Biochemical Genetics, Vol. 38, Nos. 7/8, 2000

Absence or Reduction of Carbonic Anhydrase II in the Red Cells of the Beluga Whale and Llama: Implications for Adaptation to Hypoxia

Hong Yang,[1,2] David Hewett-Emmett,[3] and Richard E. Tashian[1,4]

Received 13 July 1999—Final 13 April 2000

Carbonic anhydrase (CA) expression was examined in the red cells of two mammals that have adapted to low oxygen stress: the llama, which has adapted to high altitudes, and the beluga (or white) whale, which routinely dives for extended periods. Immunodiffusion analyses of their Hb-free hemolysates and partial amino acid sequencing of their HPLC-separated nonheme proteins indicate that the low-activity CA I isozyme is the major nonheme protein in erythrocytes of both the beluga whale and the llama. The high-activity CA II isozyme was not detected in the whale red cells but was present at low levels in erythrocytes of the llama. These results suggest that the absence or decrease in the expression of the high-activity CA II isozyme may be advantageous under hypoxic conditions.

KEY WORDS: hypoxia; red cell carbonic anhydrases; beluga whale; llama.

INTRODUCTION

The carbonic anhydrases (CA) are zinc metalloenzymes that catalyze the reversible hydration of CO_2. In mammals (and probably all amniotes), 11 active CA isozymes and 3 inactive CA isoforms have now been identified that exhibit highly diverse functions and patterns of expression (for recent reviews see

[1] Department of Human Genetics, Buhl 4909, University of Michigan Medical School, Ann Arbor, Michigan 48109-0618.
[2] Department of Science and Technology, Bryant College, Smithfield, Rhode Island 02917.
[3] Human Genetics Center, University of Texas Health Science Center at Houston, P.O. Box 20334, Houston, Texas 77225.
[4] To whom correspondence should be addressed.

0006-2928/00/0800-0241$18.00/0

Tashian, 1992; Hewett-Emmett and Tashian, 1996; Sly and Hu, 1996; Hewett-Emmett, 2000; Tashian *et al*., 2000). Three of the active isozymes, CA I, CA II, and CA III, are variously expressed in mammalian red cells, and the genes encoding them are closely linked on chromosome 8 in humans and chromosome 3 in mice (Tashian, 1992). Both CA I and CA II are usually expressed concurrently in erythrocytes, where the protein levels of the low-activity CA I isozyme are most often found at higher levels than those of the high-activity CA II isozyme. For example, in humans the protein levels of CA I and CA II are ~13 and ~2 μg/mg Hb, respectively (Tashian and Carter, 1976). However, in members of the cat family (Felidae) and the artiodactyl suborder, Ruminantia (e.g., ox, sheep, goat), to date only the high-activity CA II isozyme has been reported in their red cells (Ashworth *et al*., 1971; Tashian, 1977). While expressed abundantly in red skeletal muscle, the CA III isozyme has been detected only at very low levels (i.e., ~150 μg/g Hb) in erythrocytes; however, an early report suggested that red cell CA III levels were elevated to ~330 μg/g Hb in an individual with inherited CA II deficiency syndrome (Carter *et al*., 1984).

The biochemical properties of red cell CAs have been investigated in several species of ruminants: ox (Satore *et al*., 1969; Kloster *et al*., 1970; Ashworth *et al*., 1971; Bouquet and Van de Weghe, 1972), bison (Satore *et al*., 1969), sheep (Ashworth *et al*., 1971; Tanis *et al*., 1974; Wang *et al*., 1996), goat (Ashworth *et al*., 1971), deer (Ashworth *et al*., 1971), and moose (Carlsson *et al*., 1973). However, ruminants in the infraorder Pylopoda, such as the camel and llama (Camelidae), have not been examined. Also, except for a report of what appeared to be a high-activity CA purified from the red cells of a blue–white dolphin (Shimizu and Matsuura, 1962), little is known about the red cell CA isozymes of cetaceans (whales and dolphins).

Three types of mammals face the challenges of adjusting to low-oxygen (hypoxic) environments: those living at high altitudes or underground and prolonged-diving marine mammals. The biochemical aspects of adaptation to low-O_2 environments have traditionally focused on hemoglobin (cf. Ridgway, 1972), whereas the role of nonheme proteins (NHP) in red cells has been infrequently examined. In a recent investigation of NHPs in the subterranean mole rat (*Spalax ehrenbergi*), we found that a selenium-binding protein and the low-activity CA isozyme, CA I, were the only major NHPs expressed in their red cells (Yang *et al*., 1998). Because the high-activity CA II isozyme was not detected, we hypothesized that the absence of CA II might provide a physiological advantage for living under hypoxic conditions. To test this hypothesis further, here we have examined the protein levels of red cell CA I, CA II, and CA III in two other mammalian species that live at low oxygen levels: the llama (*Llama glama*), which has adapted to high altitudes, and the beluga (or white) whale (*Delphinapterus leucas*), which routinely engages in prolonged-diving activity.

MATERIALS AND METHODS

Preparation of Blood Samples

Whole-blood samples were collected from a domestic dromedary camel (*Camelus dromedarius*) from China, two domestic llamas (*Llama glama*), a male and female, from a llama farm in Michigan, and three beluga whales (*Delphinapterus leucas*) from the Naval Command, Control and Ocean Surveillance Center (San Diego, CA). Hemolysates from human and domestic sheep (*Ovis aries*) and purified bovine (*Bos taurus*) CA I (rumen) and CA III (red cells) were used as controls (Tanis *et al*., 1974; Tashian *et al*., 1980). Preparation of the hemolysates, and their Hb-free extracts, was carried out as described previously (Tashian *et al*., 1968; Tashian and Carter, 1976; Yang *et al*., 1998).

Electrophoresis and Immunodiffusion

Hemolysates and Hb-free extracts were examined by electrophoresis in 12% vertical starch gels (Electrostarch Co., Carrborro, NC), and the cyanogen bromide CNBr-digested fragments were separated in 10% SDS-PAGE gels. The CA activity in starch gels after electrophoresis was detected with bromthymol blue staining, and the proteins were visualized by staining with 0.1% nigrosin. These electrophoretic and staining procedures were essentially those described elsewhere (Tashian and Carter, 1976; Tashian *et al*., 1968; Yang *et al*., 1998). Antibodies were prepared in rabbits against purified bovine CA I (rumen), sheep CA II (red cell), and bovine CA III (muscle) antigens. Hemolysates, and Hb-extracted hemolysates, were tested against specific antisera by immunodiffusion analyses on 0.4% agar plates. Immunodiffusion tests were repeated at least three times for each sample, with reproducible results. These purification and immunological procedures were carried out as described previously (Tashian *et al*., 1968; 1980; Tanis *et al*., 1974; Carter *et al*., 1981).

Chromatography

Reverse-phase high-performance liquid chromatography (HPLC) was used to separate, and then to quantify, the specific CA isozymes as described elsewhere (Yang *et al*., 1998). Standard buffer A (0.1% TFA) and buffer B (80% MeCN/ 0.1% TFA) were used in a 130 ABI HPLC apparatus to develop a 45–75% gradient over a retention time of 60 min. Major peaks were collected and subsequently digested with 10 mg/ml CNBr in 70% TGA for 4 hr.

Identification of the CA Isozymes

To identify the peaks separated by HPLC, amino acid residues from selected CNBr-digested fragments from these peaks were sequenced in the protein sequencing core at the University of Michigan. BLAST searches for these sequences were conducted against existing protein databases, and aligned with other α-CA sequences (Hewett-Emmett and Tashian, 1996).

RESULTS

Electrophoresis and Immunological Analyses

Starch gel electrophoresis of nonheme proteins (NHP) from hemolysates and Hb-free extracts of the hemolysates revealed either one or two major NHP bands (Fig. 1). Only a single major protein band was detected in the extracts from whale and llama and the sheep control, whereas two bands were seen in the camel. As shown in Fig. 2, the NHPs from whale, llama, camel, and human all cross-reacted with rabbit anti-bovine CA I sera. The camel, human, and sheep controls all cross-reacted with the CA II antisera (data not shown). Cross-reactivity with anti-bovine CA III was seen with the whale, but not with extracts from llama, camel, and sheep (Fig. 2B). We have demonstrated previously that purified mammalian (i.e., rat, mouse, ox, sheep, human) CA I, CA II, and CA III isozymes show pronounced cross-reactivity, on immunodiffusion and immunoblot analyses, only with their corresponding intra- and interspecific antisera (cf., e.g., Spicer *et al*., 1979, 1990; Carter *et al*., 1981).

High-Performance Liquid Chromatography

The samples from camel (and human control) resembled the typical pattern (CA I $>$ CA II) seen in most mammals, with the CA I and CA II peaks at retention times of about 28 and 17 min, respectively (Figs. 3A and B), whereas samples from llama, whale, and sheep (control) are characterized by a single major peak at retention times of about 15, 22, and 16 min, respectively, along with one or more minor peaks (Figs. 3C, D, and E). However, only the secondary peak in the llama pattern was putatively identified as CA II by partial amino acid sequence analyses (Fig. 4C). Amino acid sequences were not determined for the secondary peak of the whale (Fig. 3D).

Cyanogen Bromide Digestion Patterns. SDS electrophoresis of the dominant HPLC peaks after CNBr digestion for the camel (28 min), llama (22 min), and whale (15 min) displayed similar patterns of three major bands, with molecular weights of 10, 25, and 30 kDa (data not shown). These patterns resemble those seen for CA I from other mammalian species (Yang *et al*., 1998,

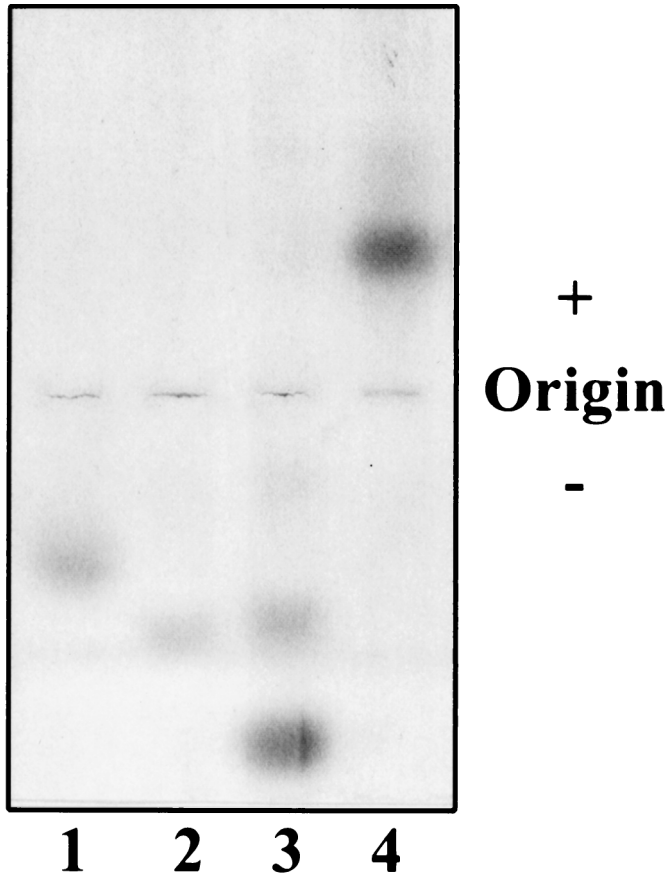

Fig. 1. Protein-stained starch gel electrophoresis patterns of ethanol/chloroform extracts of hemolysates from (1) beluga whale, (2) llama, (3) camel, and (4) sheep. Vertical electrophoresis was carried out in 20 m*M* sodium borate buffer, pH 8.6, for 21 hr at 8 V/cm. Gels stained with 0.4% nigrosin in methanol-acetic acid-H_2O.

unpublished data). The digestion pattern of the major peak from sheep was remarkably different with six major fragments grouped around 4 and 20 kDa.

Sequence Analyses

Partial amino acid sequences were determined from selected CNBr-digested fragments from the major HPLC peaks of camel, llama, and whale, and the minor peaks from camel (17 min) and llama (13 min), and aligned with the corresponding sequences of other mammalian CAs (Fig. 4).

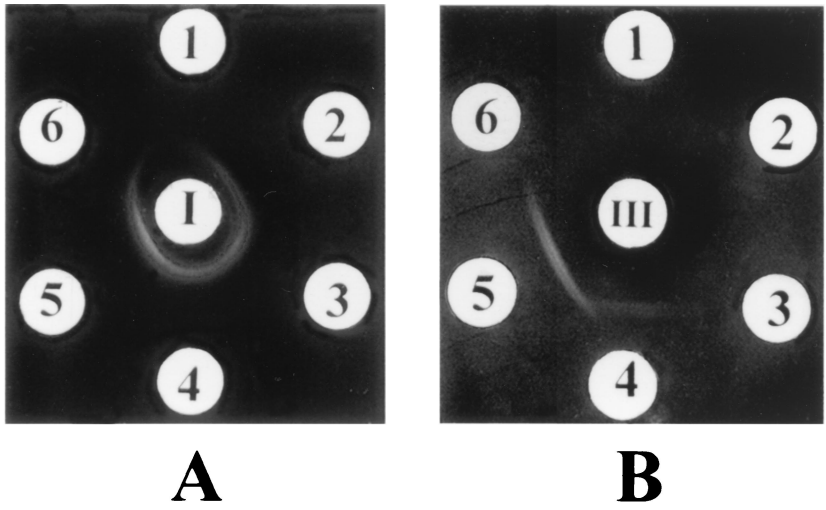

Fig. 2. Immunodiffusion patterns between rabbit anti-CA I and -CA III sera and Hb-free extracts of hemolysates from (1) sheep, (2) camel, (3 and 6) llama, (4) beluga whale, (5A) human, and (5B) bovine CA III. Antisera in center wells: (A) rabbit anti-bovine CA I, and (B) rabbit anti-bovine CA III.

In Fig. 4A, two sequences of 39 and 41 amino acids, respectively, were determined from a 10-kDa fragment of the camel 27-min peak (Fig. 3B) and llama 22-min peak (Fig. 3C), which corresponded to positions 148–186 and 188 [based on standard human CA I numbering (Hewett-Emmett and Tashian, 1996)]. A sequence of 21 amino acids (positions 148–168) was also obtained from the 10-kDa CNBr fragment of the whale 15-min HPLC peak (Figs. 3D and 4A). On alignment with the CA I, CA II, and CA III sequences of other mammals, the partial sequences from the three major peaks of camel, llama, and whale are clearly more similar to the CA I sequences than they are to those of CA II and CA III, especially the combination of Met-148, Asn-154, and Leu-161.

The amino acid sequences of two CNBr fragments corresponding to positions 23–33 and 42–54 of human CA I, CA II, and CA III sequences were determined from CNBr-digested 25- and 21-kDa bands derived from the minor HPLC peak of the camel (Figs. 3B and 4B). Although these sequences are not extensive, the pattern of Arg-27 and Leu-47 appears to support the identification of the minor HPLC peak of the camel as CA II.

The 7-kDa band from the CNBr digestion of the minor HPLC peak (13 min) of llama (Fig. 3C) yielded an 18-amino acid sequence between position 241 and position 258 that contained two residues, Met-241 and Gln-255, that appear to be unique to most mammalian CA IIs and indicate that the minor HPLC peak from

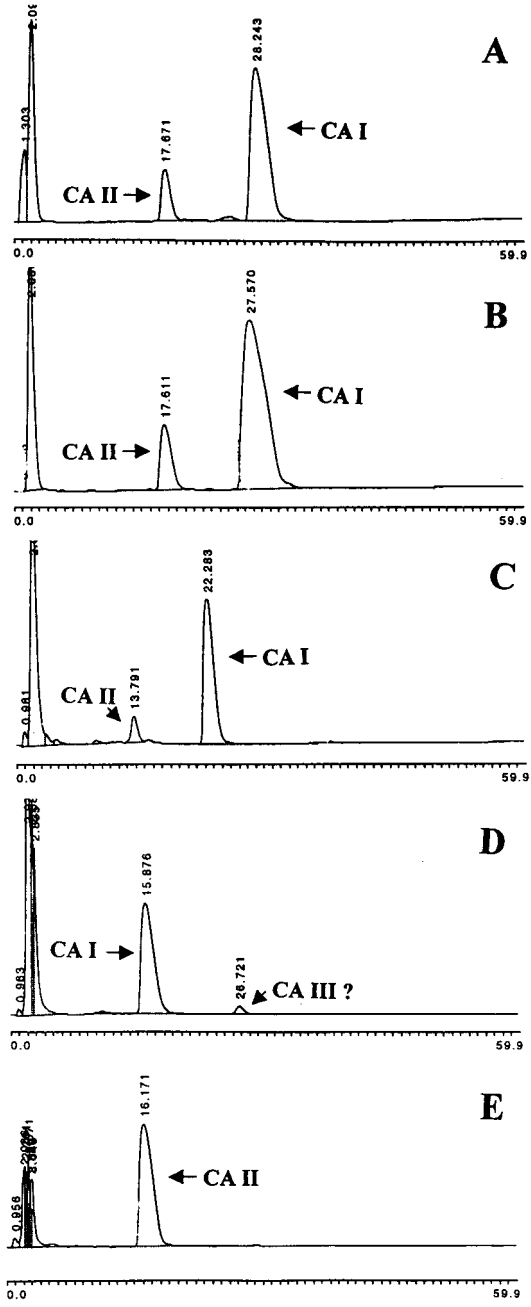

Fig. 3. HPLC profiles of Hb-free hemolysates from human (A), camel (B), llama (C), beluga whale (D), and sheep (E). Putative identification of peaks based on immunodiffusion analyses, amino acid sequences, and electrophoretic patterns of CNBr-digested peaks. The whale peak at 26.7 min was not identified.

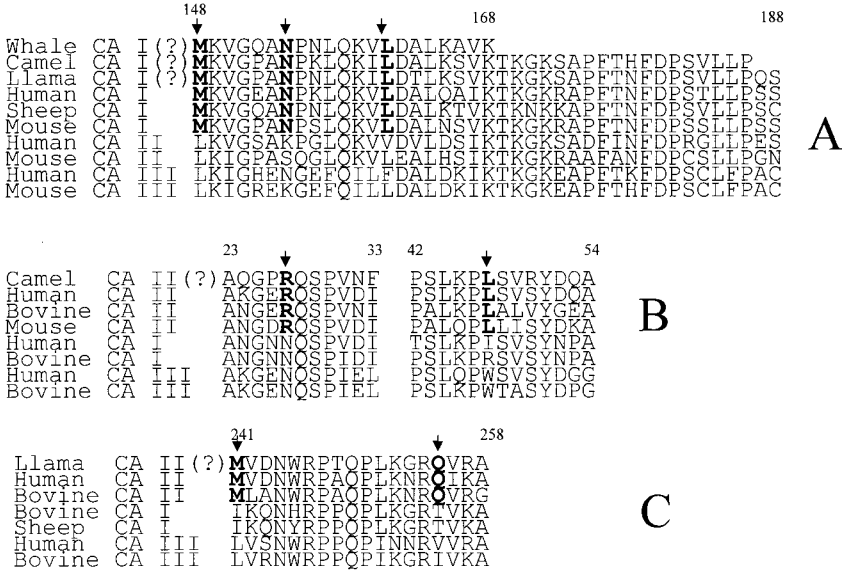

Fig. 4. Alignment of whale, llama, and camel amino acid sequences of CNBr fragments with those of human, mouse, sheep, and bovine CA I, CA II, and CA III. (A) Putative CA I residues of whale, camel, and llama compared to CA I, CA II, and CA III sequences of other mammals between position 148 and position 188; (B) putative CA II residues of camel compared to CA I, CA II, and CA III sequences of other mammals at positions 23–33 and 42–54; (C) putative CA II residues of llama compared to CA I, CA II, and CA III sequences of other mammals at positions 241–258. Residues in boldface type are invariant in most mammalian CA I (A) and CA II (B, C) isozymes.

the llama is CA II (Fig. 4C). In summary, the evidence from the comparative sequence analyses supports the identity of the major HPLC peaks of camel, llama, and whale as CA I and the minor peaks of camel and llama as CA II.

DISCUSSION

The CO_2 hydration activity of CA II, with a turnover number of $\sim 10^6$ sec^{-1} mol^{-1}, is $\sim 10\times$ and $\sim 100\times$ more active than CA I and CA III, respectively (cf. Lindskog, 1983). The finding that CA II was not detected in the red cells of the beluga whale, and is greatly reduced in the llama, suggests that a reduction in CA activity may represent an adaptation to hypoxia. For example, if CA II were absent in the kidney, the reabsorption of bicarbonate would be greatly reduced, producing a metabolic acidosis that could result in a right-shifted Hb-O_2 dissociation curve, which would increase O_2 delivery to the tissues (Turek *et al*., 1973; Poyart *et al*., 1992; Hsia, 1998). Renal tubular acidosis is symptomatic in humans and mice with inherited CA II deficiency (Sly *et al*., 1983; Brechue *et al*., 1991; Lien and Lai, 1998).

Several lines of evidence suggest that the high-activity CA II isozyme is absent in beluga whales and is greatly suppressed in llamas. The immunodiffusion results showed that the NHPs of both whale and llama cross-reacted with the bovine CA I antisera (Fig. 2). Partial amino acid sequence analyses of the single major peaks of both whale and llama indicated that their sequences were more similar to the CA I than to CA II. In fact, the sequences of the whale fragments were identical to the bovine CA I sequences in the regions examined (Tashian *et al*., 1980). In addition, the CNBr digestion pattern of the major HPLC peak from the beluga whale showed a characteristic mammalian CA I pattern with three major fragments of 10, 25, and 30 kDa (cf. Yang *et al*., 1998).

Beluga whales are known to be exceptional divers that can routinely dive to depths of up to 500 m (max. 647 m) and can remain submerged for as long as 16 min (Ridgway *et al*., 1984). It is also known that the blood of marine mammals during dives becomes more acidic because of the buildup of CO_2 and lactic acid, which results in an increase in the dissociation of O_2 from hemoglobin in tissues (Ridgway, 1972). Recently, Shaffer *et al*. (1997) measured pO_2, pCO_2, and pH in the blood of a trained beluga whale during a prolonged dive. While submerged for 16 min, the whale's blood pH dropped from 7.26 to 7.17, and its blood pCO_2 increased from 61 to 83 mm Hg without a noticeable change in hematocrit.

Examination of the red cell protein levels of CA I and CA II in the camel, a species closely related to the llama but that lives at lower altitudes, showed a typical CA I > CA II mammalian CA I:CA II ratio, whereas the llama exhibited low levels of CA II (Figs. 3B and C). Previously, ruminants tested for red cell CA I and CA II exhibited only the CA II isozyme; it now appears that this deficiency of CA I may be restricted to the noncamelid ruminants, since both camel and llama (infraorder, Pylopoda) express both CA I and CA II, with CA I the dominant form. It has been hypothesized that llamas, vicuñas, guanacos, and alpacas have adapted to high altitudes because of the high oxygen binding capacity of their hemoglobins (Poyart *et al*., 1992). Interestingly, the lowland camel hemoglobin also exhibits an increased oxygen affinity, suggesting that since all camelid species were probably derived from a common ancestor with a high oxygen affinity, they may have been, in a sense, "preadapted" to high altitudes.

The identification of the minor HPLC peak of the beluga whale has not been determined. It could be either CA II or CA III, but the fact that the Hb-free extracts of whale cross-react with both CA I and CA III, but not CA II, antisera (data not shown) suggests that this peak may be CA III, which has been reported to occur at low levels in some mammalian erythrocytes (Carter *et al*., 1984).

Inherited CA II deficiencies have previously been reported from humans (Sly *et al*., 1983; Sly and Hu, 1996) and mice (Lewis *et al*., 1988). And recently, the red cells of a mole rat species were found to be deficient in CA II (Yang *et al*., 1998), a feature which has now been found in the beluga whale. In all these

cases, the lack of CA II does not appear to impair the respiratory role of CA in the red cell (cf. Dodgson *et al*., 1988). Furthermore, the seemingly important role of red cell CA II in binding to band III in the cell membrane, where it is postulated to act as a chloride/bicarbonate exchanger, does not appear to be critical (Vince and Reithmeier, 1998). Interestingly, CA II-deficient red cells of the mole rat uniquely express a 56-kDa selenium binding protein (SeBP) at approximately the same levels as CA I and, also, low levels (~46 g/L) of Se (Yang *et al*., 1998). No such SeBP was found in the red cells of the llama or the beluga whale. Furthermore, red cell Se levels of 820 g/L for the beluga whale (as measured at the National Medical Service Laboratories, Willow Grove, PA) were at the high end of the range found in the red cells of other mammals, e.g., 120 g/L (rat) to 950 g/L (dolphin) (H. Yang, unpublished data).

It will, of course, be important to determine the expression of CA I and CA II in the red cells of other ruminants that live at high altitudes. For example, camelid species closely related to the llama (vicuña, alpaca, and guanaco), mountain goats, and, especially, the yak, which is found at higher altitudes than any other mammal. It will also be important to examine other diving marine mammals, as well as any mammal living under hypoxic conditions.

ACKNOWLEDGMENTS

We thank William and Sue Griffin (Chelsea, MI), Drs. John Lane and Arthur Tremper (Animal Clinic, Chelsea, MI), Douglas Doop (University of Michigan), and Drs. Eric Jensen and Samuel Ridgway (Naval Research and Development Veterinary Laboratory, NCCOSC, San Diego, CA) for providing blood samples. We are also grateful to Ya-Shiou L. Yu and Charles Mitchell for their help in the experimental analyses and to Dr. Erik Swensen (University of Washington, Seattle) for his valuable comments and advice. The research was supported by NIH Grant GM-24681 to R.E.T. and a Bryant College course release and summer stipend and a China Bridge International Fellowship to H.Y.

REFERENCES

Ashworth, R. B., Brewer, J. M., and Sanford, R. L. (1971). Composition and carboxyl-terminal amino acid sequences of some mammalian erythrocyte carbonic anhydrases. *Biochem. Biophys. Res. Commun.* **44:**667.

Bouquet, Y., and Van De Weghe, A. (1972). Some genetic systems with physiological properties in the blood of cattle. I. Carbonic anhydrase. *Vlaams Diergeneeskd. Tijdschr.* **41:**185.

Brechue, W. F., Kinne-Saffran, E., Kinne, R. K., and Maren, T. H. (1991). Localization and activity of renal carbonic anhydrase (CA) in CA-II deficient mice. *Biochim. Biophys. Acta* **1066:**201.

Carlsson, U., Hannestad, U., and Lindskog, S. (1973). Purification and some properties of erythrocyte carbonic anhydrase from the European moose. *Biochim. Biophys. Acta* **327:**515.

Carter, N. D., Hewett-Emmett, D., Jeffrey, S., and Tashian, R. E. (1981). Testosterone-induced,

sulfonamide-resistant carbonic anhydrase isozyme of rat liver is indistinguishable from skeletal muscle carbonic anhydrase III. *FEBS Lett.* **128:**114.

Carter, N. D., Heath, R., Welty, R. J., Hewett-Emmett, D., Shiels, A., and Tashian, R. E. (1984). Red cells genetically deficient in carbonic anhydrase II have elevated levels of a carbonic anhydrase indistinguishable from muscle CA III. *Ann. N.Y. Acad. Sci.* **429:**284.

Dodgson, S. J., Forster, R. E., Sly, W. S., and Tashian, R. E. (1988). Carbonic anhydrase activity of intact carbonic anhydrase II-deficient human erythrocytes. *J. Appl. Physiol.* **65:**1472.

Hewett-Emmett, D. (2000). Evolution and distribution of the carbonic anhydrase gene families. In Chegwidden, W. R., Carter, N. D., and Edwards, Y. H. (eds.), *Carbonic Anhydrase: New Horizons*, Birkhäuser Verlag, Basel (in press).

Hewett-Emmett, D., and Tashian, R. E. (1996). Functional diversity, conservation, and convergence in the evolution of the α-, β-, and γ-carbonic anhydrase gene families. *Mol. Phylogene. Evol.* **5:**50.

Hsia, C. C. (1998). Respiratory function of hemoglobin. *N. Engl. J. Med.* **338:**239.

Kloster, G., Larsen, D., and Nielsen, P. S. (1970). Carbonic anhydrase polymorphisms in cattle and swine. *Acta Vet. Scand.* **11:**318.

Lewis, S. E., Erickson, R. P., Barnett, L. B., Venta, P. J., and Tashian, R. E. (1988). N-ethyl-N-nitrosourea-induced null mutation at the mosue Car-2 locus: An animal model for human carbonic anhydrawse II deficiency syndrome. *Proc. Natl. Acad. Sci. USA* **83:**1962.

Lien, Y. H., and Lai, L. W. (1998). Respiratory acidosis in carbonic anhydrase II-deficient mice. *Am. J. Phys.* **274:**L301.

Lindskog, S. (1983). Carbonic anhydrase. In Spiro, T. G. (ed.), *Zinc Enzymes*, John Wiley & Sons, New York, pp. 77–121.

Poyart, C., Wajeman, H., and Kister, J. (1992). Molecular adaptation of hemoglobin function in mammals. *Respir. Physiol.* **90:**3.

Ridgway, S. H. (1972). Homeostasis in the aquatic environment. In Ridgway, S. H. (ed.), *Mammals of the Sea: Biology and Medicine*, Charles C Thomas, Springfield, IL, pp. 590–747.

Ridgway, S. H., Bowers, C. A., Miller, D., Schultz, M. L., Jacobs, C. A., and Dooley, C. A. (1984). Diving and blood oxygen in the white whale. *Can. J. Zool.* **62:**2349.

Satore, G., Stormont, C., Morris, B. G., and Grunder, A. A. (1969). Multiple electrophoretic forms of carbonic anhydrase in red cells of domestic cattle (*Bos taurus*) and American buffalo (*Bison bison*). *Genetics* **61:**823.

Shaffer, S. A., Costa, D. P., Williams, T. M., and Ridgway, S. H. (1997). Diving and swimming performance of white whales, *Delphinapterus leucas*: An assesment of plasma lactate and blood gas levels and respiratory rates. *J. Exp. Biol.* **200:**3091.

Shimizu, C., and Matsuura, F. (1962). Preparation of crystalline carbonic anhydrase of ox and blue-white dolphin. *Bull. Jpn. Soc. Sci. Fish.* **28:**735.

Sly, W. S., and Hu, P. (1996). Human carbonic anhydrases and carbonic anhydrase deficiencies. *Annu. Rev. Biochem.* **64:**375.

Sly, W. S., Hewett-Emmett, D., Whyte, M. P., Yu, Y.-S. L., and Tashian, R. E. (1983). Carbonic anhydrase II deficiency identified as the primary defect in the autosomal recessive syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification. *Proc. Natl. Acad. Sci. USA* **80:**2752.

Spicer, S. S., Stoward, P. J., and Tashian, R. E. (1979). The immunohistochemical localization of carbonic anhydrase in rodent tissue. *J. Histochem. Cytochem.* **27:**820.

Spicer, S. S., Ge, Z.-H., Tashian, R. E., Hazen-Martin, D. J., and Schulte, B. A. (1990). Comparative distribution of carbonic anhydrase isozymes III and II in rodent tissues. *Am. J. Anat.* **187:**55.

Tanis, R. J., Ferrell, R. E., and Tashian, R. E. (1974). Amino acid sequence of sheep carbonic anhydrase C. *Biochim. Biophys. Acta* **371:**534.

Tashian, R. E. (1977). Evolution and regulation of the carbonic anhydrase isozymes. In Rattazzi, M. C., Scandalios, J. G., and Whitt, G. S. (eds.), *Isozymes: Current Topics in Biological and Medical Research*, Alan R. Liss, Inc., New York, Vol. 2, pp. 21–62.

Tashian, R. E. (1992). Genetics of the mammalian carbonic anhydrases. *Adv. Genet.* **30:**321.

Tashian, R. E., and Carter, N. D. (1976). Biochemical genetics of carbonic anhydrase. *Adv. Hum. Genet.* **7:**1.

Tashian, R. E., Shreffler, D. C., and Shows, T. B. (1968). Genetic and phylogenetic variation in the different molecular forms of mammalian erythrocyte carbonic anhydrase. *Ann. N.Y. Acad. Sci.* **151:**64.

Tashian, R. E., Hewett-Emmett, D., Stroup, S. K., Goodman, M., and Yu, Y.-S. L. (1980). Evolution of structure and function in the carbonic anhydrase isozymes of mammals. In Bauer, C., Gros, G., and Bartels, H. (eds.), *Biophysics and Physiology of Carbon Dioxide*, Springer-Verlag, Berlin, pp. 165–176.

Tashian, R. E., Bergenhem, N. C. H., Carter, N. D., and Hewett-Emmett, D. (2000). Carbonic anhydrase (CA)-related proteins, and transmembrane proteins with CA or CA-like domains. In Chegwidden, W. R., Carter, N. D., and Edwards, Y. H. (eds.), *Carbonic Anhydrase: New Horizons*, Birkhäuser Velag, Basel (in press).

Turek, Z., Kreuzer, F., and Hoofd, L. J. (1973). Advantage or disadvantage of a decrease of blood oxygen affinity for tissue oxygen supply at hypoxia. A theoretical study comparing man and rat. *Pflugers. Arch.* **342:**185.

Vince, J. W., and Reithmeier, R. A. (1998). Carbonic anhydrase II binds to the carboxyl terminus of human band 3, the erythrocyte Cl^-/HCO_3^- exchanger. *J. Biol. Chem.* **3:**28430.

Wang, L.-Q., Baldwin, R. L., and Jesse, B. W. (1996). Isolation and characterization of a cDNA clone encoding ovine type I carbonic anhydrase. *J. Anim. Sci.* **74:**345.

Yang, H., Nevo, E., and Tashian, R. E. (1998). Unexpected expression of carbonic anhydrase I and selenium-binding protein as the only major non-heme proteins in erythrocytes of the subterranean mole rat (*Spalax erhenbergi*). *FEBS Lett.* **430:**343.