggesting that any conformational differences that may exist between normal and SAO Band 3 are too small to be detected by this method. The membrane domains of normal and SAO Band 3 were also purified and characterized by circular dichroism spectroscopy; however, no differences were found in the circular dichroism spectra of these samples.

**Association of Band 3 with the Cytoskeleton**—SAO Band 3 has been reported to have an enhanced interaction with the cytoskeleton (Liu et al., 1990; Tilley et al., 1991; Mohandas et al., 1992). Ghost membranes prepared from control and chymotrypsin-treated cells were therefore selectively extracted with Triton X-100 or C$_2$E$_6$ to determine the proportion of Band 3 that was bound to the detergent-insoluble cytoskeleton. Triton X-100 (1%) solubilized 77% of Band 3 from control ghost membranes and 66% from SAO ghost membranes, the remainder associated with the cytoskeleton. Similarly, C$_2$E$_6$ (1%) solubilized less Band 3 from SAO ghosts (50%) than from control ghosts (66%). The results indicate that slightly more (11-16%) SAO Band 3 was bound to the cytoskeleton, but certainly not equal to the amount of mutant protein (48%).

**Oligomeric Structure**—Tetramers of Band 3 are the oligomeric form of the protein immobilized on the cytoskeleton (Casey and Reithmeier, 1991). The oligomeric structure of Band 3 in control and SAO samples was compared by size-exclusion HPLC (Fig. 3). Previous studies have shown that the major peak represented Band 3 dimers (67%) and the leading shoulder (33%) was Band 3 tetramers and higher oligomers (Casey and Reithmeier, 1991). The HPLC analysis (Fig. 3) showed that there was only a slight increase in the proportion of tetrameric Band 3 in the SAO sample (50%). This increase in tetramer may account for the slightly enhanced binding of Band 3 to the cytoskeleton. The increase in tetramer was not, however, equivalent to the amount of mutant protein.

**Circular Dichroism Spectroscopy**—The deletion of amino acids predicted to be in the first transmembrane segment suggests that the conformation of the transmembrane segment must change to span the membrane (e.g. helix to extended) or that adjacent segments of Band 3 normally exposed to water must be pulled into the membrane. The cytosolic sequence that precedes the deleted residues (Ala$^{404}-$Ala$^{408}$), if plunged into a hydrophobic environment, would assume a helical conformation. The deletion of a portion of the first transmembrane segment may also result in a more global change in Band 3 structure due to disruption of helix-helix interactions within the membrane domain. Conformational changes of this type may be detected by circular dichroism spectroscopy. The circular dichroism spectra of Band 3 purified from normal and SAO erythrocytes are presented in Fig. 4. No significant difference in the spectra were detected, suggesting that any conformational differences that may exist between normal and SAO Band 3 are too small to be detected by this method. The membrane domains of normal and SAO Band 3 were also purified and characterized by circular dichroism spectroscopy; however, no differences were found in the circular dichroism spectra of these samples.

**Inhibitor Binding**—The binding of stilbene disulfonates such as BADS to Band 3 provides a sensitive assay for the native state of the protein (Casey et al., 1989). Fig. 5 compares the BADS titration of normal and SAO Band 3. Both samples bound the inhibitor with the same affinity (1 μM); however, the maximum fluorescence signal of the SAO sample was half that of the control sample at equal protein concentrations. Similar results were obtained by exciting the probe directly at 340 nm and with the purified membrane domains. These results show that SAO Band 3 is completely incapable of binding stilbene disulfonates.

**DISCUSSION**

In this paper we have noted two dramatic alterations in SAO Band 3, both of which involve the external aspect of the protein. The first change is in the carbohydrate structure of the protein. Normal Band 3 receives the addition of a poly-lactosaminyl moiety, a process that occurs in the trans-Golgi (Tsuji et al., 1980; Fukuda et al., 1984). SAO Band 3 escapes this modification. This may be due to an altered structure of the protein such that the recognition elements for this modification are lost, or that SAO Band 3 does not reside for a sufficient length of time in the Golgi compartment responsible for the modification. The change in oligosaccharide structure would not, however, compromise the functioning of the protein as a transporter since we have shown (Casey et al., 1992) that the deglycosylated protein is fully capable of carrying out anion-exchange. It is possible that SAO Band 3 is not glycosylated. Attempts to separate normal and SAO Band 3 by lectin affinity chromatography have not been successful. This
FIG. 3. Band 3 pooled from all Band 3 containing fractions eluted from the aminoethyl-Sepharose column was subjected to size exclusion HPLC. Samples (5 μg) of normal Band 3 (upper panel) and SA0 Band 3 (lower panel) were resolved using a 30 x 0.75-cm SEC4000 size exclusion column, run at room temperature in 0.1% (v/v) C2E8, 0.1 M NaCl, 5 mM sodium phosphate, pH 7.0. The major peak eluting at 7.3 ml corresponds to Band 3 dimers and the leading edge to Band 3 tetramers. Shown at the bottom are the elution positions for the standard proteins T, thyroglobulin (Rn = 86 Å), F, ferritin (63 Å), C, catalase (52 Å), and A, aldolase (46 Å). The void volume, V0, was determined from the elution position of Blue Dextran 2000 (average molecular weight 2 x 10^6) and the total volume, Vt, was determined from the elution position of 2-mercaptoethanol.

is likely due to the heterogeneous nature of the oligosaccharide on normal Band 3, which does not permit complete removal of the glycosylated protein by lectin binding.

The second alteration is more serious. The stilbene disulfonate-binding site in SA0 Band 3 has been destroyed by the SAO mutation. This site faces the cell exterior. Since the deletion is in the first transmembrane helix, this suggests that this helix may form a part of the inhibitor binding site. Alternatively, the structure of the protein may be changed to such an extent that the stilbene site is altered indirectly by the change in the first transmembrane helix. It is significant that the deletion encompasses a portion of the cytosolic domain and the first quarter of the first transmembrane segment, yet the effects are felt on the cell exterior. The deletion must cause a transmembrane effect and suggests that portions of the protein other than the first helix are affected by the mutation. The loss of the stilbene disulfonate-binding site accounts for the inability to covalently label Band 3 with tritiated DIDS (Schofield et al., 1992a).

The mutation does not, however, affect the secondary structure of Band 3 as determined by circular dichroism measurements of the purified protein. The same conclusion was reached by Moriyama et al. (1992) by comparison of the circular dichroism spectra of normal and SAO inside-out vesicles which are enriched in Band 3. In addition, the SAO Band 3 forms dimers and higher oligomers, and it binds to the cytoskeleton. The regions of Band 3 involved in dimer and tetramer formation and cytoskeleton binding are not altered greatly by the SAO mutation. The membrane domain of Band 3 is dimeric and, due to its high helical content, the interactions responsible for dimer formation likely involve helix-helix interactions. Since SAO Band 3 forms oligomers, it is likely that the first transmembrane helix is not involved in dimer formation. SAO Band 3 is present in about equal amounts as normal Band 3 in SAO ovalocytes. This indicates that the biosynthesis, movement from the endoplasmic reticulum through the Golgi to the plasma membrane, and the stability of the mutant protein are quite normal. One issue of importance is the possibility that SAO Band 3 forms heterodimers or higher oligomers with normal Band 3. Attempts to
The excitation wavelength was 280 nm and the emission wavelength was as normal Band 3 (solid line) and SAO Band 3 (dashed line), measured at room temperature. Band 3 (pooled peak fractions from aminoethyl-Sepharose column) in 0.1% C12E10 (v/v), 0.1 M NaCl, 5 mM sodium phosphate, pH 8.0, was diluted 10-fold into distilled water, to a final protein concentration of 60 µg/ml.

Separate normal and SAO Band 3 by lectin or inhibitor affinity chromatography have not been successful, suggesting that hetero-oligomers may exist. The fact that SAO Band 3 affects the thermal transition of normal Band 3 supports this view (Moriyama et al., 1992). If heterodimers form, then the mutant protein may "piggyback" its way to the cell surface using the normal protein. The stiibine binding results suggest that if heterodimers exist, then the ability of normal Band 3 to bind the inhibitor or transport is not seriously compromised when bound to a totally defective protein.

Acknowledgments—Ovalocytes were kindly donated by “B.S.”, with the assistance of Dr. D. Amato, Mount Sinai Hospital, Toronto. Dr. J. Palek, Division of Hematology/Oncology, St. Elizabeth’s Hospital, Boston, is thanked for encouraging us to undertake the structural characterization of Band 3 from ovalocytes.

REFERENCES

Bannister, L. H., and Dhuzewski, A. R. (1966) Blood Cells 16, 257-292
Bennett, V. (1990) Physiol. Rev. 70, 1029-1065
Cabantchik, Z. I., and Greger, R. (1992) Am. J. Physiol. 31, C803-C827
Casey, J. R., and Reithmeier, R. A. F. (1991) J. Biol. Chem. 266, 15726-15737
Casey, J. R., LaBerge, D. M., and Reithmeier, R. A. F. (1989) Methods Enzymol. 175, 494-512
Casey, J. R., Furgal, C. A., and Reithmeier, R. A. F. (1992) J. Biol. Chem. 267, 11340-11348
Fukuda, M., Dell, A., Dines, J. E., and Fukuda, M. N. (1984) J. Biol. Chem. 259, 8260-8273
Grinstein, S., Ship, S., and Rothstein, A. (1979) Biochim. Biophys. Acta 507, 294-304
Hadley, T., Saul, A., Lamont, G., Hudson, D. E., Miller, L. H., and Kidson, C. (1983) J Clin. Invest. 71, 780-782
Jarolim, P., Palek, J., Amato, D., Hassan, K., Sapak, P., Nurse, G. T., Rubin, H. L., Zhai, S., Zahr, E. R., and Liu, C.-S. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 11022-11026
Kidson, C., Lamont, G., Saul, A., and Nurse, G. T. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 5529-5532
Kopito, R. R., and Lodish, H. F. (1985) Nature 316, 234-238
Laemmli, U. K. (1970) Nature 227, 680-685
LeMaire, M., Aggerbeck, L. P., Monteilhet, C., Andersen, J. P., and Moller, J. V. (1986) Anal. Biochem. 154, 525-535
Lepke, S., Becker, A., and Passow, H. (1992) Biochim. Biophys. Acta 1106, 13-16
Lin, S.-C., Zhai, S., Palek, J., Golan, D. E., Amato, D., Hassan, K., Nurse, G. T., Banbio, D., Coetzer, T., Jarolim, P., Zaiik, M., and Borwein, S. (1990) New Engl. J. Med. 323, 1530-1538
Lowry, O. H., Roseborough, N. J., Parr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
Merkle, R. R., and Cummings, R. D. (1987) J. Biol. Chem. 262, 8179-8189
Mirendado, N., Lee-Jin, L. E., Freedman, L. M., and Mak, J. W. (1984) Blood 63, 1385-1392
Moriyama, R., Igeuchi, H., Lombardo, C. R., Van Dort, H. M., and Low, P. S. (1992) J. Biol. Chem. 267, 25792-25797
Mueller, T., and Morrison, M. (1977) J. Biol. Chem. 252, 6573-6576
Palatnik, M., Simoes, M. L. S., Alves, Z. M. S., and Laranjeira, N. M. S. (1990) Hum. Genet. 86, 129-130
Pinnell, S., Lamont, G., Sawyer, W. H., and Kidson, C. (1984) J. Cell. Biol. 98, 1348-1354
Schofield, A. E., Reardon, D. M., and Tannor, M. J. A. (1992a) Nature 355, 836-838
Schofield, A. E., Tannor, M. J. A., Pinder, J. C., Cough, B., Bayley, P. M., Nash, G. B., Dhuzewski, A. R., Reardon, D. M., Cox, T. M., Wilson, R. J. M., and Glatzer, W. B. (1992b) J. Biol. Chem. 267, 949-958
Tanner, M. J. A., Martin, P. G., and Hugh, S. (1988) Biochem. J. 256, 703-712
Tanner, M. J. A., Bruice, L. M., Martin, P. G., Reardon, D. M., and Jones, G. L. (1991) Blood 78, 2768-2778
Tilley, L., Nash, G. B., Jones, G. L., and Sawyer, W. H. (1991) J. Membr. Biol. 125, 99-66
Tsui, T., Izumi, T., and Osewa, T. (1980) Biochem. J. 187, 677-686
Yang, J. T., Wu, C.-S. C., and Martinez, H. M. (1986) Methods Enzymol. 130, 289-309
Yamazaki, D., Veasey, C., Driancourt, C., Blouquit, Y., DeLuna, J., Wajnrman, H., and Bousca, E. (1991) Blood 78, 1117-1120

FIG. 4. Far-ultraviolet circular dichroism spectra of normal Band 3 (solid line) and SAO Band 3 (dashed line), measured at room temperature. Band 3 (pooled peak fractions from aminoethyl-Sepharose column) in 0.1% C12E10 (v/v), 0.1 M NaCl, 5 mM sodium phosphate, pH 8.0, was diluted 10-fold into distilled water, to a final protein concentration of 60 µg/ml.

FIG. 5. Binding of the anion-exchange inhibitor, BADS, to normal Band 3 (filled circles) and SAO Band 3 (open circles), as determined by fluorescence enhancement titration. The excitation wavelength was 280 nm and the emission wavelength was 450 nm. Purified Band 3 (100 µl) in 0.1% C12E10 (v/v), 0.1 M NaCl, 5 mM sodium phosphate, pH 8.0, was diluted into 2 ml of 28.5 mM sodium citrate, pH 7.0, at 28 °C, to a final protein concentration of 50 µg/ml.