VARIATION IN THE PRIMARY STRUCTURE OF CARBONIC ANHYDRASE B IN MAN, GREAT APES, AND OLD WORLD MONKEYS*

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Summary--The amino acid compositions and sequences of 15 homologous tryptic peptides of carbonic anhydrase B from man and 7 Old World primates were compared. Of the 133 residues examined, differences were noted at 8 presumably homologous sites. Chimpanzee and man differed at only one site, whereas orangutan and man differed at four sites. The four monkey species differed from man by four to 6 residues. The fixation rates for mutations of these carbonic anhydrases appear to be similar to those found for the hemoglobin chains of the same species.

Two genetically distinct isozymes of erythrocyte carbonic anhydrase, designated CA B and CA C, or CA I and CA II, have now been purified from the hemolysates of man (cf. 1,2) and a number of other primate species (3,4). Because the primary amino acid sequence of the human CA B isozyme has been determined for about 205 of its approximately 265 residues (2,5), it is now possible to begin to compare the sequences of the homologous CA B enzymes from different primates. In the present communication, we have compared the amino acid sequences or compositions in 15 homologous tryptic peptides of CA B purified from man, the great apes--chimpanzee (Pan troglodytes) and orangutan (Pongo pygmaeus), and the Old World monkeys--green monkey (Cercopithecus aethiops), common baboon (Papio cynocephalus), rhesus macaque (Macaca mulatta), and cynomolgous macaque (Macaca irus). These peptides were composed of 133, or about 50%, of the approximately 265 residues which make up the single polypeptide chain of human CA B.

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MATERIALS AND METHODS

Several inherited electrophoretic variants are known for the CA B (or CA I) of all but one (chimpanzee) of the species analyzed in the present study; however, only the common electrophoretic phenotypes, designated for these species as CA Ia in previous studies (4,6), were analyzed.

The carbonic anhydrases were separated from the various primate hemolysates by column chromatography on DEAE-Sephadex, and the peptides were purified from the tryptic digests by a combination of the paper chromatography, high voltage electrophoresis, and peptide elution procedures described elsewhere (7).

The amino acid composition of the peptides was analyzed on a Technicon automatic analyzer, and subtractive Edman sequential degradation of some of the tryptic peptides was carried out essentially by the procedure of Konigsberg (8).

RESULTS AND DISCUSSION

The amino acid compositions and sequences of the tryptic peptides of human CA B are listed in Table I. The tryptic peptides have been arbitrarily numbered on the basis of their relative order of migration from the cathodal end, after electrophoresis at pH 6.5; i.e., peptides T-4 to T-12 (basic), peptides T-14 to T-19 (neutral), and peptides T-20 to T-24 (acidic). Except for peptide T-19 which was sequenced in our laboratory for both human and baboon CA B, the sequences for the other non-human primate peptides were determined by sequence homologies with the known sequences for human CA B from the literature (2,5), a personal communication from Dr. P. O. Nyman, and from our own work.

In 6 of the 15 peptides that were compared, amino acid differences were observed at 8 homologous positions (Table I) for the 7 primate carbonic anhydrases examined. In Table II, the amino acid residues at these positions are listed for each species. In baboon, rhesus macaque, and cynomolgous

TABLE I

Sequences or Compositions of Some Tryptic Peptides From Human Red Cell Carbonic Anhydrase B That Were Used to Compare With the Homologous Tryptic Peptides of CA B From Various Non-Human Primates[1]

T-24 Acetyl-Ala-Ser-Pro-Glu-Asp-Trp-Gly-Tyr-Asp-Asp-Lys[2,3]
1 5 10

T-15 Asn-Gly-Gln-Pro-Glu-Trp-Ser-Lys[2,3]
15

T-20 Leu-Tyr-Pro-Glu-Ala-Asp-Gly-Asn-Asn-Gln-Ser-Pro-Val-Asp-Ilu-Lys[2]
20 25 30

T-19 Thr-Ser-Glu-Ala-Thr-Lys[2,4]
35

T-11 His-Asp-Thr-Ser-Leu-Lys-Pro-Val-Ser-Val-Ser-Tyr-Asn-Pro-Ala-Thr-Ala-Lys[2]
40 45 50 55

T-8 Leu-Gln-Lys[4]

T-10 Ser-Val-Leu-Lys[3,4]

T-14 Val-Leu-Gly-Ala-(Asp,Glx,Pro,Lys)[4]

T-16 (Asx_3,Ser,Glx,Pro,Gly,Ala,Val,Ilu,Leu,Lys)[4]

T-18 Val-Leu-Asp-Ala-His-Ala-Leu-Ilu-Lys[4]

T-23 (Asx_2,Glx-Pro,Gly,Ser,Ala,Lys)[4]

} Positions 77-175

T-12 Ser-Leu-Leu-Ser-Asn-Val-Glu-Gly-Asp-Asn-Pro-Ala-Val-Pro-Ilu-Met-Glx-Arg-His-Asn-Asn-Arg-
235 240 245 250

-Pro-Thr-Gln-Pro-Leu-Lys[2,5]
255

T-4 Gly-Arg[2,3,4]
258

T-9 Thr-Val-Arg[2,3,4]
260

T-17 Ala-Ser-PheOH[2,3,4]
265

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[1] See text for list of these species. The underlined residues were found to differ in at least one species (see Table II); the substituted residue is shown above.

[2] Sequence and residue numbers from Andersson et al. (5) and P. O. Nyman (personal communication).

[3] Sequence and residue numbers from Derrien and Laurent (2).

[4] Present study.

[5] Sequence of chymotryptic peptide, Ser(236)...Met(246), from Funakoshi and Deutsch (11)

TABLE II

Differences Between the Chains of Carbonic Anhydrase B of Primates From a Comparison of the 133 Homologous Sites Shown in Table I

Tryptic Peptide	T-24	T-19	T-11	T-14*	T-18*	T-12		
Position**	4	38	47			243	246	248
Man	Asp	Thr	Ile	Val	Gln	Ala	Met	His
Chimpanzee	Glu	Thr	Ile	Val	Gln	Ala	Met	His
Orangutan	Glu	Ala	Val	Val	Gln	Ala	Ile	His
Green Monkey	Glu	Thr	Ile	Val	His	Pro	Ile	His
Common Baboon	Asp	Ala	Ile	Leu	His	Pro	Ile	Arg
Rhesus macaque	Asp	Ala	Ile	Val	His	Pro	Ile	Arg
Cynomolgous macaque	Asp	Ala	Ile	Val	His	Pro	Ile	Arg

*Peptides located between positions 77 and 175.

**Positions based on known sequence of human CA B (see Table I); residues at 125 remaining sites assumed to be similar for all species.

macaque, the presence of Arg in the place of His at position 248 forms (in the place of T-12) the two tryptic peptides, Ser(233)...Arg(248) and Asn(249)...Lys(257), in their two-dimensional peptide maps. The Glu/His change in T-18 has the effect of moving the homologous peptide to a more basic position in the tryptic peptide maps of the monkeys: C. aethiops, P. cynocephalus, M. mulatta, and M. irus. All of the other peptides in which changes were noted showed no differences in their migration patterns after chromatography and electrophoresis at pH 6.5.

As would be expected, there were very few differences between closely related species such as man and chimpanzee, or baboon and the macaque monkeys. The fact that orangutan differed from chimpanzee by three residues, and man by four residues resembles the number of differences found between their hemoglobin chains (9) and suggests that the lines

leading to man and chimpanzee on the one hand and to orangutan on the other, probably diverged very early in their evolutionary history. Four to 6 residue differences were noted between the four monkey species and man.

It appears that the rate of incorporation of mutational changes for 133 residues of CA B in these species is similar to the residue changes that have been reported for the 140-145 residues of the hemoglobin chains of these same primate species. For example, between the hemoglobins of man and orangutan, 5-6 differences were noted for the 140-141 residues of their α-chains and 6 differences for the 145-146 residues of their β-chains (9), or approximately four differences per 100 residues; our data for the CA B of these two species showed four differences for 133 residues, or three differences per 100 residues.

As noted, there is a second carbonic anhydrase isozyme, CA C. Because the two isozymes, CA B and CA C, appear to have been formed from a common ancestral gene by a duplication process (10), they provide an ideal pair of enzymes with which to compare the fixation rates for mutations occurring in two related gene products. Since there is now some evidence from comparative genetic and structural studies which suggests that the evolution of CA B may have proceeded at a faster rate than CA C during the evolution of mammals (4,10), it will be especially interesting to determine whether this hypothesis will hold true when the complete primary sequences for both CA B and CA C have been compared for a number of species. Such studies are presently under way in our laboratory.

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