Why Bicarbonate?

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Abstract

Bicarbonate is a simple single carbon molecule that plays surprisingly important roles in diverse biological processes. Among these are photosynthesis, the Krebs cycle, whole body and cellular pH regulation and volume regulation. Since bicarbonate is charged it is not permeable to lipid bilayers. Mammalian membranes thus contain bicarbonate transport proteins to facilitate the specific transmembrane movement of HCO_3^-. This review provides a wide-ranging view of the biochemistry of bicarbonate and its membrane transporters, revealing what makes the study of bicarbonate transport such a rewarding activity.

Introduction

This article will summarize my laboratory's studies of bicarbonate transport proteins, in the broader context of the literature. This seems an appropriate time also to pause and ask how my research got here. Why study the biochemistry of bicarbonate?

Since the start of my research career my focus has been the biochemistry and physiology of mammalian bicarbonate transport. Although bicarbonate is tremendously important in biology (especially in plants), the focus here will be on bicarbonate in mammals, in particular the transmembrane transport of the membrane impermeant anion, bicarbonate.

Biochemistry of bicarbonate

Chemistry of bicarbonate - There was considerable chance involved in deciding to study transmembrane movement of bicarbonate. Knowing what I know now, I have to wonder whether I would have chosen to study bicarbonate as a transport substrate. The chief difficulty in studying bicarbonate transport is that bicarbonate is a labile substrate. That is, the reaction $CO_2 + H_2O \leftrightarrow H_2CO_3$ occurs both spontaneously and catalyzed by carbonic anhydrase enzymes (see below). Further complexity is added by the acid/base conversion properties of bicarbonate: $H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + H^+$, reactions governed by pK_as of 6.4 and 10.3, respectively. This chemistry makes it challenging to study bicarbonate transport since the substrate under investigation changes form, and alters pH as it does so. The situation becomes more complex because CO_2 (gas) $\leftrightarrow$ CO_2 (dissolved), whose equilibrium varies with partial pressure of CO_2, temperature and pH. Bicarbonate is indeed a "slippery" substrate to study, but this tendency to change chemical form is what makes bicarbonate biologically important, as described below.

Mitochondrial carbon dioxide production - The additional complexity to the study of bicarbonate transport is the fact that our cells continuously produce metabolic CO_2 as a waste product. Catabolism of proteins, carbohydrates and lipids ultimately results in formation of acetyl-CoA, which feeds into the Krebs cycle (Lehninger 1982). The Krebs cycle, the primary source of energy production in mitochondria, effectively oxidizes acetyl-CoA to carbon dioxide (Lehninger 1982). CO_2 is thus the primary waste product of respiratory oxidation.

RuBisCO and photosynthetic CO2 fixation - Although the biochemistry of bicarbonate does not immediately seem of dramatic importance, in fact bicarbonate biochemistry is central to virtually all life. Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO, EC 4.1.1.39) catalyzes the first reaction of photosynthetic CO_2 fixation: D-ribulose 1,5-bisphosphate + $CO_2 + H_2O \leftrightarrow$ 2 3-phospho-D-glycerate. Thus, RuBisCO provides the energy source for most other organisms, whose metabolism is based on consumption of plants/algae. In light of this central biochemistry RuBisCO comprises 30-50% of soluble protein in leaves (Dhingra *et al.* 2004) and has been suggested to be the most abundant protein of life.

Combustion and global warming - The chemistry, if not biochemistry, of CO_2/HCO_3- is very much in the news with the unfortunate arrival of carbon dioxide induced global warming. Virtually all organisms ultimately rely on the energy provided by the fixation of CO_2 by RuBisCO (*i.e.* plants/algae fix the CO_2 and other creatures eat the plants). So too, our society's need for energy is in large part satisfied by oxidation of fossil carbon that was originally fixed by RuBisCO tens of millions of years ago. The overwhelming consensus is that the resulting elevation of atmospheric CO_2 traps heat, reducing nighttime loss and elevating mean earth surface temperature (Friedlingstein and Solomon 2005). There was hope that elevated atmospheric CO_2 levels would increase photosynthetic CO_2 fixation, thus mitigating some of the CO_2 rise. Unfortunately the most careful study to date suggests that there will be at best a modest increase of plant CO_2 fixation with increased levels of atmospheric CO_2 (Long *et al.* 2006). Engineering of RuBisCO to enhance CO_2 fixation in crop plants is an active area of investigation (Parry *et al.* 2003). With the advent of global warming as a significant threat to human survival, it is likely that the biochemistry of bicarbonate/carbon dioxide will receive increased attention in the future.

Physiology of bicarbonate

Bicarbonate and whole body pH regulation - Biochemical processes occur within narrow optimal pH range. Consequently our bodies have extensive pH buffering to restrict changes of intra and extracellular pH. Principal among these is the CO_2/HCO_3^- system. As mentioned above CO_2/HCO_3^- inter-convert; CO_2 is a conjugate acid and HCO_3- is a base. While CO_2 is membrane permeant by diffusion across the lipid bilayer, HCO_3^- is charged and moves across membranes only with the assistance of specific transport proteins (see below). Because of the acid/base properties of CO_2/HCO_3^- movement of CO_2 out of the cell will alkalinize the cell, while HCO_3^- efflux acidifies the cell. Under physiological conditions the majority of CO_2/HCO_3^- is found as HCO_3^- (at levels around 25 mM) and metabolic acid will readily be consumed by conversion of HCO_3^- to CO_2. Thus, CO_2/HCO_3^- acts as a major physiological buffering system.

Excretion of CO_2/HCO_3^- - CO2 is produced as a metabolic waste product by the Krebs cycle. It is essential to continued body function that this acid load be excreted as the acid form, CO_2. Yet the body has a problem in that the blood that flows through our kidneys contains high levels HCO_3^-. Secretion of renal HCO_3^- must be prevented as loss of this base from the body would cause dramatic, life-threatening acidosis. Not surprisingly our kidneys reabsorb virtually all of the HCO_3^- (amounting to about 500 g $NaHCO_3$/ day in each individual) that passes through them, using a series of bicarbonate transport proteins (see below). Rather than secrete HCO_3^-, our bodies exhale gaseous CO_2 using a remarkable and highly conserved system.

In the secretion of CO_2 from our bodies there is one major difficulty: CO_2 is poorly soluble in the aqueous medium of our blood. To maximize CO_2/HCO_3^- carrying capacity, metabolically produced CO_2 diffuses out of our cells into the blood (Fig. 1). It then diffuses across the red blood cell (erythrocyte) membrane. Inside the erythrocyte the enzyme, carbonic anhydrase, is localized to convert CO_2 to HCO_3^-. HCO_3^- levels cannot be allowed to rise in the erythrocyte because the process would shut down, as a result of mass action. To prevent this erythrocytes express exceptionally high levels (about half the integral mem-

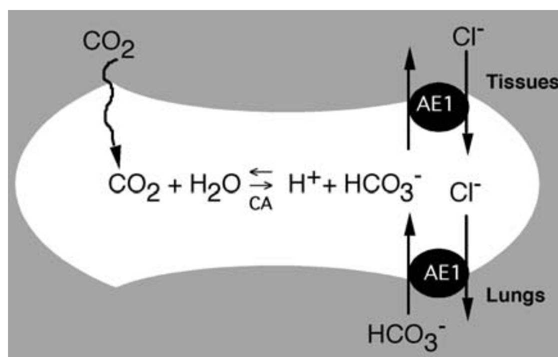

Figure 1 The role of AE1 in facilitating CO_2 efflux from the body. The body's tissues produce CO_2 as a waste product. CO_2 diffuses across membranes into the plasma and across the erythrocyte membrane. Inside the erythrocyte the enzyme, carbonic anhydrase (CA) catalyzes the conversion of CO_2 to HCO_3^-. The integral membrane transport protein, AE1, moves the HCO_3^- out of the cell into the plasma, in which the HCO_3^- is carried to the lungs. AT the lungs the process reverses: AE1 carries HCO_3^- into the erythrocyte, where it is converted to CO_2, and subsequently exhaled.

brane protein) the protein AE1 (Anion Exchanger 1). AE1 exchanges one intracellular HCO_3^- for one extracellular Cl^-, thus effluxing HCO_3^- into the plasma in an electroneutral manner. Upon reaching the lungs, the erythrocyte confronts an environment with low CO_2 levels. This drives dissolved CO_2 to leave the plasma, become gaseous and leave the body through exhalation. In turn, the low plasma CO_2 levels drive conversion of HCO_3^- to CO2: HCO_3^- is moved back into the erythrocyte in exchange for intracellular Cl-, mediated again by AE1. Carbonic anhydrase converts the HCO_3^- to CO_2, which diffuses across the erythrocyte membrane into the plasma, whereupon it is exhaled out through the lungs. With the assistance of AE1 secretion of acidic CO_2 is thus accomplished.

Bicarbonate transporters

Phylogeny of bicarbonate transporters - The examples of renal HCO_3^- reabsorption and the role of erythrocytes and the lungs in ridding the body of CO_2 illustrate two very different function of bicarbonate transport proteins. These proteins facilitate the movement of membrane-impermeant HCO_3^- across biological membranes. In mammals about 13 different genes encode bicarbonate transporters (Fig. 2), which function via a range of catalytic mechanisms, including Cl^-/HCO_3^- exchange (e.g. AE1), Na^+/HCO_3^- co-transport and Na^+-dependent Cl^-/HCO_3^- exchange. These bicarbonate transporters cluster into three separate branches upon phylogenetic analysis (Fig. 2): classical Cl^-/HCO_3^- exchangers of the "AE" family, Na^+/ bicarbonate co-transporters of the NBC family and members of the SLC26 (Solute Carrier sub-family 26) family.

Cellular roles of bicarbonate transport - Bicarbonate transporters are involved in three fundamental processes: HCO_3^- metabolism/excretion, regulation of pH, and regulation of cell volume. By facilitating the movement of bicarbonate across membranes, bicarbonate transporters drive HCO_3^- metabolism. A good example of this function is the role of erythrocyte AE1, as described above. Since movement of HCO_3^- will acidify the region it leaves and alkalinize the opposite side of the membrane, HCO_3^- transporters are clearly involved in regulation of pH. A prime example of this activity is the role of the AE2 Cl^-/HCO_3^- exchanger in the basolateral surface of acid-secret-

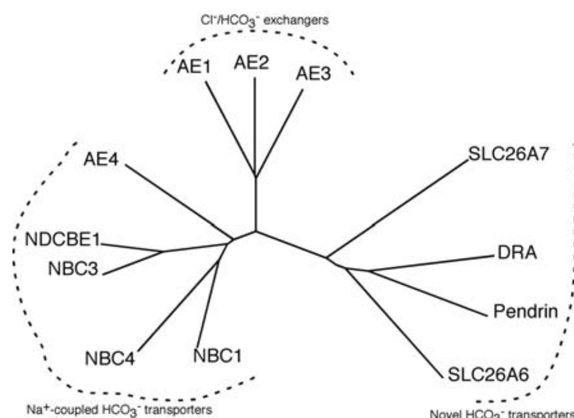

Figure 2 Phylogenetic relationships of human HCO_3^- transporters. Amino acid sequences for human bicarbonate transporters were analysed with the program Phylip. The degree of sequence similarity is represented by the length of line between proteins. The dashed curves enclosing the transporters indicate the three different clusters of bicarbonate transporters. Transporters analysed are: AE1 (Kopito and Lodish 1985); AE2 (Alper et al. 1988); AE3 (Kudrycki *et al.* 1990), NBC1 (Burnham *et al.* 1997), (Romero *et al.* 1997); NBC3 (Ishibashi *et al* 1998); NBC4 (Pushkin *et al.* 2000); AE4 (Tsuganezawa *et al.* 2001), NDCBE1 (Romero et al. 2000), SLC26A3 (Schweinfest *et al.* 1993); SLC26A4 (Scott *et al.* 1999); SLC26A6 (Waldegger *et al.* 2001); and SLC26A7 (Lohi *et al.* 2002).

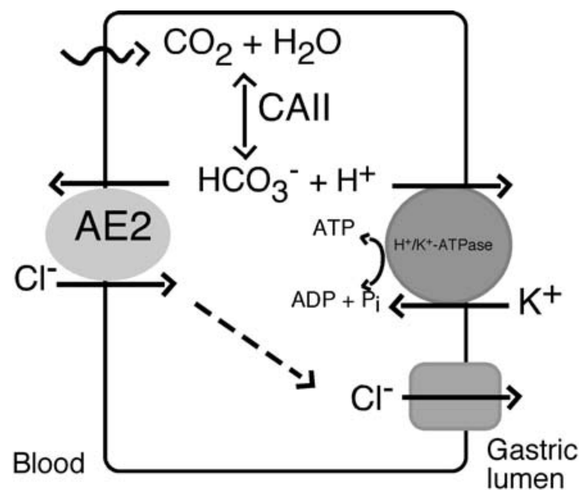

Figure 3 Essential role for Cl^-/HCO_3^- exchange in parietal cell acid secretion. Basolateral AE2 provides both H^+ and Cl^-, required for HCl secretion. Similar physiology occurs for osteoclast acid secretion.

ing gastric parietal cells (Fig. 3). By efflux of HCO_3^- in exchange for influx of Cl^-, AE2 provides both the H^+ equivalent and Cl^- required for HCl secretion into the stomach lumen at the apical surface of the cell. The role of Cl^-/HCO_3^- exchangers in volume regulation was first studied in lymphocytes (Mason *et al.* 1989), where it was found that cells exposed to hyper-osmotic challenge restore their fluid volume by loading with NaCl. They do so by coordinated activation of a Cl^-/HCO_3^- exchanger and a Na^+/H^+ exchanger (which moves Na^+ in and H^+ out). Working together these transporters result in no change of cell pH, since the acid resulting from HCO_3^- efflux is balanced by H^+ efflux, so the net effect is cell loading with NaCl; osmotic water movement restores cell volume.

Bicarbonate transport and the heart - Contractile activity of the heart is critically sensitive to pH. Consequently the heart expresses a wide range of pH-regulatory transport proteins, including HCO_3^- transporters (Alvarez *et al.* 2004). In addition to the need for HCO_3^- transporters to deal with the metabolic HCO_3^- load, HCO_3^- transporters have importance in two other physiological settings that have been studied by my laboratory: recovery from ischemic acidosis and cardiac hypertrophy. In ischemia reduced blood flow to cardiac muscle results in accumulation of waste products, including acid, and a shift to anaerobic metabolism. Upon restoration of blood flow, pH needs to be recovered to normal and HCO_3^- transporters are responsible for about 50% of pH recovery, through alkaline HCO_3^- influx (Vandenberg *et al.* 1993). Rapid recovery of pH by the cardiac NHE1 Na^+/H^+ exchanger can result in heart cell death, so that understanding the mechanisms of cardiac pH regulation is of great interest (Karmazyn 1988; Karmazyn 1996; Myers and Karmazyn 1996). A broad survey of bicarbonate transporter expression in the heart revealed that several HCO_3^- transporters are expressed in the heart (Alvarez *et al.* 2004). Predominant among these is the SLC26A6 protein, which carries out both Cl^-/HCO_3^- and Cl^-/OH^- exchange (Alvarez *et al.* 2004). The role of AE in the heart is generally thought to be cellular acidification, by the efflux of HCO_3^- (Vaughan-Jones 1986). Indeed our recent data, using an inhibitory anti-AE3 antibody, showed that pHi rose in rat cardiomyocytes treated with anti-AE3, leading to an increase of contractile force (Cingolani *et al.* 2003). This suggests that under normal conditions AE3 significantly acidifies myocytes through HCO_3^- efflux.

We have proposed that cardiomyocyte acidification plays a significant role in the progression of cardiac hypertrophic, the enlargement of cardiomyocytes, leading to the compromised cardiac function found in heart failure. Our recent studies of Cl^-/HCO_3^- exchange in the heart have led us to hypothesize that AE proteins contribute to the development of cardiac hypertrophy. Heart failure is marked by progressive enlargement of the heart, which contributes to the loss of cardiac function (Frey *et al.* 2004). Hypertrophic growth of individual cardiomyocytes underlies the heart expansion. Understanding the processes that regulate cardiomyocyte hypertrophic growth is thus of considerable importance. The cardiac NHE1 protein has a central role in the development of cardiac hypertrophy (Cingolani and Camilion De Hurtado 2002; Engelhardt *et al.* 2002) (Fig. 4). Hypertrophic signaling pathways converge by activation of NHE1; inhibition of NHE1 activation either through blockade of the signaling pathways that activate NHE1 (e.g. angiotensin converting enzyme inhibition (Camilion de Hurtado *et al.* 2002; Ennis et al. 1998; Fortuno et al. 1997)) or direct inhibition of NHE1 (e.g. with cariporide (Ennis *et al.* 2003; Kusumoto et al. 2001; Yoshida and Karmazyn 2000)) results in amelioration of cardiac hypertrophy. However, the fact that NHE1 action alkalinizes the cell and that NHE1 auto-inhibits at alkaline pH is under-appreciated (Counillon and Pouyssegur 2000). Thus, hyperactivation of NHE1 cannot be sustained in the absence of a balancing acid. Interestingly, under hypertrophic stimulation cardiomyocytes do not show an increase in steady-state pH (Perez *et al.* 1995), yet the increase of $[Na^+]_{cytosolic}$ verifies that NHE1 is hyperactivated (Cingolani and Camilion De Hurtado 2002; Perez *et al.* 2001). How is NHE1 hyperactivated without alkalinizing the cell? A parallel acidifying pathway, such as Cl-/HCO_3^- exchange, must be activated to balance the activity of NHE1 (Perez *et al.* 1995). We have found that $AE3_{fl}$ is the only AE protein whose activity is activated by PKC, the major kinase that integrates hypertrophic pathways (Alvarez *et al.* 2001) (Fig. 4). We thus hypothesize that $AE3_{fl}$ is essential for hypertrophic signaling pathways that act through NHE1. The mechanisms that couple NHE1 activation to hyper-

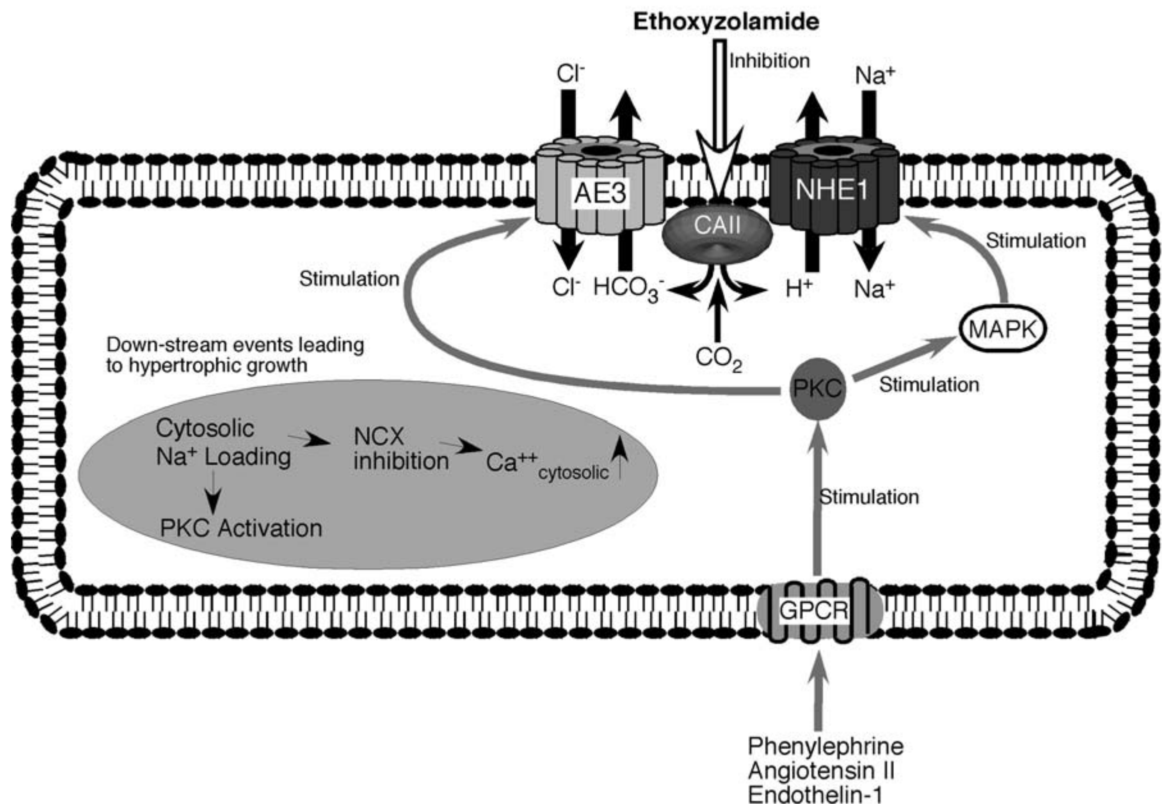

Figure 4 Hypertrophic growth in cardiomyocytes and the Hypertrophic Transport Metabolon. We propose that co-activation of the AE3 Cl-/HCO_3^- exchangers and the NHE1 Na^+/H^+ exchanger results in cellular NaCl loading, leading to stimulation of hypertrophic growth. CAII inhibition with ETZ limits substrate availability for AE3 and NHE1, thereby decreasing NaCl accumulation. MAPK= mitogen activated protein kinase; PKC= protein kinase C; GPCR= G-protein coupled receptor; CAII= carbonic anhydrase II.

trophic growth are not firmly established. The rise of cytosolic Na^+ associated with NHE1 activation will, however, inactivate the Na^+/Ca^{++} exchanger and cause cytosolic Ca^{++} elevation. In turn, elevated cytosolic Ca^{++} activates the calcineurin/NFAT/transcription factor pathway to induce hypertrophic gene expression (Frey and Olson 2003).

We found that the cytoplasmic enzyme, carbonic anhydrase II (CAII) has a key role in cardiac pH regulation. Through hydration of CO_2, CAII produces H^+ and HCO_3^-(Pastorekova *et al.* 2004). We have established that both AE proteins (Sterling *et al.* 2001b) and NHE1 (Li *et al.* 2002) bind CAII and that this binding event accelerates the respective transport rates of NHE1 and AE proteins, by maximizing the local concentration of transport substrates. We have termed the complex of a carbonic anhydrase with a bicarbonate transporter, the Bicarbonate Transport Metabolon, for the linkage of metabolism and membrane transport (Sterling and Casey 2002; Sterling *et al.* 2001a; Sterling et al. 2001b). In a related way CAII, NHE1 and AE3 are linked (Fig. 4). The products of CAII action are effluxed by NHE1 and AE3, for a net cell-loading with NaCl. Co-activation of NHE1/CAII and AE3 is pathological as it is self-sustaining and NHE1 is not subject to inhibition by alkaline pH, since the co-activated transporters do not change pH_i. We propose that AE3, CAII and NHE1 form a functional and physical complex, which pathologically stimulates hypertrophic heart growth. We call this complex the Hypertrophic Transport Metabolon (Fig. 4).

Structure of bicarbonate transporters

The basic structure shared by bicarbonate transporters is a large cytoplasmic N-terminal domain of 40-80 kDa, followed by a membrane domain of

about 55 kDa and a variable length cytoplasmic C-terminal region (Fig. 5). Erythrocyte AE1 has been subjected to an enormous number of studies since the protein's identification about 30 years ago (Cabantchik and Rothstein 1974) (reviews (Reithmeier *et al.* 1993; Salhany 1990)). Little work on the structure of other bicarbonate transporters has been reported, but topology of NBC1 has also been studied (Tatishchev *et al.* 2003). Most significantly a negative stain electron microscopy structure was determined for dimeric AE1MD at 20 Å resolution (Wang *et al.* 1993; Wang et al. 1994), which revealed each AE1MD as a 40x50 Å rectangle. Many aspects of AE1 N-terminal cytosolic domain biology were explained by the 2.6 Å resolution crystal structure for the domain (Zhang *et al.* 2000).

Topology - The topology of AE1MD, in particular the C-terminal portion, remains controversial (Fig. 5). Hydropathy analysis for the first 9 transmembrane segments (TMs) is relatively clear and has been verified in large part experimentally (outlined in (Tang *et al.* 1998; Zhu *et al.* 2003)). However, the remainder of AE1MD is not readily amenable to hydropathy analysis and experimental evidence has been difficult to interpret. We performed a systematic scan of the AE1 TM8 region, clearly delineating the boundaries of the TM and identifying a two turn extension of helical structure into the cytoplasm (Tang et al. 1998). Insertion of glycosylation acceptor sites revealed the presence of a re-entrant "T-loop" (transport loop) between TM9 and 10 (Popov et al. 1999), and helped to define topology in the remainder of the C-terminal region of AE1 MD. We mapped topology of the AE1MD by measurement of chemical reactivity of a series of individual introduced Cys mutants to membrane permeant and impermeant sulfhydryl reagents (Fujinaga *et al.* 1999; Zhu *et al.* 2003). This revealed the last two TMs as short (16 residues), suggesting that they are inserted into the protein's core structure and do not interact with lipid. N-terminal to the last two TMs is a region with puzzling structure. Much of the region is accessible to the extracellular medium, but it is difficult to model as either a conventional helical TM or as an extended β-structure (Fig. 5) (Zhu *et al.* 2003). We concluded that the region is extremely flexible, undergoing conformational transitions that allow it to extend across the AE1 transmembrane permeability barrier; the region likely forms a central portion of the transport site of AE1.

Oligomeric state - In the erythrocyte, AE1 exists as a mixture of dimers and tetramers (60:40 ratio) (Casey and Reithmeier 1991). The tetramers associate with the cytoskeleton, while the dimers do not. Proteolytic cleavage of the N-terminal cytoplasmic domain from AE1 reveals that the cyto-

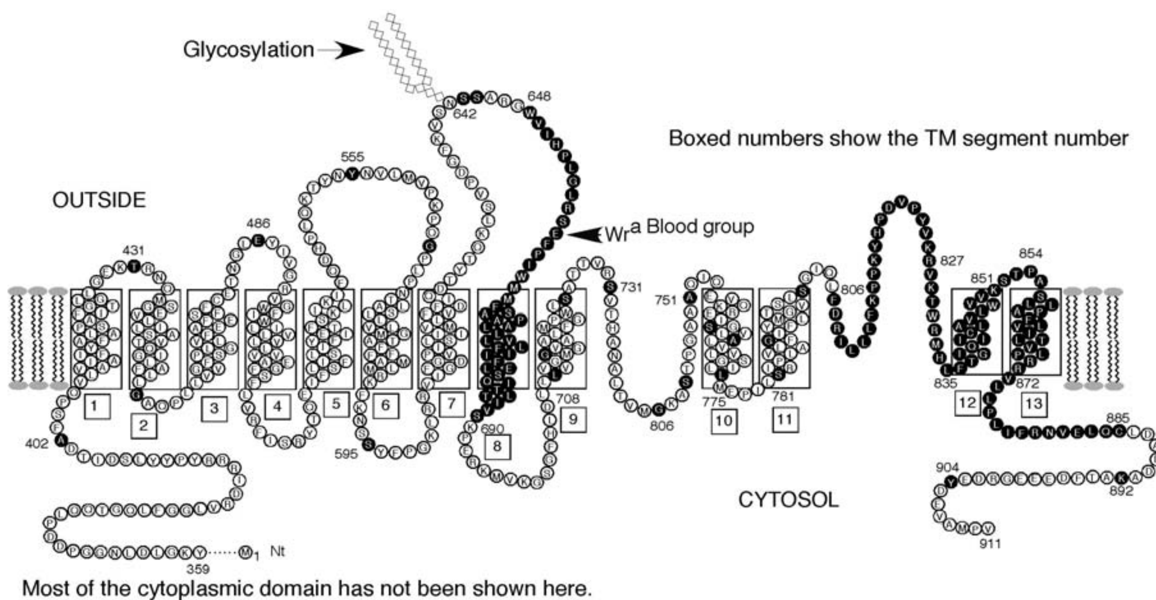

Figure 5 AE1 topology model, showing introduced cysteine mutants already constructed (filled circles). Topology model of AE1 was developed from several lines of evidence (summarized in Zhu, Lee and Casey, 2003).

plasmic domain is dimeric, as is the membrane domain (Casey and Reithmeier 1991; Zhang et al. 2000). Denaturation is required to separate AE1MD dimers to monomers, indicating a very strong association (Boodhoo and Reithmeier 1984). AE1 monomers function independently and each monomer has its own anion translocation pore (Jennings *et al.* 1998; Taylor *et al.* 2001). Oligomerically pure dimeric AE1MD can be readily purified following proteolytic separation from the cytoplasmic domain (Casey and Reithmeier 1991; Lemieux *et al.* 2002).

TM helical packing - Little data is available on the packing of AE1 TMs to form the membrane domain. Two studies designed to examine the region forming the AE1 dimeric interface developed limited models for AE1 TM packing. Tanner co-expressed separate portions of AE1MD to identify regions that were dispensable and those which could co-associate to form a functional transporter (Groves and Tanner 1999; Groves *et al.* 1998a; Groves *et al.* 1998b), which allowed him to propose a model for AE1MD helical packing (Fig. 6). Similarly, we cross-linked AE1 monomers to dimers, using AE1 introduced Cys mutants and a range of cross-linking reagents to determine a model for AE1MD (Fig. 6) (Taylor *et al.* 2001). The anion exchange inhibitor, H_2DIDS, covalently cross-links K539 and K851, and thus localizes these residues within 20 Å of each other (Okubo *et al.* 1994).

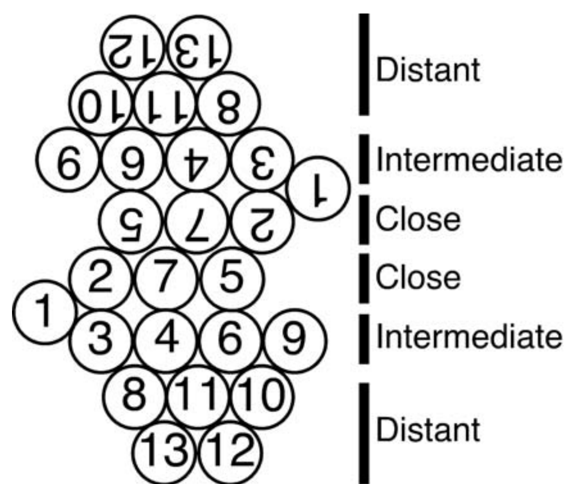

Figure 6 AE1 transmembrane segment packing in the dimeric unit. Numbers refer to transmembrane segment numbers. Adapted from (Taylor et al., 2001).

Carbonic anhydrases and bicarbonate transporters

The bicarbonate transport metabolon- A metabolon is a physical complex of enzymes in a linked enzymatic pathway (Srere 1987). Flux through the enzymatic pathway is accelerated by enzymatic co-localization, which increases the local concentration of substrate at the active site as the product of one reaction feeds into the next enzyme in the pathway. Likewise, flux is driven by reduced concentration of enzymatic product, when that product is removed into the active site of the next enzyme in the pathway. In the HCO_3^- transport literature, the initial indication of metabolon phenomena was the observation that carbonic anhydrase II (CAII) binds to the cytoplasmic C^- terminus of the erythrocyte AE1 Cl^-/HCO_3^- exchanger (Vince and Reithmeier 1998), mediated by an interaction between an acidic motif on AE1 (hydrophobic residue, followed by four residues, with at least two acidic) (Vince and Reithmeier 2000) and the basic N-terminus of CAII (Vince *et al.* 2000). We examined the functional significance of the AE1/CAII interaction and found that the direct interaction was essential for maximum transport activity, with transport activity ~40-60% lower if CAII was free in the cytosol, rather than tethered to AE1 (Sterling *et al.* 2001b). This led us to introduce the concept of a "transport metabolon," the complex between a transporter and the enzyme that produces or consumes the transport substrate (Fig. 7). Interestingly, the CAII binding motif is conserved among bicarbonate transporters (Sterling *et al.* 2001b), except for the SLC26A3 (DRA) transporter (Sterling et al. 2002b). Consistent with the observation that SLC26A3 does not have a CAII binding motif, DRA does not bind CAII and is not inhibited by dominant negative CAII (Sterling *et al.* 2002b). Subsequently we found that AE1 interacts with the enzyme, CAIV, which is anchored to the extracellular surface via a glycosylphosphatidyl inositol anchor (Sterling et al. 2002a); this interaction, too, accelerates the transport rate and is mediated by the fourth extracellular loop of AE1. Similarly, we have found that the Na+/HCO_3^- co-transporter (NBC) isoforms, NBC1 and NBC3, interact with and require CAII for full HCO_3^- transport activity (Alvarez *et al.* 2003; Loiselle *et al.* 2004).

The large and immediate impact of CAII upon

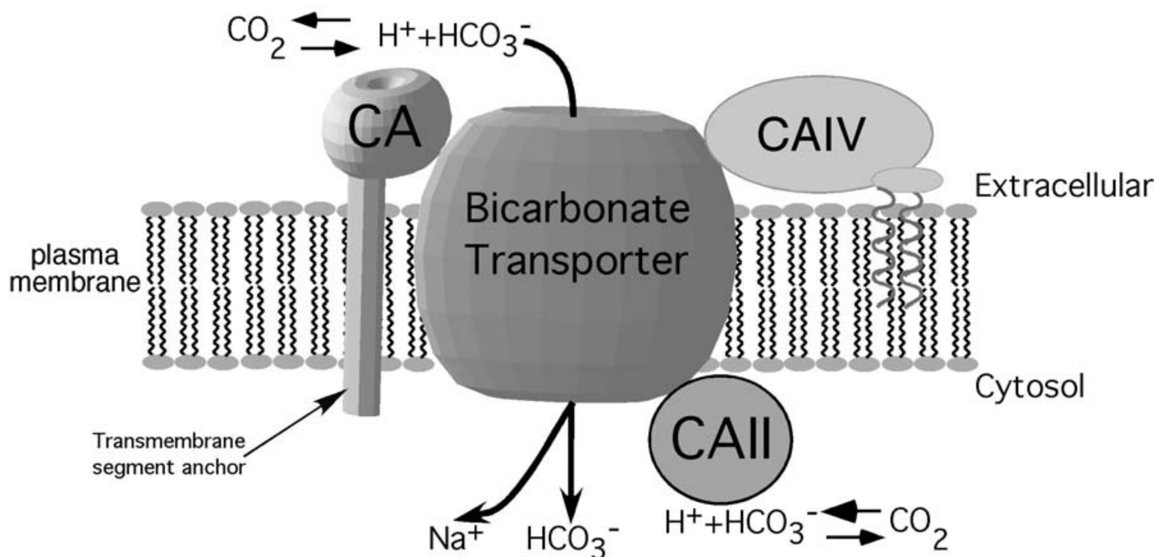

Figure 7 The Bicarbonate Transport Metabolon. We have shown that some Na^+/bicarbonate co-transporters and Cl^-/HCO_3^- exchangers directly bind both the soluble cytosolic enzyme, CAII, and the extracellular enzyme CAIV, anchored to the cell surface via a glycosylphosphatidyl inositol linkage.

HCO_3^- transport rate has led us to hypothesize that transport could be regulated acutely by modulation of the CAII/AE1 interaction, perhaps by phosphorylation. Recently this has been borne out; SLC26A6 transport activity is acutely inhibited, following protein kinase-C mediated phosphorylation, leading to displacement of CAII from a site on SLC26A6 (Alvarez *et al.* 2005). This finding led us to introduce the concept of "metabolon disruption" as a mechanism that regulates transport. The combined action of intracellular CAII and extracellular CAIV work to maximize the size of the transmembrane [HCO_3^-] gradient, which is the driving force for transport (Fig. 7). CAIX has a single TM, anchoring the catalytic site on the extracellular surface. Recently we showed that CAIX binds AE1-3 and accelerates the rate of cellular acid loading. The finding is significant as it reveals that CAIX /AE2 interaction plays a fundamental role in gastric acid secretion (Fig. 3) (Morgan *et al.* 2006).

Conclusions

So why study bicarbonate and its transport? HCO_3^- is involved in a very broad range of biochemical and physiological processes. This has allowed me to follow a broad range of fascinating projects from structural biology to physiology, even disease processes. It has allowed me to interact with a wide range of outstanding scientists, which has been the best part!

Acknowledgements

I would like to thank my Ph.D. supervisor, Reinhart Reithmeier, for setting me down the path to study bicarbonate transport. Research in my laboratory has been possible only because of an amazing group of students, post-doctoral fellows and technicians who have worked with me. I also thank my collaborators for fun and inspiration! Generous support from the Alberta Heritage Foundation for Medical Research, Canadian Institutes of Health Research, Heart and Stroke Foundation of Alberta and the Canadian Cystic Fibrosis Foundation has made this work possible. Finally, I thank CSBMCB and Merck-Frosst for this award. This work has been previously published in Biochem. Cell Biol. (2006) 84, 930-939.

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