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The Carbonic Anhydrases: Widening Perspectives on Their Evolution, Expression and Function

Richard E. Tashian

Summary

Now, some 55 years after its discovery in bovine red cells, carbonic anhydrase (CA), in all its varied forms, continues to challenge and intrigue physiologists, biochemists and molecular geneticists. This is so because of an increasing awareness of the many apparently diverse functions of the different CA isozymes encoded by this large multigene family, the continuing discovery of new CA, or CA-related, genes, and the extensive variation in their hormonal control, cellular expression and subcellular localization.

Introduction

The carbonic anhydrases (CA) are zinc metalloenzymes that catalyze the simple interconversion of CO_2 and HCO_3^- ($CO_2 + O_2 \rightleftharpoons HCO_3^- + H^+$). They are found in almost all organisms, and are notable for the extremely high turnover numbers (some exceeding 1×10^6 sec^{-1}) of the high-activity forms, ranking them along the most efficient enzymes known. They are also distinctive because of the extensive diversity in both the cellular distribution and in the putative or established biological functions of the seven CA isozymes now known to occur in higher vertebrates. Some of the CA genes are expressed in certain cells of nearly all tissues, whereas others appear to be more limited in their distribution. In view of such diversity in expression and function, the CA isozyme system is an excellent model for the study of molecular processes and patterns underlying the evolution and expression of genes derived from a common ancestor. (for recent accounts, see papers in refs. 1 and 2.)

Evolutionary Patterns

Since the reaction catalyzed by carbonic anhydrase may well have been of widespread adaptive value to the earliest organisms, it is possible that it was among the first enzymes to appear. Since what seem to be evolutionarily related (homologous) forms of carbonic anhydrase are found in certain bacteria and algae,[3] the original gene probably arose before the divergence of prokaryotes and eukaryotes more than one and a half billion years ago. And during their long evolutionary history the CA genes have undergone many rounds of duplications resulting in new genes, the products of which have been implicated in such diverse processes as calcification, photosynthesis, respiration, acid-base homeostasis, bone resorption, formation of aqueous humor and gastric juice, and the synthesis of urea, glucose and lipids.[1,2]

The Genes

In terrestrial vertebrates (i.e. amniotes) the carbonic anhydrase system comprises at least 7 genes (Table I). Four of these (coding for CA I, CA II, CA III and CA VII) have now be fully or partially characterized,[4–8] and with the exception of human CA I, which has a large intron in the 5′ untranslated region,[8] they all appear to have seven exons, with the six introns at the same positions, and range in size from 9·8 kb (human CA VII) to 17 kb (chicken and

*TABLE I. Summary of selected features of the mammalian CA isozymes and viral CA-like proteins**

	Amino acid residues	Activity (mol sec^{-1})	Chromosome position	Distribution	Some established and purported functions
CA Isozymes					
Cytosolic					
CA II	259	High ($\sim 1 \times 10^6$)	Human 8q22 Mouse 3	Widely distributed in secretory and absorbing epithelia. Certain cells of virtually all tissues [e.g. bone (osteoclasts), brain† (oligodendrocytes), eye (ciliary body, lens, Müller cells of retina), liver (perivenous cells), kidney (tubules), salivary glands (acinar cells), pancreas (ductal cells), stomach (parietal cells) and uterus]. Red cells and platelets. Undetected in granulocytes, monocytes, fibroblasts, osteoblasts, and CNS neurons	Promotion of gas, fluid and ion transfer. Maintenance of acid-base homeostasis. H^+ secretion (gastric juice production, renal acidification, bone resorption). HCO_3^- secretion (production of pancreatic juice, CS fluid, acqueous humor, saliva). HCO_3^- reabsorption, (proximal renal tubules). CO_2 excretion and exchange. Ca^+ transport (placenta). Fatty acid and amino acid synthesis. Cl^- exchange (colon)?
CA I	260	Low ($\sim 1 \times 10^5$)	Human 8q22 Mouse 3	Primarily in red cells, gastrointestinal epithelia and vascular endothelium. Also in such tissues as: eye (corneal endothelium, lens and ciliary body epithelium), sweat glands, salivary glands, adipose cells and myoepithelial cells	Probably similar to CA II functions. Possible unique roles in endothelial cells of capillaries and eye (cornea and lens). Absorption of NH_3 in rumen and colon?
CA III	259	Low ($\sim 1 \times 10^4$)	Human 8q22	Mainly in red skeletal muscle (type 1). Also in liver of non-primates (esp. male rat), salivary glands, smooth muscle (uterus), red cells, prostate gland, lung, kidney, brain colon, testis, mammary gland, and white fat cells	Facilitation of CO_2 diffusion. Role in muscle contraction? Acid-base homeostasis (liver)
CA VII	263	?	Human 16q21–23	Salivary gland (?)	?
Membrane-bound					
CA IV (glycoprotein)	?	High	?	Lung (capillary endothelium), kidney (brush border of tubular cells). Probably also in other tissues (e.g. muscle, liver, gastrointestinal mucosa)	Reabsorption of HCO_3 in proximal renal tubules. Transport of H^+ and HCO_3^- in capillary endothelium of lung, and facilitation of CO_2 transfer. Ca^{2+} exchange (muscle)?
Mitochondrial					
CA V	?	High	?	Kidney and liver. Probably in many other tissues (e.g. muscle, gastric mucosa)	Provide HCO_3^- for initial steps in gluconeogenesis (e.g. liver & kidney), and ureagenesis (liver)
Secreted					
CA VI (glycoprotein	314	High and low	Human 1pt36	Salivary glands (acinar cells)	Regulation of salivary pH?
Unknown					
CA 'Y'	262	?	?	Mouse liver (possibly CA V)	?
Viral CA-like proteins					
D8 product	304	—	—	Vaccinia virus (transmembrane protein)	?
erb-A (domain 2) product‡	164	—	—	Avian erythroblastosis virus	c-*erb-A* (thyroid hormone receptor)

* Sources for this table compiled largely from refs. 1 and 2 and citations therein. Additional data for CA isozymes: CA I[8,9], CA II[10,24,42], CA III[6,9,24,42], CA IV (W. S. Sly, unpublished), CA VI[37,41], CA VIII[7], CA 'Y' is a tentative designation, sequence derived from mouse liver mRNA (M. Amor & T. Meo, unpublished). Viral sources: AEV *erb-A*,[16,17] vaccinia D8[14,15]. † 50% membrane-bound (rat). ‡ Homology to CA genes uncertain.

human CA II). The human CA I gene contains an unusually long 5′ non-coding region of about 37 kb, making it about 3–5 times larger than the other CA genes. Because the evolutionary origin of the intron which interrupts this 5′ region is of interest, it will be useful to know to what extent it is also characteristic of the CA I genes from other, especially non-mammalian, sources.

The human CA I, CA II and CA III genes are linked on chromosome 8,[9,10] whereas the genes for CA VI and CA VII are located on chromosomes 1 and 16, respectively.[7,11] Since the CA I, CA II and CA III genes are present in birds and mammals,[12] they must have been formed prior to the divergence of reptiles and mammals about 300 million years ago, and have remained linked in the mammalian lineage since that time. In view of the dispersion of the CA genes to at least three chromosomes, it

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TABLE II. Matrix comparing amino acid sequences of the carbonic anhydrases (CA) and CA-like proteins

	CA II's	CA III's	CA VII	CA 'Y'	D8 (Vaccinia)	CA VI	CA IV	*erb-A* (*AEV*)
				Percent identity*				
CA I's	59	55	52	47	36	32	32	16
CA II's	—	56	56	52	35	33	32	20
CA III's	—	—	51	46	37	34	31	15
CA VII	—	—	—	52	35	36	33	15
CA 'Y'	—	—	—	—	34	34	32	17
D8 (vaccinia)	—	—	—	—	—	29	29	13
CA VI	—	—	—	—	—	—	28	15
CA IV	—	—	—	—	—	—	—	9

* Determined from arithmetic means of pairwise comparisons of 6 mammalian CA I's and CA II's, 5 CA III's, human CA IV and mouse CA 'Y', sheep CA VI, human CA VII, vaccinia D8, and human c-*erb-A*. Sequences for CA I, CA II and CA III isozymes are from ref. 11, plus mouse CA I[38], mouse CA III (Y. Edwards, unpublished) and horse CA III[39]. References for other sequences are: human CA VII,[7] mouse CA 'Y' (M. Amor & T. Meo, unpublished), vaccinia virus D8,[13] sheep CA VI,[37] human CA IV (W. S. Sly & C. X. L. Zhu, unpublished) and *erb-A*, domain 2.[16,17] Insertions and deletions were introduced to maximize the number of matches in the comparisons.

will now obviously be of interest to determine the chromosomal locations of the membrane-bound CA IV and mitochondrial CA V genes. Possibly, detailed analyses at the DNA level of the flanking regions of the CA I, II and III genes will eventually provide clues concerning the importance of common regulatory elements as selective factors in maintaining gene linkages. Preliminary studies in our laboratory indicate that in humans these genes are arranged in the 5′ to 3′ order (CA I, CA III, CA II) within a span of less than 180 kilobases (P. J. Venta, unpublished, and ref. 13).

Although the structures of CA genes from plants and lower organisms have not been characterized, it is interesting to note that a CA-like gene (D8), devoid of introns, and encoding 304 amino acids, has been found in vaccinia virus.[14] When the derived amino acid sequence is compared to the sequences of the cellular CA isozymes, the percent identity ranges from 37% to 29% (Table II). This viral gene product, however, probably lacks CA activity (see discussion of active sites below). Recent studies have demonstrated that the product of the D8 gene is a transmembrane protein with a major extraviral, CA-like domain which is non-essential for viral propagation.[15] It will be especially interesting to dissect the evolutionary history of the vaccinia D8 gene by examining other pox viruses. Another virus which possibly contains a CA-related gene is avian erythroblastosis virus (AEV),[16] in which the 3′ portion (domain 2) of its *erb-A* gene may be homologous to a region spanning residues 10–183 (based on CA I numbering)[12] of the 261 residues characteristic of the CA I, CA II and CA III isozymes.[12] It is now known that the human and chicken cellular c-*erb-A* gene encodes a thyroid hormone receptor,[17] the C-terminal, hormone-binding segment of which shows some apparent similarity (not statistically significant) to the region (positions 10–183) of the CA sequences just discussed. Thus, c-*erb-A* may be a hybrid gene deriving its 3′ hormone-binding half from a portion of a CA gene, and its 5′ DNA-binding region from some other source.

Phylogenetic Relationships

To date, amino acid sequences have been determined, or inferred from DNA sequences, for the CA I, CA II and CA III isozymes from a number of species,[12] as well as sequences from single species for the more recently characterized CA isozymes, CA VI, CA VII and CA 'Y'. CA 'Y' is a tentative designation for a CA isozyme whose sequence was derived from mouse-liver mRNA (M. Amor & T. Meo, unpublished). Because its sequence does not closely resemble any of the other mouse CA isozymes (Table III), it is possible that it represents the mitochondrial isozyme, CA V (see below).

A matrix has been prepared comparing the consensus amino acid sequences for CA I, CA II and CA III, along with those for CA IV, CA VI, CA VII, CA 'Y' and the two viral CA-like genes (Table II). As can be seen, there is a 59% percent identity between the CA I and CA II sequences, with an apparent decrease in evolutionary relatedness when comparisons are made with other CA and CA-like sequences in the order:

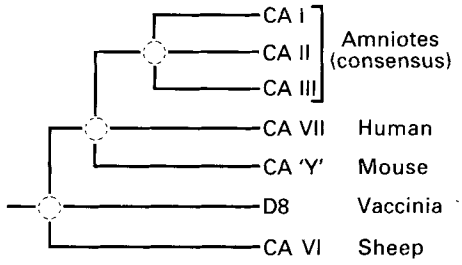

Fig. 1. *Generalized phylogenetic branching scheme for the CA isozymes of amniotes and the vaccinia D8 protein. Based on amino acid sequences cited in Table II.*

CA III, CA VII, CA 'Y', vaccinia D8, CA VI, CA IV and *erb-A*. With the exception of the latter two, these CA and CA-related sequences were also used to construct the generalized branching scheme shown in Fig. 1 utilizing a maximum parsimony algorithm for determining ancestral sequences.[12] As shown with the matrix analysis, there is a similar order of evolutionary relatedness of the CA genes, with the salivary CA VI gene appearing to have diverged first from the ancestral root.

The Active Sites

Because the three-dimensional structures of human CA I and CA II and bovine CA III have been determined,[18,19] it is possible to compare residues presumed to be located within the active sites of the different CA isozymes (Table III). Of the 36 residues at homologous positions, 17 (47%) are invariant. Of the other residues, all expect the tiger shark red-cell CA have at least one residue that seems to be unique and invariant for that isozyme. To what extent these residues contribute to the catalytic variation noted among the different isozymes is difficult to assess. For example, CA III is a low-activity isozyme which is not strongly inhibited by sulfonamides,[20] and it is tempting to attribute these feature to one or more of its eight unique residues, especially the basic residues, Arg and Lys, at positions 64, 67 and 91. However, when His-64 was replaced by Lys in human CA II by site-directed mutagenesis, no appreciable reduction in its CO_2 hydrase activity at pH 8·8 was observed.[21] This was unexpected, because His-64 was thought to play an important role in the transfer of protons from the active site in the interconversion of CO_2 and HCO_3^-.[22] Another possibility is that the unique Phe-198 residue may modify the function of the Thr-199/Glu-106 complex which has been strongly implicated in the catalytic process.[22] Also, the unique

TABLE III. Residues postulated to occur within the active sites of animal carbonic anhydrases compared with vaccinia D8 protein[a]

CA Forms[c]	Residue number[b]																																		
	7	29	61	62	64	65	66	67	69	91	92	94	96	106	107	117	119	121	131	141	143	145	192	194	198	199	200	201	202	204	206	207	209	211	244	246
	*	*			*			*			*	Zn	Zn	*	*	*	Zn							*		*	*						*		*	*
Human CA I	Y	S	N	V	H	S	F	H	N	F	Q	H	H	E	H	E	H	A	L	L	V	G	W	Y	L	T	H	P	P	Y	S	V	W	I	N	R
Rabbit CA I	Y	S	N	V	H	S	F	H	N	S	Q	H	H	E	H	E	H	V	I	L	I	A	W	Y	L	T	H	P	P	H	S	V	W	I	N	R
Mouse CA I	Y	S	N	V	H	S	F	H	I	T	Q	H	H	E	H	E	H	V	A	L	I	G	W	Y	L	T	H	P	P	H	S	V	W	I	N	R
Ox CA I	Y	S	N	V	H	S	F	H	N	F	Q	H	H	E	H	E	H	V	F	L	L	G	W	Y	L	T	H	P	P	L	S	V	W	I	N	R
Horse CA I	Y	S	N	V	H	S	F	Q	K	V	Q	H	H	E	H	E	H	V	F	L	I	G	W	Y	L	T	H	P	P	Y	S	V	W	V	N	R
Turtle CA I	Y	S	N	V	H	S	F	H	N	Q	Q	H	H	E	H	E	H	V	F	L	L	G	W	Y	L	T	H	P	P	F	S	V	W	I	N	R
Human CA II	Y	S	N	N	H	A	F	N	E	I	Q	H	H	E	H	E	H	V	F	L	V	G	W	Y	L	T	T	P	P	L	C	V	W	V	N	R
Rabbit CA II	Y	S	N	N	H	S	F	N	E	I	Q	H	H	E	H	E	H	V	F	L	V	G	W	Y	L	T	T	P	P	L	C	V	W	V	N	R
Mouse CA II	Y	S	N	N	H	S	F	N	E	V	Q	H	H	E	H	E	H	V	F	L	V	G	W	Y	L	T	T	P	P	L	C	V	W	V	N	R
Ox CA II	Y	S	N	N	H	S	F	N	E	I	Q	H	H	E	H	E	H	V	F	L	V	G	W	Y	L	T	T	P	P	L	S	V	W	V	N	R
Horse CA II	Y	S	N	N	H	S	F	N	E	I	Q	H	H	E	H	E	H	V	F	L	V	G	W	Y	L	T	T	P	P	L	C	V	W	V	N	R
Chick CA II	Y	S	N	N	H	S	F	N	E	V	Q	H	H	E	H	E	H	V	F	L	V	G	W	Y	L	T	T	P	P	H	C	V	W	V	N	R
Human CA III	Y	S	N	N	K	T	C	R	V	R	Q	H	H	E	H	E	H	V	F	I	V	G	W	Y	F	T	T	P	P	E	C	I	W	L	N	R
Mouse CA III	Y	S	N	N	R	T	C	R	V	R	Q	H	H	E	H	E	H	V	F	I	V	G	W	Y	F	T	T	P	P	E	C	I	W	L	N	R
Ox CA III	Y	S	N	N	K	T	C	R	V	R	Q	H	H	E	H	E	H	V	F	I	V	G	W	Y	F	T	T	P	P	E	C	I	W	L	N	R
Horse CA III	Y	S	N	N	R	T	C	R	V	R	Q	H	H	E	H	E	H	V	Y	I	V	G	W	Y	F	T	T	P	P	E	C	I	W	L	N	R
Shark CA	X	S	X	X	X	X	X	X	X	R	Q	H	H	E	H	E	H	V	F	L	V	G	W	Y	L	T	T	P	P	L	S	V	W	V	N	R
Mouse CA "Y"	X	S	N	V	H	Q	F	Q	V	K	Q	H	H	E	H	E	H	F	Y	L	V	G	W	Y	L	T	T	P	P	A	S	V	W	V	N	R
Human CA VII	Y	S	N	N	H	S	V	Q	D	K	Q	H	H	E	H	E	H	V	F	L	V	G	W	Y	L	T	T	P	P	S	N	V	W	V	N	R
Sheep CA VI	Y	S	N	N	H	T	V	N	S	K	Q	H	H	E	H	E	H	V	Y	L	V	A	Y	Y	L	T	T	P	P	T	N	V	W	V	N	R
Human CA VI	Y	S	N	N	H	T	V	Q	G	Q	Q	H	H	E	H	E	H	V	Y	L	V	A	Y	Y	L	T	T	P	P	T	N	V	W	V	N	R
Vaccinia D8		S	N	T	K	L	V	R	N	S	S	H	Y	M	N	E	N	V	Y	L	I	S	F	Y	-	T	T	I	N	S	D	A	W	I	N	R

[a] Based on tertiary structures of human CA I, CA II and bovine CA III.[17,18]

[b] Based on CA I numbering.[12]

[c] Sequence sources are as in legend for Table II. Tiger-shark sequence from N. Berhenhem & U. Carlsson (unpublished). Residues common to all animal sequences (including vaccinia) have been boxed. Other boxes indicate invariant and unique residues for CA isozymes of aminiotes. Dashed box at position 64 for CA III's indicates unique basic residues. Residues forming hydrogen-bond network to Zn-bound solvent molecule, or to the three Zn-liganded His residues (designated, Zn), in human CA I and CA II and bovine CA III (cf. refs. 17, 18) are designated by an asterisk (*).

Cys-66 residue could conceivably contribute to the characteristic properties of the CA III isozymes. Almost certainly, results from other site-directed mutageneis studies will provide further insights into the active site mechanisms of the CA isozymes.

The antiquity of the active-site structure is suggested by an examination of the residues found in the red-cell CA of the tiger shark (Table III), a cartilaginous fish which arose around 450 million years ago. Although the residues at positions 64–69 have not been determined, the shark CA has retained an active-site structure differing little from those of the cystolic CA isozymes of amniotes. However, since this represents the first structure of a non-amniotic CA, it would be premature to say that the active-site residues of the shark CA are representative of the ancestral structure.

Unfortunately, no clues are evident concerning the evolution of the active sites by simply examining the structural regions coded by exons. As shown in Table III and Fig. 2, residues associated with the active sites are coded on all but one exon, and the three His residues binding the essential zinc ion are coded on exons 2 and 3. In view of the observations from several studies that exons encode various domains of proteins, it is notable that no similar correlation is evident when the exonic regions of mammalian and avian CA II genes are compared with the structural or functional features of the CA II molecules (Fig. 2).

It is also noteworthy that the CA-related gene, D8, from the vaccina virus has retained 60% of the active site residues found in the cellular CA isozymes (Table III). However, because certain crucial residues are not present, such as the zinc-binding His residues at positions 96 and 119, the possibility that this viral protein has CA activity is extremely remote.

Hormonal and Neuronal Control

Early studies indicated that the activities or levels of CA I and CA II in various mammalian tissues could be influenced by thyroxine (red blood cells), sex hormones (endometrium, accessory sex glands), cyclic AMP-mediated action of epinephrine (red blood cells), and prolactin (pituitary gland).[3]

Recently, it was shown that pituitary growth hormone (GH) has a differential effect on the levels of CA III in rat liver, where the amount of CA III in the male can be 15–20 times greater than in the female.[23] Initially, this was thought to be due to the induction of CA III by testosterone; however, recent studies indicate that CA III is suppressed in the female liver by the continuous action of GH, whereas the pulsatory release of GH in the male does not noticeably suppress the levels of CA III.[23] No similar effect of GH on CA III was seen in muscle where the levels of CA III are the same in males and females. It is noteworthy that CA III (as well as CA II) in rat liver as determined by immunofluorescence is concentrated in hepatocytes surrounding the central vein.[24] In these perivenous cells, the staining intensity of CA III is markedly higher in males than in females, whereas the

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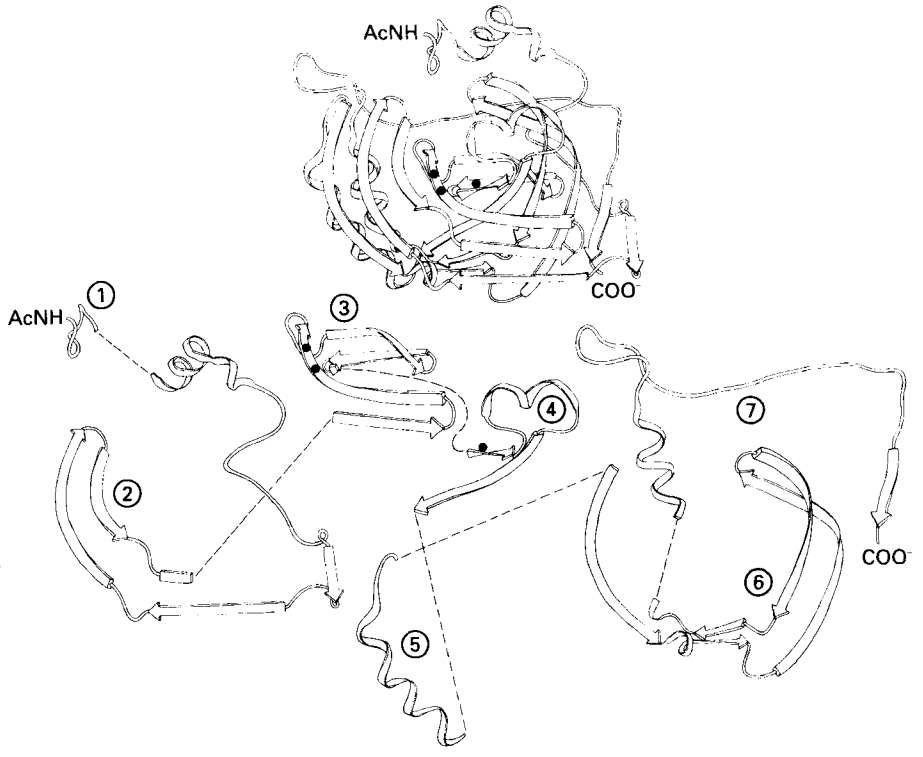

Fig. 2. *Schematic representation of the three-dimensional structure of human carbonic anhydrase II (above), and separated structures of the segments encoded by the seven exons of human and mouse CA II genes (below). Beta strands are designated by arrows. Based on the human CA I sequence,[12] the numbering of the residues encoded by these exons are: exon 1 (residues 1–12), exon 2 (12–78), exon 3 (78–117), exon 4 (118–149), exon 5 (150–170), exon 6 (171–222) and exon 7 (223–260). The His residues at 94, 96 and 119 forming ligands to the active-site Zn ion are indicated by solid circles (●). The tertiary structure of CA II is based on Fig. 3 in ref. 22 originally drawn by Anders Liljas.*

opposite is true for CA II, which stains more intensely in females than in males.

Changes in the levels of CA III in rat skeletal muscle brought about by hypo- and hyperthyroid conditions seem to depend on the type and amounts of the different fibers. The increase in CA III resulting from thyroidectomy was due not only to an increase in the slow-twitch type-1 fibers (which normally express CA III), but also in a subtype of type 2A fibers (which do not normally express CA III).[25] Treatment with thyroid hormone brought about a decrease of CA III in type-1 fibers, and an increase in CA III in type 2A fibers.[26] These variations in the expression of CA III in the different fiber types to varying thyroxine levels are difficult to interpret at present. It is of interest that a similar response to thyroxine levels has been noted for CA I in human and mouse red cells in that hyper- and hypothyroidism results in increased and decreased levels, respectively, of CA I.[3]

Neuronal control of CA III in rat and rabbit skeletal muscle has also been shown by the demonstration of increased levels of CA III after denervation,[27] or the continuous stimulation of an innervating nerve.[20] Interestingly, the elevated levels are more pronounced in type-2 fibers, where CA III is not normally expressed, than in type 1 fibers, where they are normally present. Possibly type 2 fibers are replaced by type 1 and superimposed on this may be the induction of CA III in pre-existing fibers. Although the mechanism for this increase in CA III is not clear, it is possible that CA III is suppressed to some extent by a growth factor secreted by nerve endings, and in the absence of this factor, CA III levels are increased in a manner similar to the increase of CA III in female rat liver on hypophysectomy.[23]

Other examples of hormonal control of CA have been the recent demonstration of androgen regulation of CA II in rat prostate,[28] and the modulation of CA II by calcium-regulating hormones (calcitonin and parthyroid hormone) in human red blood cells.[29]

Diversity in Expression and Function

Although the CA isozymes are known to possess activities other than the reversible hydration of CO_2 (e.g. carboxylesterase, aldehyde hydratase, phosphatase),[3] physiological roles, if any, for these activities have not been established. Thus it is the simple reversible hydration of H^+ and HCO_3^- from CO_2 and H_2O which is the basis for the numerous putative and established physiological roles of the CA isozymes (Table I). This functional diversity appears to be mostly associated with the widely distributed high-activity CA II isozyme and to some extent with CA I. Although CA III is found mainly in skeletal muscle, it is also expressed in hepatocytes (as discussed above) and at low levels in certain other cells. In muscle, CA III has been implicated in the facilitated transport of CO_2,[20] and is also thought to have a role in the slow-twitch contractile apparatus of type-1 fibers.[26] CA III does not seem to be associated with any subcellular structures of the muscle cell such as mitochondria, nuclei, triads and Z and M bands.[30] It is difficult to understand why the low-activity CA III isozyme is needed in muscle and liver; however, because CA III possesses low acid phosphatase activity, it is possible that it may function in a role different from, or in addition to, that of facilitating CO_2 diffusion.[20] The clustering of CA II and CA III around the venous wall in liver suggests that the acid-secreting hepatocytes regulate protein and HCO_3^- secretion into the blood, implicating a role in acid-base balance.[24] The other isozymes (CA IV, CA V, CA VI and CA VII) seem to be more restricted in their cellular or subcellular expression. Although the membrane-bound CA IV form is known to occur in kidney (brush border of luminal cells) and lung (endothelium), it seems likely that it will be found in other tissues as well. As of now, the greatest number of expressed CA genes that have been reported are apparently expressed in the salivary glands. These are: the cytosolic CA I, CA II and CA III forms, the secreted CA VI form, and the newly discovered isozyme CA VII,[7] previously designated CA 'Z' (Table I). Why are four, non-secreted, cytosolic forms of CA needed to carry out their putative roles in fluid and ion transport? Of course, a similar question can be asked about the roles of the same CA I, II and III isozymes in such tissues as liver and muscle, which incidentally probably also express a total of at least five CA genes.

Metabolic Roles

The presence of carbonic anhydrase in liver mitochondria has been confirmed in guinea pig and rat, and more recently reported from the mitochondria of rat

(but not guinea pig) kidney.[19,31] It also may be present in the mitochondria of muscle fibers, oligodendrocytes, and gastric mucosa. The partial amino acid sequence of a CA isozyme (termed CA V) purified from guinea-pig mitochondria has been reported,[32] and an examination of the sequence indicates that it is coded by a CA gene distinct from those coding for the other CA isozymes.

Because CA inhibitors (e.g. acetazolamide) diminish the production of urea and glucose in hepatocytes, it appears that a role for CA V in mitochondria (which are permeable to CO_2, but not HCO_3^-), is to provide HCO_3^- ions for the synthesis of carbamyl phosphate and oxaloacetate formed in the first, intramitochondrial steps, respectively, of the urea cycle and gluconeogenesis. Possible roles for mitochondrial CA in muscle, brain and gastric mucosa have yet to be fully explored.

In addition, the production of HCO_3^- by CA is probably utilized for other anabolic syntheses requiring carboxylation reactions such as fatty-acid synthesis as well as the synthesis of certain amino acids.[33]

Inferring Function from Carbonic Anhydrase Deficiencies

We might expect to learn something about function of an enzyme if its absence results in an abnormality in the cell or tissue where it is normally expressed. Therefore it was somewhat unexpected to find that the inherited deficiency of CA I in humans and pigtail macaques (homozygous for the deficiency gene) produced no detectable defect.[3] Although seemingly not as widely distributed as the CA II isozyme, CA I is nevertheless the second most abundant protein in red cells next to hemoglobin, and is found in the cells of a number of different tissues (Table I). Among the mechanisms which could compensate for the loss of the CA I activity would be that CA II is probably present in most cells where CA I is expressed and could substitute for the role of CA I. Because individuals deficient in CA I exhibit no detectable clinical symptoms on routine physical examination, nothing was learned about any specific function of CA I. On the other hand, a deficiency of CA II in humans was more informative, as its pleiotropic effects include impairments to growth, kidney function, bone resorption, and brain development.[34] Even this was not anticipated, because the wide distribution of CA II (Table I) would suggest that its deficiency would result in a syndrome of even greater severity. Again, as in the CA I deficiency, the presence of CA I or other CA isozymes in a cell deficient in CA II probably compensates for the absence of CA II. This was nicely demonstrated in studies on intact erythrocytes from humans deficient in CA II, where the CA I isozyme which is normally present at high levels appears to function quite adequately in carrying out the important respiratory role of CA in the red cell.[35] In all likelihood, alternative biochemical pathways or mechanisms compensate for the loss of CA II in those cells. With this in mind, we might speculate on the consequences of deficiencies in the non-cytoplasmic CA isozymes such as the membrane-bound CA IV and mitochondrial CA V forms, where the probability of a 'back-up' CA seems less likely. Possibly, CA V deficiency would result in hyperamoniaemia, and the absence of CA IV would produce critical dysfunctions in kidney and lung.

An important finding from studies on the human CA II-deficiency syndrome was that the osteopetrosis produced by a reduction in bone demineralization confirmed that the CA II normally present in osteoclasts is involved in bone resorption. However, osteopetrosis was not observed in mice deficient in CA II,[36] although CA II is known to be expressed in murine osteoclasts.

So far as kidney function is concerned, the renal tubular acidosis appears to be produced by an impairment of renal acidification and HCO_3^- reabsorption in both CA II-deficient humans and mice, and is probably due to the absence of soluble CA II in the luminal cells of the distal and proximal tubules, which impedes the reclamation of HCO_3^-.

The cerebral calcification associated with humans deficient in CA II has not been observed in CA II-deficient mice. However, older CA II-deficient mice do exhibit calcified plaques in the choroid plexus, in addition to rather extensive medial calcification of small arteries in a number of organs[40]. Whether the cerebral calcification found in older CA-II-deficient humans arises from vascular calcinosis has yet to be determined. CA II appears to be the most prevalent CA isozyme in brain and is characteristically found in oligodendrocytes. The association between CA II deficiency and mental impairment in humans is not understood. Although some degree of mental impairment is usually associated with the CA II-deficiency syndrome, some affected individuals have been reported with seemingly normal mental function in spite of extensive cerebral calcification[34].

The differences in the pleiotropic human and mouse phenotypes serve as a warning that species-specific CA isozyme expression, and differences in interacting gene products, may compromise the general use of animal models.

As yet the molecular lesions responsible for the human and mouse CA deficiencies have not been determined. In the mice deficient in CA II the levels of CA II mRNA were not found to deviate from normal in all tissues examined, including brain and kidney, suggesting that a translational defect is responsible for the deficiency. No major deletion in the CA II gene from CA II-deficient humans was detected by Southern blot analysis, and although a considerable portion of the defective genes found in patients from Belgium and Kuwait[34] has been sequenced in our laboratory, no splicing or coding errors, or alternations in the promoter regions, have as yet been detected (P. J. Venta, unpublished).

Finally, with regard to the control of gene expression, several features of the inherited CA deficiencies might be expected to repay detailed analyses at the DNA level. One of the most intriguing is that the reduction of red-cell CA I to trace levels in pigtail macaques deficient in CA I is accompanied by a 60% reduction in the closely linked CA II gene product.[3,13] It would obviously be of considerable interest to determine the molecular defect responsible for this 'double deficiency' such as the possibility of a mutation in a common regulatory *cis*-element. Another type of 'double deficiency' is the possibility of *trans*-acting regulation implicated in the report of a deficiency of salivary CA VI in CA II-deficient humans[41] whose CA II and CA VI genes are known to be on chromosomes 8 and 1, respectively.[10,11]

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Note added in proof

Results of recent ^{18}O-exchange, activity measurements on human CA II in which Ala and Lys have been substituted for His at position 64 by site-directed mutagenesis now indicate that His 64, as predicted, does contribute to the catalytic mechanism through its role in the proton transfer pathway (D. N. Silverman and S. Lindskog, personal communication; Tu, C. K. *et al.*, 1989, Biochemistry, in the press).

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