THE ROLE OF BAND 3 PROTEIN IN OXYGEN DELIVERY BY RED BLOOD CELLS

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ABSTRACT

The synergistic effects of hemoglobin, carbonic anhydrase and the band 3 protein make red blood cells the ideal vehicle for oxygen delivering to the tissues. As long as oxygen is supplied by these ideal vehicles, oxygen intoxication of the tissues is precluded. Band 3 protein mediates the "Chloride-Shift", i.e., the anion exchange of Cl^-/HCO_3^-. Because of the Chloride-Shift, red blood cells are able to recognize metabolically active tissues and to supply the minimum amount of oxygen to the tissues. Investigation into the molecular mechanisms of the anion exchange mediated by the band 3 protein was introduced.

I INTRODUCTION

Oxygen is essential for any form of life, but too much oxygen is harmful and can elicit tissue damage. Living creatures, therefore, have a tightly regulated system delivering the necessary amount of oxygen to specific tissues at the right time. Red blood cells play an important role in this system and provide a vehicle for delivering oxygen to tissues, depending on their metabolic activity, utilising the synergistic effects of hemoglobin, carbonic anhydrase and the anion exchange activity of band 3 protein

Red blood cells lack intracellular organelles normally found in cells, such as the nucleus, microsomes, mitochondria etc. and instead contain a very high concentration of hemoglobin.

Red blood cells are quite flexible, allowing the cells to penetrate rapidly through the capillaries to carry oxygen throughout the body Circulation of red blood cells through the capillaries is very rapid of the order of a millisecond for one cell to pass through the lung capillary vessels.

The life span of red blood cells varies among species, However, the number of recirculations through the heart is virtually identical in rat, dog, and the human being, approximately 2×10^5 (1). After this number of passages the cells are targeted for cell death The signal for cell death probably results from alterations in the red blood cell membrane, either through accumulated damage leading to membrane stiffness or through exposure of antigens that bind immunoglobulins, making the red blood cells target for macrophages (2,3). This review describes the role of red blood cells in oxygen transport and how their membrane proteins, especially band 3 protein, are involved

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in the oxygen delivering system.

II What Types of Membrane Proteins Exist in Red Blood Cells ?

Proteins which are co-fractionated with biological membranes have been defined as the membrane proteins. The membrane proteins can be further classified into two classes, integral membrane proteins and peripheral membrane proteins. Integral membrane proteins are incorporated into the membrane lipid bilayers, surrounded by boundary lipids, while peripheral membrane proteins interact with the surface of membrane lipid bilayers through electrostatic interactions. Membranes play pivotal roles in biological cell functions. Apart from separating the cell from the surrounding environment, and regulating the entry and exit of molecules into the cells, membranes also partition the cell components into the organelles such as the nucleus etc. Furthermore, membranes play important roles not only in ligand-receptor interactions and signal transduction, but also in cell development and differentiation.

Membrane proteins interact with both the lipid bilayer and each other, and through these interactions regulate these membrane functions. Integral membrane proteins often act as receptors, channels and transporters. Some interact with the underlying cytoskeleton and others act as anchors for signal messenger proteins which transduce extracellular signals into the cytosol. Peripheral membrane proteins often form part of the cytoskeleton which supports the membrane integrity flexibility. Some peripheral membrane proteins behave as signal messenger proteins by shuttling between the membrane and

the cytosol.

Fig. 1 shows the red blood cell membrane proteins separated by SDS- polyacrylamide gel electrophoresis. These membrane proteins were named after their electrophoretic mobility. Bands 1, 2, 2.1, 4.1, 4.2, 5 and 6 are the peripheral membrane proteins, while bands 3, 4.5 and 7 are the integral membrane proteins. Fig. 2 shows a schematic model of the red blood cell membrane. The integral membrane proteins, band 3 and band 4.5, act as transporters for anions and glucose, respectively, while the peripheral membrane proteins, bands 1, 2, 2.1, 4.1 and 4.2 form the cytoskeletal network which interacts with the cytosolic domain of band 3 and glycophorin C.

III Synergistic Effects of Hemoglobin, Carbonic Anhydrase and Band 3 Protein in Oxygen Delivery

Fig. 3a shows the red blood cell Red blood cells are unique cells which have no organelles as explained above. Instead, red blood cells contain a very high concentration of hemoglobin to carry oxygen from the lungs to the tissues. Provided the peripheral membrane protein network is correctly organized, red blood cells are highly deformable both in the circulation and *in vitro*. Because of this deformability, red blood cells can quickly pass through the capillaries whose diameter is narrower than the diameter of the cell (Fig. 3b), and deliver oxygen from the lungs to the tissues. When arterial blood arrives in the peripheral capillaries, red blood cells pass through the narrow capillaries one by one and can distinguish metabolically active cells from inactive cells by a combination of three mechanisms : the oxy/deoxy conversion of
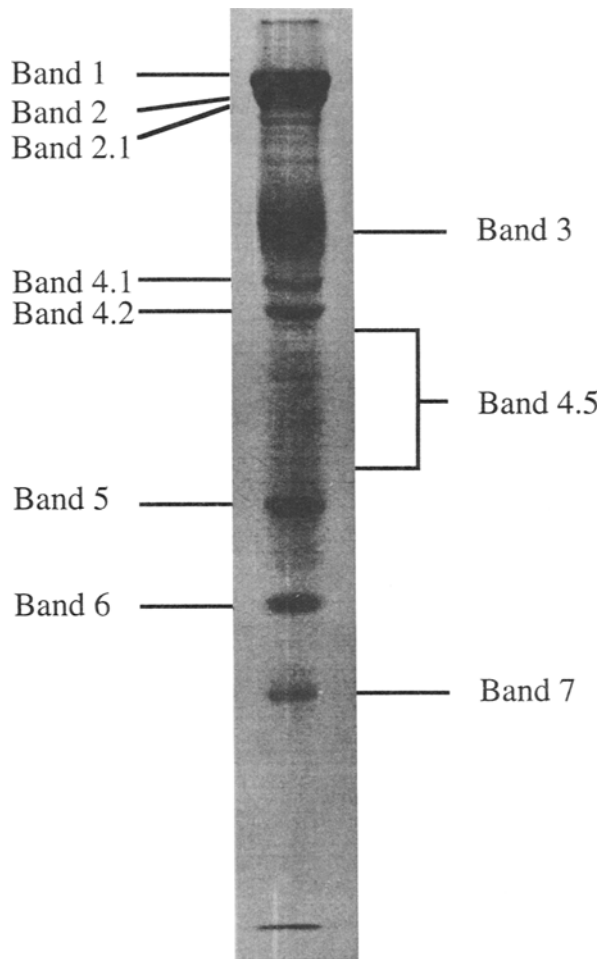
Peripheral   Integral

Band 1 ——
Band 2 —
Band 2.1 ——

Band 3

Band 4.1 ——
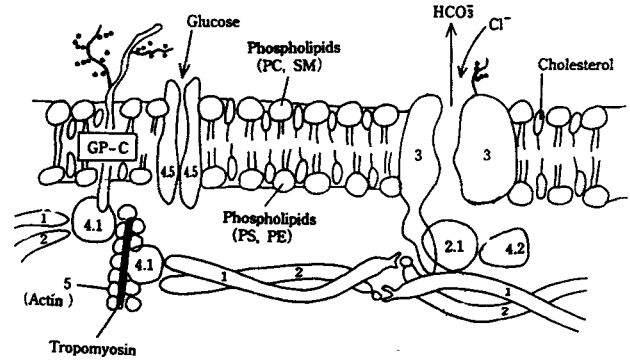Band 4.2 ——

Band 4.5

Band 5 ——

Band 6 ——

Band 7

Fig. 2. Schematic diagram for the proposed mode of interactions between integral and peripheral membrane proteins: Spectrin (bands 1, and 2) is linked to band 3 protein and Kyrin (band 2.1) / band 4.2, and to glycophorin C (GP-C) by band 4.1, which also binds an actin (band 5) filament containing tropomyosin. Cytoskeletal network composed of the peripheral membrane proteins is attached to the red blood cell membrane with the band 3 protein and GP-C sites. Bands 3, 4.5 and 7 are the integral membrane proteins and function as anion exchanger, glucose transporter and water channel, respectively. Glycophorins (GP-A~E) are visualized only when the SDS-polyacrylamide gel (Fig. 1) was stained with PAS staining.

Fig. 1. Red blood cell membrane proteins: Red blood cell membrane proteins were separated by SDS-polyacrylamide gel electrophoresis and stained with Coomassie Brilliant Blue. Bands 1, 2, 2.1, 4.1, 4.2, 5 and 6, and bands 3, 4.5 and 7 are defined as the peripheral membrane protein, and the integral membrane protein, respectively (see text).

hemoglobin, the carbonic anhydrase reaction and the Cl^-/HCO_3^- exchange by band 3 protein (4).

a) Discocyte. Normal biconcave red blood cell.

b) Knizocyte. Abnormal triconcave red blood cell due to an abnormal band 3 protein.
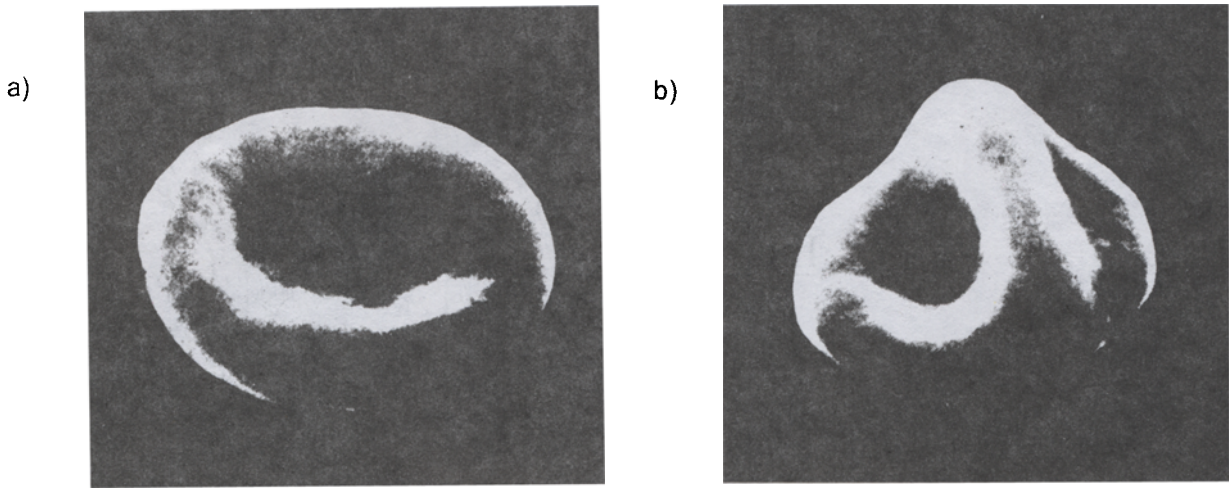
a)

b)

Fig 3 Scanning electron micrograph of red blood cells: From Red Cell Shape, Eds Bessis, M , Weed, R.I., Leblond, P.F. (1973) Springer Verlag New York, Heidelberg, Berlin

As shown in Fig. 4, CO_2 produced in peripheral cells diffuses into the red blood cells as they pass through the capillaires. The diffusing CO_2 is rapidly hydrated to H_2CO_3 inside the red blood cells, by the action of carbonic anhydrase, and the H_2CO_3 promptly dissociates into H^+ and HCO_3^- Band 3 protein exchanges the cellular HCO_3^- with Cl^- in plasma, which has

Metabolically Inactive Cell
(less oxygen)

Metabolically Active Cell
(more oxygen)
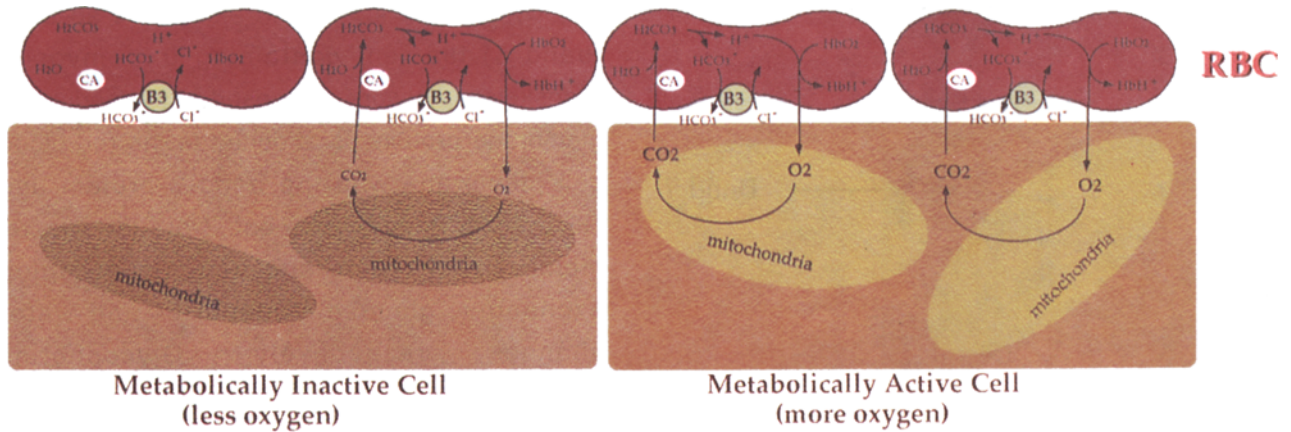
Oxygen Delivering System

Fig. 4. Role of band 3 protein in oxygen delivery by red blood cells: Red blood cells are the ideal vehicles for the oxygen deliverying system to tissues as a result of synergistic effects of hemoglobin, carbonic anhydrase and band 3 protein. As long as oxygen is supplied by these ideal vehicles, oxygen intoxication to tissues is precluded. Band 3 protein mediates the "Chloride-Shift", i.e., the anion exchange of Cl^-/HCO_3^- Because of the Chloride-Shift, red blood cells are able to recognize metabolically active tissues and to supply the minimum amount of oxygen to the tissues as described in the text (partially modified from Hamasaki, N. and Okubo, K. (4) Band 3 protein : physiology, function and structure Cell. Mol Biol 42, 1025-1039.

been known conventionally as the "Chloride Shift". As the result of the anion exchange, the weak acid H_2CO_3 is converted to the strong acid HCl and consequently the intracellular pH of the red blood cells is rendered acidic. This acidification is the trigger for the dissociation of O_2 from oxyhemoglobin (HbO_2) and the dissociated O_2 is supplied to the tissues which metabolically produce CO_2. Protons formed in the red blood cells are accepted by reactive groups of deoxyhemoglobin (HbH^+) participating in the Bohr effect and the pH within the red blood cells is restored in order to prevent the further dissociation of oxygen from HbO_2. By means of the transient acidification triggered by the band 3 protein anion exchange activity, cells producing excess CO_2 are supplied with more O_2 from HbO_2. In this manner, red blood cells can discriminate the metabolically active cells from inactive cells and deliver oxygen particularly to metabolically active cells.

Accordingly, as long as oxygen is delivered by red blood cells, a minimum but sufficient amount of oxygen is supplied to tissues, and as such, oxygen intoxication never occurs. The chain of reactions, ensuring appropriate oxygen supply to metabolically active tissues, should be completed in 0.3~0.7 seconds (5-7). Band 3 protein plays a pivotal role as a metabolic sensor in facilitating the oxygen allocation from hemoglobin to the tissues, by mediating the chloride-bicarbonate exchange across the red blood cell membrane.

IV   Band 3 Protein : Structure and Function

1.   General Structure of Band 3 Protein

Band 3 protein is the major integral membrane protein of human red blood cells and consists of 911 amino acids The NH_2-terminal 360 amino acid residues form a water soluble, highly elongated domain (the 40-kDa domain) that serves as an attachment site for the binding of the membrane skeleton and other cytoplasmic proteins. The remainder of the protein is a 55-kDa hydrophobic domain (the 55-kDa domain) that is responsible for the anion exchange activity. The abundance of this protein has made it a useful model for the study of structure/ function relationship in the transport protein. Instead of involving a ternary complex with an anion to be transported, the transport process can be explained by the ping-pong mechanism, that is, band 3 protein assumes two conformational states during the exchange process, the inward facing form which can bind an anion from the intracellular surface and the outward facing form which can bind an anion from the extracellular surface [reviewed by Passow, (8)]. Over the past few years we have focused our attention on the study of the structure of the active center for anion exchange

2.   Active Center for Anion Exchange

Unlike other organic phosphates, pyridoxal phosphate (PLP) and phosphoenolpyruvate can be transported across the red blood cell membrane by the anion exchange system (9,10). PLP forms a Schiff-base with the lysine residue of the protein and the linkage is stabilized by reducing it with sodium borohydride (11). Using these characteristic features of PLP, we tried to isolate the anion binding site of band 3 protein after treating the cell membrane with PLP (12,13). The specific Schiff-base formation was stabilized and labeled with [^{3}H] sodium

borohydride. The radiolabeled peptide contained 78 amino acids and could be aligned with amino acid residues 834 to 911 of the human erythrocyte band 3 protein. The 78 amino acid-peptide was designated 8.5-kDa peptide (13). The PLP binding site was identified as Lys-851 in the deduced sequence. The anion exchange activity was quantitatively inhibited by modifying Lys-851 with PLP (10), suggesting that the 8.5-kDa peptide resides in a portion of the anion exchange channel. Studies of naturally occurring band 3 mutations also suggest that this region of the band 3 protein is involved in anion exchange activity. The Diego (Di^a) antigen results from the amino acid substitution Pro-854 to Leu. Band 3 protein with this Pro-854-> Leu substitution is more readily labeled by [^{3}H]H$_2$DIDS(4,4'-diisothio-cyanodihydrostilbene-2, 2'-disulfonic acid), suggesting that this substitution alters the environment of Lys-851, the H$_2$DIDS crosslinking site (see below). Band 3HT (high transport) results from the amino acid substitution Pro-868 to Leu. This substitution causes the mutant band 3 protein to be less readily labeled by [^{3}H]H$_2$DIDS. Band 3HT transports sulphate ions 1.5 times more rapidly than normal band 3 protein showing that Pro-868 is involved in the anion exchange activity (14,15). It also suggests the importance of the role of the affinity labeled 8.5-kDa peptide region for the anion exchange activity

Band 3 protein has a single high affinity site for the reversible binding of the stilbenedisulfonate derivatives DIDS (4,4'-diisothiocyanostilbene-2,2'-disulfonic acid) and H$_2$DIDS. which are potent inhibitors of the anion exchange activity (16). It has been known for years that the single bound H$_2$DIDS molecule can

react covalently at neutral pH with a lysine residue in the NH$_2$-terminal chymotryptic fragment (17). Biochemical studies have shown that this rapid reaction must take place with either Lys-539 or Lys-542 of human band 3 protein [reviewed by Passow (8)]. At alkaline pH, the other isothiocyanate group on H$_2$DIDS (not on DIDS) can react with a lysine residue in the COOH-terminal 35-kDa chymotryptic fragment (Met-559~Val-911), forming an intramolecular cross-link between the two major chymotryptic fragments, the NH$_2$-terminal 60-kDa fragement (Met-1~Tyr-553) and the COOH-terminal 35-kDa fragment (18). We determined that the H$_2$DIDS cross-linked sites were Lys-539 and Lys-851 (19), indicating that the two lysine residues, Lys-539 and Lys-851, are located in situ in sufficiently close proximity to react with the same H$_2$DIDS molecule (ca.15A).

3. Conformation Change during the Anion Exchange Process

Amino acid residues of band 3 protein known to be essential for the anion exchange activity are lysine, arginine and glutamic acid (6-8, 20-24). In addition to these amino acids, we have shown, by pH titration and diethyl pyrocarbonate (DEPC) modification experiments, that an intracellular histidine residue of band 3 protein also participates in anion exchange (25). The inhibition of anion exchange activity by DEPC was prevented in the presence of external DNDS, a reversible inhibitor of anion exchange. DEPC exerts its action only after penetration into the red blood cell and subsequently reacts with the inward- facing substrate binding site of the band 3 protein (25-27). It is conceivable that the protection of the functional histidine against

DEPC modification was due to the conformational change of the band 3 protein induced by DNDS. The functional histidine residue would be hidden from the intracellular surface in the outward facing conformation of band 3 protein (26-28).

Passow and co-workers (20) confirmed our data using site-directed mutagenesis of histidine residues in mouse band 3 protein. According to Muller-Berger et.al. (29) substitution by site-directed mutagenesis of any one of the histidine residues His-721 (human His-703), His-837 (human His-819), and His-852 (human His-834) to glutamine, or of His-752 (human His-734) to serine, inhibited Cl⁻ flux mediated by band 3 protein expressed in *Xenopus* oocytes. They also suggested that the formation of a hydrogen bond between His-752 (human His-734) and Glu-699 (human Glu-681) was essential for the decrease of band 3 protein-mediated Cl⁻ transport at low pH (30) Glu-681 has been shown to be essential by Jennings and Fritz (31) in anion exchange and is probably located at the intracellular side of the membrane. Accordingly, both amino acid residues Glu-681 and His-734 are also presumed to be in the anion channel.

V   Topogenic Activity of Transmembrane Segments of Band 3 Protein

Membrane proteins on the secretory pathway in eukaryotic cells are integrated into the membrane in the endoplasmic reticulum and acquire their membrane topology. This integration normally occurs during protein synthesis. Specific sequences of membrane proteins regulate the co-translational insertion and define the final membrane topology. Such specific sequences have been called "topogenic sequences" (32-33). Hydrophobic signal sequences are responsible for targeting membrane proteins to the endoplasmic reticulum and for initiating translocation. Although the topogenic functions of these sequences in simple single spanning bitopic membrane proteins have been established, their contribution to the membrane topogenesis of polytopic membrane proteins remains to be clarified. Our recent results, on the membrane structure of band 3 protein, indicate that hydrophobicity is not an absolute requirement for the formation of transmembrane segments in multi-spanning polytopic membrane proteins and suggest that polytopic membrane proteins may contain hydrophilic transmembrane segments with considerable freedom of movement in relation to the membrane (34, 35). These flexible regions must play important roles in the anion transport function. Hamasaki et.al. (36) have proposed recently new concept for polytopic membrane proteins.

REFERENCES

1.   Landaw, S.A. (1988) Factors that accelerate or retard red blood cell senescence. Blood Cells. 14, 47-52.

2.   Turrini, F., Arese, P., Yuan, J. and Low, P.S. (1991) Clustering of integral membrane proteins of the human erythrocyte membrane stimulates autologous IgG binding, complement deposition, and phagocytosis. J. Biol. Chem. 266, 23611-23617.

3. Beppu, M., Ando, K. and Kikugawa, K. (1996) Poly-N-accetyllactosaminyl saccharide chains of band 3 as determinants for ant-band 3 autoantibody binding to senescent and oxidized erythrocytes. Cell. Mol. Biol. 42, 1007-1024.

4. Hamasakı, N and Okubo, K. (1996) Band 3 protein : physiology, function and structure. Cell. Mol. Biol. 42, 1025-1039.

5. Wagner, P.D. (1977) Diffusion and chemical reaction in pulmonary gas exchange. Physiol. Rev. 57, 257-312.

6. Wıeth, J.O., Anderson, O.S., Brahm, J., Bjerrum. P.J. and Borders, Jr. C.L. (1982a) Chloride-bicarbonate exchange in red blood cells : Physiology of transport and chemical modification of bınding sites. Phil. Trans. R. Soc. Lond. B 299, 383-399.

7. Wıeth, J O , Bjerrum, P.J. and Borders, C.L. (1982b) Irreversible inactivation of red cell chloride exchange with phenylglyoxàl, an arginine-specific reagent J. Gen. Physiol. 79, 283-312.

8. Passow, H (1986) Molecular aspects of band 3 protein-mediated anion transport across the red blood cell membrane. Rev. Physiol. Biochem. Pharmacol. 103, 61-223.

9   Nanri, H , Hamasaki, N. and Minakami, S. (1983) Affinity labeling of erythrocyte band 3 protein wıth pyridoxal 5-phosphate. Involvement of the 35,000-dalton fragment in anion transport. J. Biol. Chem. 258, 5985-5990.

10. Hamasakı, N., Matsuyama, H. and Hirota-Chigita, C. (1983) Characterization of phosphoenolpyruvate transport acrosss the erythrocyte membrane. Evidence for involvement of band 3 ın the transport system Eur. J. Biochem. 132, 531-536.

11. Means, G.E. and Feeney, R.E. (1971) Eds. Chemical Modification of Proteins. Holden-Day, Inc., San Francısco, USA.

12. Kawano, Y. and Hamasaki, N. (1986), Isolation of a 5,300-dalton peptide containing a pyridoxal phosphate binding site from the 38,000-dalton domain of band 3 of human erythrocyte membranes. J. Biochem. (Tokyo) 100, 191-199.

13. Kawano, Y., Okubo, K., Tokunaga, F., Miyata, T., Iwanaga, S. and Hamasakı, N. (1988) Localızation of the pyridoxal phosphate binding site at the COOH-terminal region of erythrocyte band 3 protein. J. Biol. Chem. 263, 8232-8238.

14. Bruce, L.J., Kay, M.M.B., Lawrence, C. and Tanner, M.J.A. (1993) Band 3HT, a human red-cell variant associated with acanthocytosis and increased anion transport, carries the mutation Pro-868-> Leu in the membrane domain of band 3. Biochem. J. 293, 317-320.

15. Bruce, L.J., Anstee, D.J., Spring, F.A. and Tanner, M.J.A. (1994) Band 3 Memphis variant II. Altered stilbene disulfonate binding and the Diego (Di^a) blood group antigen are associated with the human erythrocyte band 3 mutation Pro-854->Leu. J. Biol. Chem. 269, 16155-16158.

16. Cabantchik, Z.I. and Greger, G. (1992) What do chemical probes tell us about anion transporters of mammalian cell membranes? Am. J. Physiol. (Cell Physiol), 262, c803-c827

17. Cabantchik, Z.I. and Rothstein, A. (1974) Membrane proteins related to anion permeability of human red blood cells. I. Localization of disulfonic stilbene binding sites in proteins involved in permeation. J. Membr. Biol. 15, 207-226.

18. Jennings, M.L. and Passow, H. (1979) Anion transport across the erythrocyte membrane, in situ proteolysis of band 3 protein and cross-linking of proteolytic fragments by 4,4'-diisothiocyano-dihydrostilbene-2,2'-disulfonate. Biochim. Biophs. Acta. 554, 498-519.

19 Okubo, K., Kang, D., Hamasaki, N. and Jennings, M.L. (1994) Red blood cell band 3. Lysine 539 and lysine 851 react with the same H$_2$DIDS (4,4'-diisothiocyanodihydrostilbene-2,2'-disulfonic acid). J. Biol. Chem. 269, 1918-1926.

20. Passow, H., Fasold, H., Gartner, M., Legrum, B., Ruffing, W. and Zaki, L. (1980) Anion transport
 •   across the red blood cell membrane and the conformation of the protein in band 3. Ann NY. Acad. Sci. 341, 361-383.

21. Wieth, J.O. and Bjerrum, P.J. (1982) Titration of transport and modifier sites in the red cell anion transport system J. Gen. Physiol. 79, 253-282.

22. Zaki, L. (1981) Inhibition of anion transport across red blood with 1, 2-cyclohexanedione. Biophys. Res. Commun. 99, 243-251.

23. Jennings, M.L. and Anderson, M.P. (1987) Chemical modification of glutamate residues at the stilbene disulphonate site of human red blood cell band 3 protein. J. Biol. Chem. 262, 1691-1697.

24. Julien, T. and Zaki, L. (1988) Studies on inactivation of anion transport in human red blood cell membrane by reversible and irreversible acting arginine-specific reagents. J. Membr. Biol. 102, 217-224.

25. Matsuyama, H., Kawano, Y. and Hamasaki, N. (1986) Involvement of a histidine residue in inorganic phosphate and phosphoenolpyruvate transport across the human erythrocyte membrane J Biochem. (Tokyo) 99, 495-501.

26. Izuhara, K., Okubo, K. and Hamasaki, N. (1989) Conformational change of band 3 protein induced by diethyl pyrocarbonate modification in human erythrocyte ghosts. Biochemistry 28, 4725-4728.

27. Hamasaki, N., Izuhara, K. and Okubo, K. (1989) Involvement of an intracellular histidine residue of band 3 in the conformational changes taking place during anion transport. In: Anion Transport of the Red Blood Cell Membrane Eds., Hamasaki, N. and Jennings, M.L. Elsevier Science Publishers B.V. Amsterdam p. 47-58.

28. Hamasaki, N. Okubo, K. and Kang, D. (1992) Protein chemistry of the anion transport center of erythrocyte band 3. Progr. Cell Res. 2, 65-71.

29. Muller-Berger, S., Karbach, D., Koning, J., Lepke, S., Wood, P.G., Appelhans, H. and Passow, H (1995a) Inhibition of mouse erythroid band 3-mediated chloride transport by site-directed mutagenesis of histidine residues and its reversal by second site mutation of Lys 558, the locus of covalent H_2DIDS binding. Biochemistry 34, 9315-9324.

30 Muller-Berger, S., Karbach, D., Kang, D., Aranibar, N., Wood, P.G., Ruterjans, H. and Passow, H. (1995b) Roles of histidine 752 and glutamate 699 in the pH dependence of mouse band 3 protein-mediated anion transport. Biochemistry 34, 9325-9332.

31. Jennings, M.L. and Smith, J.S. (1992) Anion-proton cotransport through the human red cell band 3 protein : role of glutamate 681. J.Biol. Chem. 267, 13964-13971.

32. Lingappa, V.R. and Hegde, R. (1997) Translocational pausings and the regulation of membrane protein biogenesis. In : Membrane Proteins : Structure, Function and Expression Control. Eds. Hamasaki, N. and Mihara, K., Kyushu University Press, Fukuoka, Japan / S. Karger, AG, Basel, Switzerland, p. 93-100.

33. Sakaguchi, M. (1997) Topogenic sequences which regulate the integration and topology of membrane proteins in the endoplasmic reticulum membrane. In : Membrane Proteins : Structure, Function and Expression Control. Eds. Hamasaki, N. and Mihara, K., Kyushu University Press, Fukuoka, Japan / S. Karger, AG, Basel, Switzerland p. 101-116.

34. Ota, K., Sakaguchi, M., von Heijne, G., Hamasaki, N. and Mihara, K, (1998a) Forced transmembrane orientation of hydrophilic polypeptide segments in multispanning membrane proteins. Molec. Cell 2, 495-503.

35. Ota, K., Sakaguchi, M., Hamasaki, N. and Mihara, K, (1998b) Assessment of topogenic functions of anticipated transmembrane segments of human band 3. J. Biol. Chem. 273, 28286-28291.

36. Hamasaki, N., Kuma, H., Sakaguchi, M. and Mihara, K. (1998) A new concept in polytopic membrane proteins following from the study of band 3 protein. Biochem. Cell Biol. (in press).