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VOLUME 2

THE BAND 3 PROTEINS: ANION TRANSPORTERS, BINDING PROTEINS AND SENESCENT ANTIGENS

EDITORS

E. BAMBERG
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Progress in Cell Research

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The band 3 proteins: Anion transporters, binding proteins and senescent antigens

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The band 3 proteins: Anion transporters, binding proteins and senescent antigens

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Preface

The present book contains a collection of papers on the molecular biology of the band 3 proteins and their various functions: as anion transporters; binding proteins for membrane skeleton; hemoglobin and glycolytic enzymes; and as a recognition signal for the removal of senescent cells by the immuno system of the body. The papers were written to provide an overview of the work done during the past 5 years or so in most of the laboratories engaged in research on band 3. They serve to give the reader factual information on nearly all aspects of band 3 research, to introduce him to the current literature and to give him a feeling for the philosophy behind the approaches chosen in the various laboratories. The papers are introduced by a brief review of the field which is hoped to give the newcomer some of the background information required to estimate the significance of the individual chapters on specific subjects. The book also contains tables showing the amino acid sequences deduced from the 14 hitherto known band 3 species and their cDNA sequences, and the sequence homologies existing amongst them. This may be useful for easy reference.

The information on band 3 is supplemented by a few selected studies on anion transport systems other than band 3. This may help to put the work on band 3 into some more general perspective.

The contributions were made by the participants of the Second Band 3 Meeting at Schloss Ringberg near München. We thank the Volkswagen Foundation and the Max-Planck-Society for the financial support that enabled us to organize the meeting and to gain the participants as authors of this book.

Frankfurt, January 1992

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The band 3 proteins. An introduction

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The present book is concerned with a family of closely related, integral membrane proteins of ubiquitous occurrence: the band 3 proteins. The name of these proteins is derived from the fact that the first member of the family was discovered on SDS polyacrylamide gel electropherograms of the plasma membranes of human red blood cells as the third major band from the top (Fairbanks et al., 1969; Steck, 1974). Its location indicated a molecular weight of about 96 000, its diffuseness pointed to the presence of carbohydrates (approx. 7%) and the intensity of the Coomassie's blue staining indicated the existence of about 10^6 copies per red blood cell, which corresponds to about 25 – 30% of the total membrane protein by weight.

Using immunochemical or molecular biological techniques, it was possible to demonstrate the presence of band 3-related proteins in the epithelial cells of kidney, lung and intestine, in liver, brain and heart, in precursor cells of the erythrocytes and in the B and T cells of the immune system. The molecular weights ranged from 60 000 to 180 000 (Kay et al., 1983; Stone et al., 1983; Drenckhahn et al., 1989). More recent work has established that the erythroid and non-erythroid band 3 species are the products of three distinct genes, each of which transcribes multiple forms of mRNA (reviewed by Alper, 1991; Kopito, 1991). The products of these genes are designated AE1, AE2 and AE3. Altogehter, 14 of these products have been cloned and sequenced. The amino acid sequences deduced are listed in Wood (1992).

The calculated molecular masses of the AE1 peptides as derived from their coding cDNA's range from 93 kDa to 102 kDa; those of AE2 and AE3 peptides are rather similar and amount to about 135 kDa. Immunological methods suggested the existence of a band 3-related protein with a molecular mass of about 45 kDa (Karniski and Jennings, 1989). Cloning and sequencing have shown, however, that in at least one of these cases (a peptide from the brush border of the kidney), there was no homology to any one of the AE proteins; the protein turned out to be a dipeptidase with a short stretch of amino acid sequence accidentally identical to that of an antigenic determinant of AE1 (Igarashi and Karniski, 1991).

Genomic clones of mouse (Kopito et al., 1987) and chicken (Kim et al., 1988, 1989) AE1 have been sequenced and their intron-exon structure has been established. The human AE1 gene is located in chromosome 17 (Showe et al., 1987), the AE2 gene in chromosome 7 (Palumbo et al., 1986).

Hydrophobicity plots of the various members of the AE protein family (see Alper, 1991, 1992) indicate that all of them consist of two domains, the existence of which was known already for the human erythroid (AE1) protein on the basis of investigations with physicochemical methods (Snow et al., 1981). The hydrophobic domains contain the C-terminus. They are of about equal sizes (approx. 55 kDa). The sequence homologies of the hydrophobic domains of the erythroid band 3 proteins of mouse, rat, chicken and human exceed 90%. The trout AE1 shows a homology of only 70% (Hübner et al., 1992) and resembles in many respects the AE3 proteins. The sizes of the hydrophilic domains are variable (about 35 kDa in AE1, approx. 75 – 80 kDa in AE2 and AE3) and the sequence homologies are rather low.

The functions of the hydrophilic domain have been extensively investigated for the human erythroid band 3 protein. This domain sticks out into the cytosol and carries binding sites for ankyrin, hemoglobin and glycolytic enzymes (reviewed by Passow, 1986). Ankyrin binding leads to the attachment of spectrin and other constituents of the membrane skeleton to the lipid bilayer. A number of important glycolytic enzymes including aldolase and glyceraldehyde phosphate dehydrogenase are capable of binding near the N-terminal end of the hydrophilic domain. In the bound state, these enzymes are inactive. Phosphorylation of a tyrosine residue (Tyr 8 and possibly Tyr 21; Dekowski et al., 1983; Low et al., 1987) by a tyrosine kinase leads to their release and a corresponding increase of glycolytic activity in the red cell (Harrison et al., 1991). Hemoglobin binding takes place at a site distinct from the binding site for the glycolytic enzymes. The efficiency of binding seems to depend on the conformational state of the hydrophilic domain. Combination of a non-penetrating stilbene derivative DIDS with a specific site at the outer surface of the hydrophobic domain increases the strength of hemoglobin binding to the hydrophilic domain (reviewed by Salhany, 1990).

The hydrophilic domain contains the most effective antigenic determinants of the band 3 proteins. Due to the low sequence homology, antibodies raised against a given band 3 species may not cross-react with one another. This applies for example, to the antibodies against mouse and human band 3 (Kopito and Lodish, 1985).

The hydrophobic domains of all three members of the AE gene family are capable of executing anion exchange across the plasma membrane. This universal capacity lead to the designation AE, which stands for 'anion exchanger'. It also lead to the suggestion of the term 'capnophorin' or 'carrier of smoke' as a suitable name for band 3 (Wieth and Bjerrum, 1983). This alludes to the essential role of the band 3-mediated Cl^-/HCO_3^- exchange in the CO_2 transport by the red cells and in the pH regulation in red cells and other tissue cells.

The protein exists in the membrane in the form of monomers, dimers and tetramers. The state of aggregation is important for the binding of glycolytic enzymes and hemoglobin (see Low, 1992; Schubert et al., 1992). Although each protomer is able to act as an independently operating anion transporting unit, aggregation may lead to interactions between adjacent protomers that modulate the transport process under certain conditions (Salhany, 1990; Knauf et al., 1992; Salhany, 1992).

During senescence of the red blood cells, patches of band 3 aggregates may be formed which are recognized by the immune system and lead to the removal of the senescent cells from the circulation. Although the evidence for this mechanism looks quite convincing, there are other views on the recognition of aged cells. All agree, however, that band 3 plays an essential role (see Arese et al., 1992; Lutz, 1992; Kay, 1992).

Transport proteins may be classified into channels and carrier-like proteins. The channels form fluctuating aqueous pores through which solutes diffuse under the influence of their own electrochemical potential gradients. The carrier-like proteins do not permit free diffusion. They form complexes with their substrates at one surface of the membrane. The complex undergoes one or a sequence of several conformational changes which lead to the exposure of the bound substrate to the other surface and its release by dissociation from the protein. Usually, after release of the transported substrate, the protein is able to undergo the conformational changes necessary to get the empty substrate binding site back to the surface from where the substrate originated. There, it may pick up another substrate molecule and the cycle repeats itself.

Anions may be transported across cell membranes by channels (Gögelein, 1988) and carrier-like proteins. The band 3 protein belongs to the latter class of proteins. It has the specific feature that, in the absence of substrate, the conformational changes of the substrate protein complex that lead to the movement of the substrate from one surface to the other cannot be reversed by a conformational change in the opposite direction. Thus, there is no 'slippage' and hence the protein mediates an anion exchange that can only be measured with radioisotopes. It is not able to promote an anion net flow. This is the reason why the exchange process does not contribute to the membrane conductance. It is 'electrically silent'.

Although in the red cell membrane, the electrical field does not act as a driving force for the anion exchange, it may, nevertheless, affect the rate of the electrically silent exchange reaction by shifting the steady state distribution of inward-directed and outward-directed band 3 protein molecules. Such shift should be possible if the conformers have an electrical momentum due to an unequal distribution of charges. In the intact red cell, attempts in several laboratories failed to demonstrate an effect of the membrane potential on Cl^- equilibrium exchange. However, after expression of band 3 in *Xenopus* oocytes, a potential dependence was observed that could be interpreted by a field-induced shift of the orientation of band 3 conformers (see Donath and Egger, 1992; Schwarz et al., 1992). The discrepancy between the behavior of band 3 in red cells and oocytes is still unclear, although it has been suggested that in the oocyte, where the number of expressed band 3 molecules per μm^2 of membrane surface is much smaller than in the red cell, the band 3 molecules predominantly exist as monomers and not as oligomers, and that these monomers may be more easily susceptible to changes of conformation under the influence of the electrical field.

Although the band 3 protein mediates essentially an electrically silent anion exchange, it also allows a small anion net flux which amounts to about $1/10^4$ of the exchange flux. The nature of this process is not yet understood. It is clear, however, that it does not represent slippage (reviewed by Jennings, 1989; Knauf, 1989; Passow, 1986). According to one hypothesis, the net exchange is brought about by the occasional diffusion of a bound anion prior to the conformational change of the ion-protein complex which leads to anion exchange (Fröhlich, 1984). Another hypothesis involves the assumption that band 3 may exist in two distinct, interconvertible conformations: an exchange conformation which allows only the electrically silent transport, and a channel conformation which permits the diffusion of anions as in an aqueous pore (Passow, 1986). The latter hypothesis is supported by the observation that patch clamp experiments with single red cells show the existence of anion channels (5pS) that can be inhibited by dipyridamol at the same concentration as the inorganic anion net efflux, which is attributed to band 3 (Passow et al., 1988).

The anion exchange reaction follows ping-pong kinetics which is characterized by saturation kinetics and reorientation of the substrate binding site of the transport molecules under the influence of electro-chemical gradients across the membrane ('recruitment') (reviewed by Passow, 1986; Jennings, 1989; Knauf, 1989).