

Chimerism, point mutation, and truncation dramatically transformed mast cell δ tryptases during primate evolution

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Background: Tryptases are serine peptidases stored in mast cell granules. Rodents express 2 soluble tryptases, mast cell proteases (MCPs) 6 and 7. Human α - and β -tryptases are orthologs of MCP-6. However, much of the ancestral MCP-7 ortholog was replaced by parts of other tryptases, creating chimeric δ -tryptase. Human δ -tryptase's limited activity is hypothesized to be due to truncation and processing mutations. **Objective:** We sought to probe the origins and consequences of mutations in primate δ -tryptases.

Methods: Prosimian (lemur), monkey (macaque), great ape (orangutan, gorilla, and chimpanzee), and human δ -tryptase genes were identified by means of data mining and genomic sequencing. Resulting genes were analyzed phylogenetically and structurally.

Results: The seminal conversion event generating the δ -tryptase chimera occurred early because all primates studied contain δ -tryptase genes. Truncation, resulting from a nonsense mutation of Trp206, occurred much later, after orangutans and other great apes last shared an ancestor. The Arg-3Gln propeptide mutation occurred most recently, being present in humans and chimpanzees but not in other primates. Surprisingly, the major active tryptase in monkeys is full-length δ -tryptase, not β -tryptase, which is the main active tryptase in human subjects. Models of macaque δ -tryptase reveal that the segment truncated in human subjects contains antiparallel β -strands coursing through the substrate-binding cleft, accounting for truncation's drastic effect on activity.

Conclusions: Transformations in the ancestral MCP-7-like gene during primate evolution caused dramatic variations in function. Although δ -tryptases are nearly inactive in humans, they are active and dominant in monkeys. (J Allergy Clin Immunol ■■■■;■■■;■■■-■■■.)

Key words: Tryptase, mast cells, comparative evolution, chimpanzee, gorilla, orangutan, macaque

Human soluble tryptases, which are trypsin-like serine peptidases, are stored in the secretory granules of mast cells as

Abbreviations used

MCP: Mast cell protease
mMCP: Mouse mast cell protease

catalytically active, heparin-bound tetramers.¹ After release from degranulating mast cells, they resist inactivation by plasma antipeptidases and are implicated in mast cell–related disorders, such as anaphylaxis and asthma.^{2–4} Known human tryptase genes cluster tightly on chromosome 16p13.3.^{5,6} At the telomeric end of the cluster, *TPSG1* encodes γ -tryptase, a type I transmembrane peptidase that is thought to be proinflammatory.^{6,7} The remaining loci encode soluble peptidases lacking an anchor. From *TPSG1* towards the centromere, these loci are *TPSB2* (encodes β II- and β III-tryptase), *TPSAB1* (α - and β I-tryptase), and *TPSD1* (δ -tryptase). To date, only β -tryptases have been shown to be capable of proper processing, assembly, storage, and secretion and are therefore the dominant isoforms in tissue extracts.^{8–10} α -Tryptases, on the other hand, have an Arg-3Gln propeptide mutation, which sabotages zymogen activation, as well as a Gly215Asp catalytic domain mutation, which disturbs the active site and interferes with substrate binding.^{9,11–13} Although α is transcribed and translated in human subjects who carry the gene and can influence circulating levels of immunoreactive tryptase, it appears to be released as an inactive zymogen.^{14,15} Therefore α -tryptase is suspected to be functionless, a suspicion reinforced by absence of overt phenotype in the 20% to 30% of individuals lacking α -tryptase genes.¹⁶

Human δ -tryptases also are potentially flawed. They contain the same processing mutation as α -tryptases and might be unactivatable.⁵ Also, they feature a nonsense mutation, which ends translation 40 residues earlier than in α - and β -tryptases.⁵ Two of the 3 “specificity triad” residues that shape the substrate-binding pocket in other serine peptidases are contained in the truncated segment, inviting speculation that substrate docking is disturbed. Initially, δ -tryptase genes were predicted to be pseudogenes because of the truncated C-terminus, lack of expressed sequence tags, and paucity of δ mRNA in mast cell preparations.^{5,17} Subsequently, δ transcripts and immunoreactivity were detected in human tissues, although recombinant enzyme equipped with an activatable pseudopropeptide exhibited little activity.¹⁸ In an effort to define the relevance of human δ -tryptase, the present work explores the origins and consequences of its mutations.

METHODS

Data mining

Full amino acid sequences of some primate δ -tryptases not already published or annotated were obtained by means of data mining, including

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Basic Local Alignment Search Tool and Basic Local Alignment Search Tool-like Alignment Tool searches of high-throughput genome sequences and whole-genome shotgun databases at the National Center for Biotechnology Information by using human δ -tryptase genes and cDNAs as query sequences. Marmoset and orangutan sequences were obtained from Basic Local Alignment Search Tool searches of genomic sequences archived at the Washington University Genome Sequencing Center (<http://genome.wustl.edu>). Amino acid sequences of previously unreported δ -tryptases were predicted from genomic DNA by using existing tryptase gene structures as a guide.

Sequencing of primate tryptase genes and transcripts

Sequences of additional δ -tryptases were obtained by means of *de novo* sequencing. Primate genomic DNA from species-specific cultured fibroblasts was obtained from Primate Cell Repositories of the Coriell Institute for Medical Research (Camden, NJ). *Macaca fascicularis* lung/trachea cDNA was obtained from Cytomol (Sunnyvale, Calif). Genes and cDNA were PCR amplified with Advantage 2 (Clontech, Mountain View, Calif). For genes, primers were based on conserved parts of macaque, orangutan, gorilla, chimpanzee, and human δ -tryptase genes bracketing the 5' flank to intron 4 and intron 4 to 3' flank, with approximately 250 nucleotides of overlap. For cDNA, primers were based on conserved exonic sequence of *M fascicularis* δ - and $\alpha\beta$ -tryptases. Individual amplimers were ligated into pCR2.1-TOPO (Invitrogen, Carlsbad, Calif) for sequencing.

Phylogenetic comparisons

Multiple sequence alignments of deduced preprotryptase amino acid sequences were prepared by using Geneious software (Biomatters, Auckland, New Zealand). Aligned sequences were analyzed phylogenetically by using the unweighted pair group method with arithmetic mean and Neighbor-Joining with bootstrap resampling.

Molecular modeling

Protein databank files representing macaque δ tetramers were generated with Swiss-Model (<http://swissmodel.expasy.org>) by threading deduced δ -tryptase sequences onto each monomer of crystal-based human β II tetramer 1A0L.10 Images were created from resulting files by using Chimera (www.cgl.ucsf.edu/chimera).

Dot-plot analysis

Nucleic acid sequences of mouse mast cell proteases (MCPs) 6 and 7 and human δ - and β I-tryptase genes, including 5'- and 3'-untranslated regions, were analyzed by using the CLC DNA workbench (www.clcbio.com) with a 33-nucleotide window and stringency of 21.

RESULTS

Sequence of primate δ -tryptase genes

Sequences bracketing all protein-coding exons of primate δ -tryptase genes were obtained from approximately 10 cloned amplimers each from gorilla, chimpanzee, and macaque (cynomolgus monkey; *M fascicularis*) genomic DNA. Amplimer sequences yielding unique δ -tryptase genes were deposited into GenBank. Exonic sequence was predicted by aligning genomic DNA to human δ -tryptase cDNA. In all cases introns begin with dinucleotide GT and end with AG, and phase (0, 1, or 2) is conserved. In addition, all penultimate δ -tryptase introns are enlarged by a δ -tryptase-specific approximately 0.8-kb insertion compared with α - and β -tryptase genes. Transcription of *M fascicularis* δ -tryptase was confirmed by sequencing the full protein-coding regions of cloned cDNA amplimers, which also confirmed correct prediction

of intron-exon boundaries in corresponding gene sequences. Sequences of genes used in this article are as follows: human δ - and β I-tryptase (*Homo sapiens*, NT_03788 and AF099144); common chimpanzee δ 1-, δ 2-, and β -tryptase (*Pan troglodytes*, EU069815, EU069816, and EF206351); gorilla δ - and β I-tryptase (*Gorilla gorilla*, EU069814 and EF208020); Sumatran orangutan δ - and β I-tryptase (*Pongo pygmaeus abelii*, contig11332.1 at <http://genome.wustl.edu> and EF452227); cynomolgus monkey δ - and $\alpha\beta$ I-tryptase (*M fascicularis*, EF514222 and EF212445); common marmoset δ -tryptase and $\alpha\beta$ -like tryptase (*Callithrix jacchus*, contig10879.13 and contig12371.9 at <http://genome.wustl.edu>); Garnett's galago δ - and $\alpha\beta$ -like tryptase (*Otolemur garnetti*, AAQR01437408.1 and AAQR01303216); gray mouse lemur δ -tryptase and $\alpha\beta$ -like tryptase (*Microcebus murinus*, ABDC01528916.1 and ABDC01465079); mouse MCP-6 and MCP-7 (*Mus musculus*, M57625 and EF154453); and gray short-tailed opossum (*Monodelphis domestica*, AAFR03046243).

Protein structure of primate δ -tryptases: General features

As shown in Fig 1, the preprotryptase amino acid sequence predicted from macaque and orangutan δ -tryptase genes is 275 residues from initiator Met to stop codon, as in primate α - and β -like tryptases.⁵ However, in gorilla, chimpanzee, and human δ -tryptase genes, a nonsense mutation (TGG to TAG in gorillas and chimpanzees and TAA in humans, Table I) in the Trp206 codon causes a 40-amino-acid truncation. Consensus N-linked glycosylation sites at Asn102 and Asn203 are conserved in all δ -tryptase sequences. Because a Cys is among the residues deleted in truncated δ -tryptases, only full-length δ -tryptases (ie, from orangutan and macaque) feature all 8 Cys residues (forming 4 intramolecular disulfide pairs) at locations conserved in known murine and primate soluble tryptases. Like human δ -tryptase, all other primate δ -tryptases identified here are highly similar to α - and β -tryptases, except for residues 137 to 191 encoded by the penultimate exon, which is the exon most similar to mouse mast cell protease (mMCP) 7 (Fig 1). Thus nonhuman primate δ -tryptases, like their human counterparts, appear to be chimeric.⁵

Protein structure of primate δ -tryptases: Processing mutation

Human δ -tryptase possesses a propeptide Arg-3Gln mutation, which also is a feature of human α -tryptase, preventing the autocatalytic first step in activation by removal of the propeptide.⁹ In other primates this processing mutation was identified solely in the chimpanzee δ 1-tryptase gene (Fig 1 and Table I). The otherwise nearly identical δ 2-tryptase gene, which is presumably an allelic variant or less likely a separate gene, contains classic Arg-3. Gorilla, orangutan, and macaque δ -tryptases also feature Arg-3 and thus are not burdened with a processing defect. These findings suggest that the processing mutation arose in a shared ancestor of chimpanzees and humans, after tribe Gorillini split from tribe Hominini.¹⁹

Protein structure of primate δ -tryptases: Catalytic and specificity triads

All primate δ -tryptases possess residues His44, Asp91, and Ser195 (corresponding to His57, Asp102, and Ser195 of

TABLE I. Codons and translations of δ -trypsin mutations

	Species divergence vs human*	Gene	-3 Processing mutation		+206 Truncation mutation		+215 Specificity triad mutation	
Human	—	δ I (<i>TPSD1</i>)	CAA	Gln	TAA	Stop	GAG†	Glu
Chimpanzee	6 mya	δ 1	CAA	Gln	TAG	Stop	GAG†	Glu
		δ 2	CGA	Arg	TAG	Stop	GAG†	Glu
Gorilla	9 mya	δ	CGA	Arg	TAG	Stop	GAG†	Glu
Orangutan	16 mya	δ	CGA	Arg	TGG	Trp	GAC	Asp
Macaque	32 mya	δ	CGA	Arg	TGG	Trp	GGC	Gly
Marmoset	50 mya	δ	NA		TGG	Trp	GGC	Gly
Galago	83 mya	δ	NA		TGG	Trp	GGC	Gly
Mouse	110 mya	<i>Mcpt7</i>	CGA	Arg	TGG	Trp	GGT	Gly

mya, Million years ago; NA, not applicable, sequence unknown in this region.

Residues deviating from human δ -trypsin shown are shown in boldface and italics.

*Estimate generated at www.timetree.org.

†Codon if open reading frame is continued after premature stop codon.

	-30	-3 +1	#	62
Human δ	MLSLLLALPVLASPAYVAPAGQALQQTGIVGGQEAAPRSKWFQVSLRVGPYWMHFCGGSLLHPQWVLTAAHCVEPDIKDLAALRVQLRE			
Chimp δ 1	-----V-L-----R-----F-D-----R-----LG-EV-----Q-			
Chimp δ 2	-----V-L-----R-----F-D-----R-----LG-EV-----Q-			
Gorilla δ	-----V-----L-M-----RA-----CDQ-----G-V-----			
Orang δ	-----V-----A-----RV-----H-Q-----G-V-----			
Macaque δ	-N-----V-----HA-----RA-----LHDQ-----G-V-----N-----			
Human β I	-N-----R-A-----RV-----H-----G-V-----			
mMCP7	-K-----T-L-S-LVHA--G--*-MTRE-----HGN-----ANDT-----G--VA-PNKV-----K			
Human δ	63	#	+	154
Chimp δ 1	QHLYYQDQLLPVSRIIVHPQFYIIQTGADIALLELEEPVNISSHIHTVTLPPASETFPPGMPWCWVTGWGDVDNNVHLPPPYPLKEVEVPVVE			
Chimp δ 2	-----T-----T-----NM-----L-----			
Gorilla δ	-R-----TA-I-----V-----NM-----L-----A-----			
Orang δ	-----VM-----NV-----L-----A-T-----R-----I-----			
Macaque δ	-----AV-I-----V-----L-----T-----D-R-----I-----			
Human β I	-----TA-I-----V-----V-----DER-----F-----Q-K-----IM-----			
mMCP7	-Y-----H-H-MT--Q--T--D--V-D-----K-TN-----DYV-P-P-----S-TL-----NI--G-N-----F-----Q--II-----			
Human δ	155	%	#	+205
Chimp δ 1	NHLCNAEYHTGLHTGHSFQIVRDDMLCAGSENHDSQGDSSGGLVCKVNGTX			
Chimp δ 2	---GD-----M-----K-N-----X			
Gorilla δ	---D-----H-----K-----S-----X			
Orang δ	---I-D-----D-----K-----WLGAGVVSWEDECAQPNRPGIYTRVTYYLDWIHRYVPKPK			
Macaque δ	---Q-D-----D-R-----K-----WLGAGVVSWEDECAQPNRPGIYTRVTYYLDWIHRYVPKPK			
Human β I	---I-D-K--L-AY--DDVR-----NTRR-----WLGAGVVSWEDECAQPNRPGIYTRVTYYLDWIHRYVPKPK			
mMCP7	---DLK--K--I--DNVH-----N-G-----ED-WLGAGVVSWEDECAQPNRPGIYTRVTYYLDWIHRYVPKPK			

FIG 1. Alignment of primate δ -trypsins. Selected δ -trypsins are aligned and compared with human β I-trypsin and mouse MCP-7. —, Amino acid identity compared with human δ -trypsin; *, gap in alignment; #, conserved “catalytic triad” residue; %, conserved “specificity triad” residue; +, predicted sites of N-glycosylation.

chymotrypsinogen), which comprise the “catalytic triad” common to active peptidases of the chymotrypsin/trypsin family. However, only macaque δ -trypsin possesses the classic “specificity triad” of Asp188, Gly215, and Gly225 (Asp189, Gly216, and Gly226, in chymotrypsinogen) found in serine peptidases of tryptic specificity. These residues line and shape the pocket accommodating the substrate P1 side chain at the site of hydrolysis. Orangutan δ -trypsin has a Gly215Asp mutation, which, interestingly, is also present in chimpanzee and human α -trypsin (where it perturbs the active site and causes loss of function^{11–13}) and in macaque $\alpha\beta$ -trypsins, which share features of α - and β -trypsins of humans and great apes.²⁰ Gly215 and Gly225 are absent in gorilla, chimpanzee, and human δ -trypsins because they reside in the truncated segment. However, extension of the open reading frame past the stop codon reveals that gorilla, chimpanzee, and human δ -trypsin genes contain an untranslated Gly215-Glu mutation (Table I).

Molecular models of macaque δ -trypsin

In the present work macaque δ -trypsin is the only peptidase in primate parvorder Catarrhini (Old World monkeys and apes) with all known attributes of an activatable and functional trypsin (ie, without truncation, processing, or specificity triad mutations). Indeed, the predicted N-terminus of the macaque δ -trypsin catalytic domain matches that of active trypsin purified from 2 macaque tissue sources: the lung and cheek pouch.^{21,22} Homology models of macaque δ -trypsin in Fig 2 were compared with crystal-based models of human β II-trypsin.¹⁰ These models predict that macaque δ -trypsin is active and tetrameric with the potential for differences (in comparison to human β -trypsin) in surface charge, substrate preferences, and inhibitor susceptibility. Residues encoded by the chimeric penultimate exon contribute most of these differences. The models also reveal that the segment that is truncated in gorilla, chimpanzee, and human δ -trypsin involves the internal β -barrel structure of serine peptidases, as well

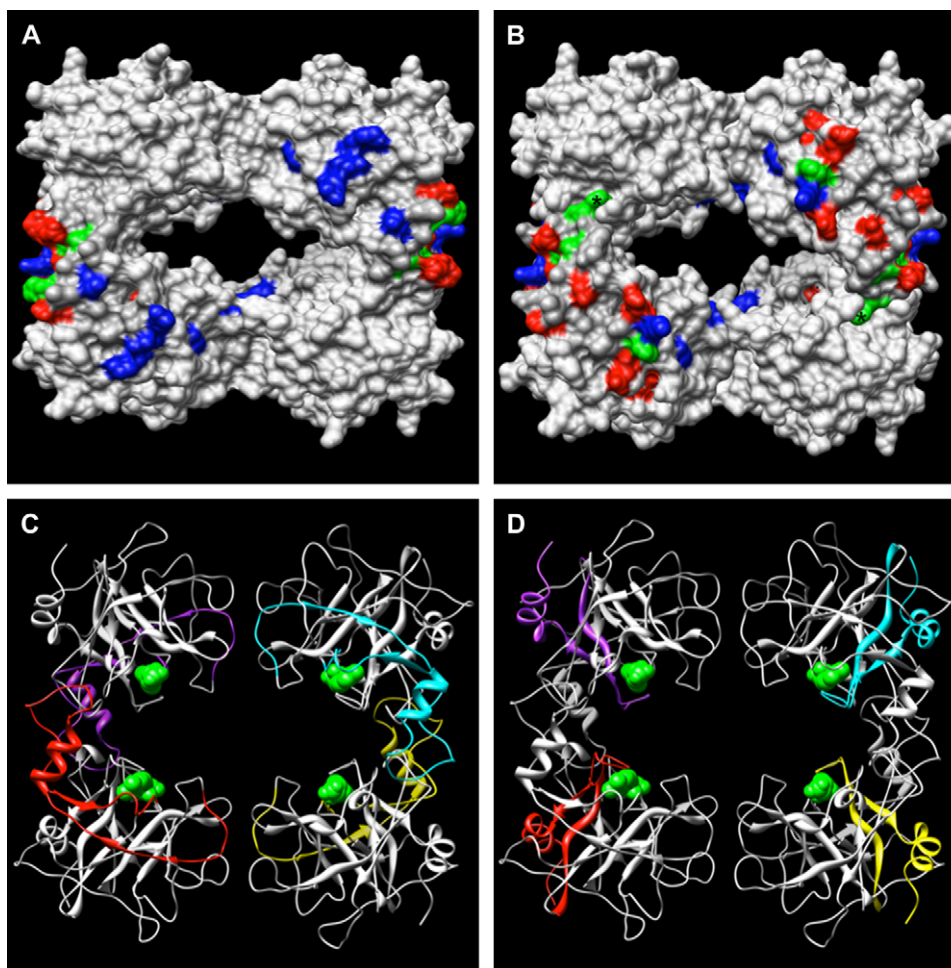


FIG 2. Models of human β II-tryptases (**A**) and macaque δ -tryptases (**B-D**). Exon 5–encoded basic, acidic, and His residues are colored blue, red, and green, respectively. His167 residues are marked with an *asterisk*. Fig 2, **C** and **D**, respectively, highlight exon 5 residues and the segment truncated in humans, chimpanzees, and gorillas. *Purple, cyan, red, and yellow* ribbons reside in monomers A, B, C, and D, respectively. Inhibitor amidinophenyl pyruvate, in *green*, occupies the active sites.

as highly conserved regions of the substrate-binding pocket facing the hollow center of the tryptase tetramer and portions of surface loops on the face and rim of the tetramer. However, the truncated segment does not contain residues forming intersubunit contacts in the human β II-tetramer. Overall, 16% of the catalytic domain in macaque δ -tryptase is truncated in gorilla, chimpanzee, and human δ -tryptases. The models predict that catalytic function of the tetramer is severely perturbed without the truncated segment.

Dot-plot analysis

A dot-plot comparison (Fig 3, A) of human δ -tryptase versus β I-tryptase gene sequence reveals great similarity, even in introns and flanking regions, up until intron 4. The offset diagonal line of identity reflects the approximately 0.8-kb insertion in the human δ -tryptase intron 4. Fig 3, A, also reveals 3'-untranslated region tandem repeats, which are extended in δ -tryptase versus β I-tryptase. When comparing human β I-tryptase and δ -tryptase genes with mouse tryptase MCP-6 and MCP-7 genes, similarity is predominantly in exons and in the intronic sequence immediately

flanking exonic sequence. The notable exception is the similarity between intron 4 of human δ -tryptase and the corresponding intron of the mouse MCP-7 gene (Fig 3, C), which is consistent with other evidence in this work that this part of the δ -tryptase chimera has MCP-7–like ancestry. The offset line of identity between human β I-tryptase and mouse MCP-7 genes (Fig 3, B) reflects an approximately 0.8 base insertion in intron 4 of the mouse gene.

Phylogenetic analysis

The human δ -tryptase gene was hypothesized to have been created by a gene conversion event because its penultimate (fifth) exon appeared to share ancestry with the penultimate (fourth) exon of the MCP-7 gene *Mcpt7*, whereas other exons share ancestry with human α - and β -tryptases and with the MCP-6 gene *Mcpt6*.⁵ To test this theory and to trace the postulated conversion event in primate evolution, we compared nucleic acid sequences of a range of murine and primate tryptase exons. In support of the hypothesis, Fig 4 demonstrates that exon 5 of primate δ -tryptases forms a clade with the corresponding exon of *Mcpt7*, whereas

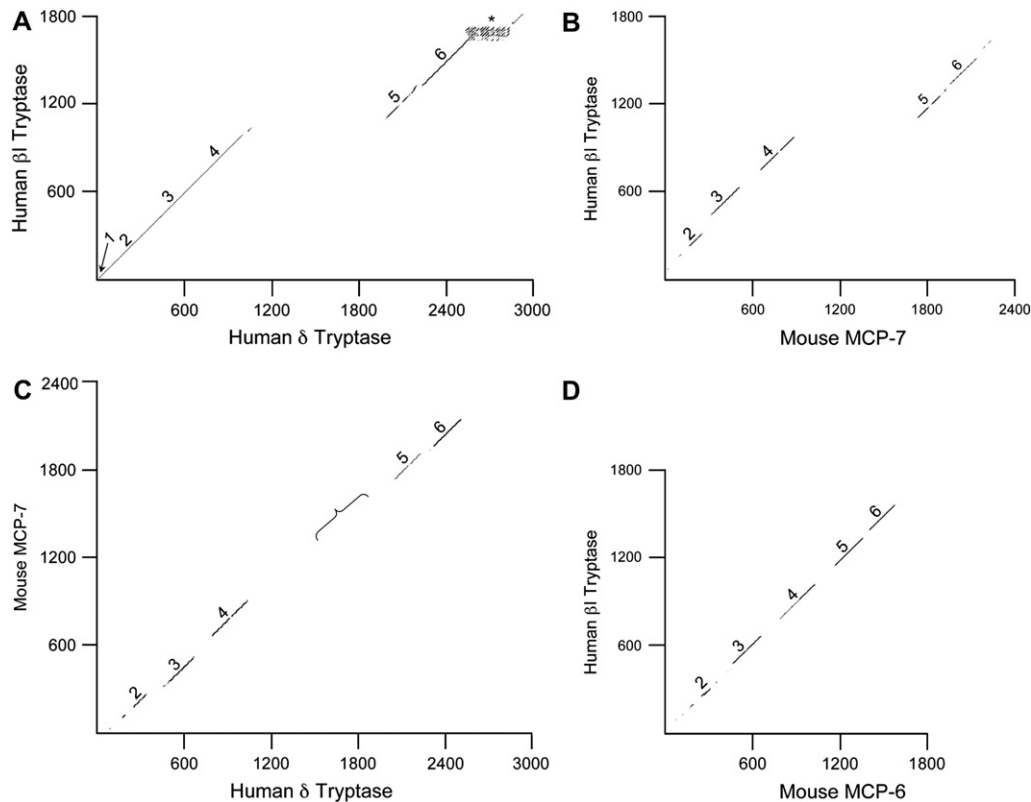


FIG 3. Dot-matrix plots comparing human and mouse tryptase genes. Human exons are marked 1 through 6. The asterisk marks short tandem repeats. The bracket marks a region of intron 4 homology between human δ -tryptase and mouse MCP-7.

exon 5 of primate α - and β -tryptases forms a clade with the corresponding exon of *Mcpt6*. As also predicted by the hypothesis, Fig 4 reveals that all primate $\alpha/\beta/\delta$ -tryptase fourth exons align more closely with the corresponding exon of *Mcpt6* than of *Mcpt7*. Additionally, all primate δ -tryptase genes identified here contain an enlarged intron 4, which is similar in size to the corresponding intron of *Mcpt7*. This evidence further supports the hypothesis that the ancestral primate δ -tryptase gene was created by a major recombination event involving an $\alpha\beta$ -like tryptase gene and an ortholog of *Mcpt7*, with the latter being substantially replaced by the first 4 exons of the former. This conversion event likely occurred early in primate evolution because all major groups of primates, including prosimians, have δ -tryptase genes, and none have been found to have full-length *Mcpt7*-like genes.

DISCUSSION

Human δ -tryptases, being encoded by genes that lack close relatives in nonprimate genomes and possessing catalytic domains that are substantially shorter than those of classic serine peptidases, are intriguingly idiosyncratic.⁴ By characterizing genes encoding δ -tryptases in a range of primates and tracing evolutionary origins, the present work reveals several genetic mechanisms by which the human enzymes acquired their present characteristics. In so doing, this work suggests that the human enzyme's most distinctive feature, its 40-residue C-terminal truncation, is a comparatively recent development in the evolution of primate tryptases, which are hyperevolving as a group.²⁰ The results also suggest that some features of human δ -tryptase, including the truncation,

can be blamed for drastic reductions in activity compared with β -tryptases, but that this cannot be assumed to be typical of primate δ -tryptases in general. Indeed, perhaps the study's most unexpected result is that macaque δ -tryptase is full length and active and that it might play a role in macaques similar to the role played by β -tryptases in humans and their closest relatives. These findings and their implications are discussed in more detail below.

Human δ -tryptase is produced from a locus (*TPSD1*) different from that of human α - and β -tryptase (*TPSAB1* and *TPSB2*).^{5,6} This contrasts with the mouse, which has only 2 homologous loci, *Mcpt6* and *Mcpt7*. Therefore it appears that a gene duplication occurred after humans and mice split from the mammalian tree. Although δ -tryptase is highly similar to α - and β -tryptase in overall structure, δ -tryptase's exon 5 resembles the corresponding exon of mMCP-7 more so than any exon 5 of α - or β -tryptase. This laboratory previously suggested that this is evidence that δ -tryptase is a chimera created by recombination between an $\alpha\beta$ -like and mMCP-7-like gene.⁵ The present work further supports this theory and provides evidence that the conversion event occurred early in primate evolution.

Our investigation of the truncation and processing mutations in human δ -tryptases shows that they were acquired at different times in primate evolution. The truncation mutation occurred between 9 and 16 million years ago, before gorillas, chimpanzees, and humans (subfamily Homininae) last shared an ancestor but after these lineages split from the ancestor shared with orangutans.^{23,24} The Arg-3Gln processing mutation occurred between 6 and 9 million years ago, after humans and gorillas last shared an ancestor but before chimpanzees and humans branched from the

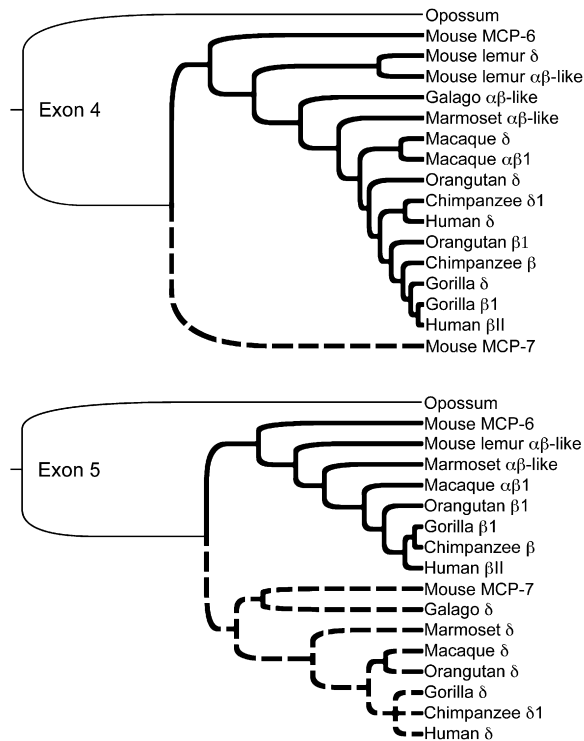


FIG 4. Dendrograms of exons 4 and 5 of soluble tryptases. *Solid lines* represent exons aligning with mouse MCP-6, and *broken lines* represent exons aligning with mouse MCP-7. Opossum tryptase exonic sequence is included as an example of a branch of mammals (Metatheria) that speciated before the rodent/primate split.

tree.²⁴ Because human, but not chimpanzee, α -tryptase has an Arg-3Gln mutation, it is likely that the human α -tryptase gene acquired this processing mutation through genetic exchange with the neighboring δ -tryptase gene after the ancestors of humans and chimpanzees split from the primate tree. In this manner δ -tryptase genes might have passed on some of their features, including flaws, to a neighboring gene. Related conversion events might have occurred more than once. Indeed, as shown by the tree in Fig 4, such events might explain the observation that macaque δ -tryptase exon 4 is more similar to macaque $\alpha\beta$ -tryptase exon 4 than to the same δ -tryptase exons from other primates. Similar considerations apply to gorilla δ - and β 1-tryptases. However, the dendrogram in Fig 4 suggests that exon 5 has not participated in recent gene-conversion events, given that its nucleotide sequence in every primate examined is more similar to exon 5 in mouse MCP-7 than to exon 5 in any primate non- δ -tryptase. We speculate that δ -tryptase exon 5 is protected from gene conversion by the neighboring α - or β -tryptase genes because of its proximity to the intron 4 sequence, which contains an approximately 0.8-kb insertion, which is absent in non- δ -tryptases and is likely to inhibit formation of errant duplexes with the neighboring tryptase gene. However, exon 6, which is highly conserved among mammalian tryptases, is a more likely candidate for exchange. Indeed, the Gly215Asp mutation in orangutan δ -tryptase (Fig 1 and Table I) might have been acquired through genetic exchange with an $\alpha\beta$ -like tryptase gene because the same mutation is present in macaque $\alpha\beta$ -tryptases but not in macaque δ -tryptases.²⁰

δ -Tryptases are not universally flawed in primates. A purified macaque tryptase is highly active (being similar to human β -tryptase in this regard), but its N-terminal sequence matches that

of macaque δ -tryptase sequenced in this work and not the $\alpha\beta$ -tryptase previously sequenced.^{21,22} Thus synthesis of past and present data suggests that the major extractable, active macaque tryptase is full-length δ -tryptase. In fact, the predominant tryptase transcript detected in macaque lung cDNA is δ -tryptase and not $\alpha\beta$ -tryptase (data not shown). When an $\alpha\beta$ -like tryptase first switched roles with δ -tryptase to become the major active tryptase is not yet clear. We speculate that the transition occurred after ancestors of macaques and orangutans split from the primate tree because orangutan δ -tryptase is flawed by a catalytic domain mutation (Fig 1 and Table I), whereas orangutan β -tryptases have the structural hallmarks of classic active tryptase.²⁰ The later appearance of the truncation mutation, which results from a 1-step nucleotide change (Table I) and is present in more closely related gorillas, chimpanzees, and humans, raises the possibility that it occurred in a peptidase with activity already reduced by catalytic domain mutation at residue 215, which subsequently was deleted in the truncated segment.

The magnitude of the effect of 40-residue C-terminal truncation on a functioning peptidase is apparent from the position of the affected segment in the molecular model of full-length macaque δ -tryptase (Fig 2, D). The truncated form contains 2 antiparallel β -strands coursing through the heart of one of the twin, 6-stranded β -barrels forming the substrate-binding cleft. These barrels and their component strands are highly conserved in active chymotrypsin family peptidases. Although the truncated segment does not contain the "catalytic triad" residues that are essential for serine peptidase activity, it does possess 2 of 3 specificity triad residues (including residue 215 mutated in full-length orangutan δ -tryptase), forming the wall of the binding pocket accommodating the side chain of the substrate amino acid at the site of cleavage.²⁵ This is readily seen in Fig 2, D, which shows an inhibitor in the pocket.

Absence of the propeptide mutation and C-terminal truncation explains why macaque δ -tryptase is much more active than human δ -tryptase. However, understanding the structural basis of differences between macaque δ -tryptase and human β -tryptase requires a more detailed examination of the model. As reported, macaque lung tryptase differs from human lung tryptase (β -tryptase) in substrate preferences, inhibitor susceptibility, and heparin-dependent stability.²¹ Both enzymes are highly active and hydrolyze bronchodilating vasoactive intestinal peptide at 2 sites, although with differing preferences for one site relative to the other. Both peptidases strongly resist inactivation by large antipeptidases, but macaque δ -tryptase is more sensitive to the small Kunitz-class inhibitor aprotinin. The most striking biophysical difference is macaque δ -tryptase's instability at neutral to alkaline pH, which is not rescued by heparin.²¹ Although the model might not explain subtle differences in substrate and inhibitor preferences, it affords potential insights into the origins of pH-dependent instability. As revealed in Fig 1, most of the amino acids in macaque δ -tryptase that differ from human β -tryptase congregate in the exon 5-encoded region. Seventeen (31%) of 55 amino acids differ in this region, whereas only 11 (6%) of 190 vary in the rest of the catalytic domain. Exon 5 encodes part of both types of subunit interfaces (A-B and A-D) and also forms part of the wall of the central cavity (Fig 2, C), where it might influence substrate and inhibitor access to the cavity and binding to the active site.¹⁰

These differences between macaque δ -tryptase and human β II-tryptase also might explain the latter enzyme's greater stability at alkaline pH. Compared with human β II-tryptase, the macaque

δ -tryptase catalytic domain contains more Glu and Asp residues with acidic side chains (28 vs 24), fewer Lys and Arg residues with basic side chains (17 vs 21), and an equal number (ie, 12) of His weak bases with a proton extractable at neutral to alkaline pH (see Fig 1 and note that human β II-tryptase is identical to human β I-tryptase, except for an Asn102Lys substitution). Thus macaque δ -tryptase is more electronegative than human β II-tryptase (net charge of -11 vs -3 at neutral pH). This difference (Fig 2, A and B) predominantly is due to exon 5 (net charge of -5 in δ -tryptase vs 0 in β II-tryptase). Additionally, in exon 5, there is net gain of 1 His (3 vs 2) in macaque δ -tryptase versus human β II-tryptase, a gain not seen in other exonic regions. At pH 8, when His is likely deprotonated, macaque δ -tryptase's exon 5 region can be expected to become even more anionic than human β II-tryptase and to bind polyanionic heparin less avidly. Thus a major cause of alkaline inactivation might be loss of intersubunit bridging and oligomer stabilization by heparin, a phenomenon observed in mouse MCP-6 and MCP-7.^{26,27} Alternatively, replacement of β II-tryptase Tyr167 by δ -tryptase His, a residue that helps to form the A-D interface in the β II-tryptase tetramer (Fig 2, B), might weaken the interface when His is deprotonated, making the tetramer prone to spontaneous dissociation.¹⁰

We conclude that δ -tryptase is a chimeric peptidase created early in primate evolution by gene conversion. Subsequent transformations led to remarkably different fates in various primate lineages. In Old World monkeys, as exemplified by macaques, δ -tryptase appears to be the main active tryptase in the lung and cheek pouch and presumably in other tissues as well, given recent evidence that macaque $\alpha\beta$ -like tryptases contain catalytic domain mutations expected to greatly limit catalytic activity.²⁰ As suggested by genomic sequence encoding key residues (Table I), it also appears likely that δ -tryptase is active in New World monkeys (eg, marmosets) and in prosimians (eg, galagos). Therefore in macaque and some other primate-based models of mast cell-related disorders, such as asthma, δ -tryptase, but not β -tryptase, is likely to be responsible for pathologic consequences of tryptase release. In chimpanzees and humans, however, β -tryptases appear to be the major active soluble tryptases. Beginning at least 16 million years ago, accumulation of point mutations in δ -tryptases that hamper activation and cripple activity greatly diminish the likelihood that they make major contributions to mast cell tryptase function and allergic inflammation in humans and great apes.

Clinical implications: δ -Tryptase probably has little influence in human mast cell-related disorders but is likely a major factor in monkey models of tryptase biology.

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