Mutation His322Asn in Human Acetylcholinesterase Does Not Alter Electrophoretic and Catalytic Properties of the Erythrocyte Enzyme

By Patrick Masson, Marie-Thérèse Froment, Robert C. Sorensen, Cynthia F. Bartels, and Oksana Lockridge

The YT blood group antigen is located on human red blood cell (RBC) acetylcholinesterase. Wild-type acetylcholinesterase, YT1, has histidine at codon 322, whereas the genetic variant of acetylcholinesterase, YT2, has asparagine. This mutation is located within exon 2 of the ACHE gene, an exon that is present in all alternatively spliced forms of acetylcholinesterase. Therefore, acetylcholinesterase in brain and muscle has the same mutation as RBC acetylcholinesterase.

We compared the electrophoretic and kinetic properties of RBC acetylcholinesterases having His 322 or Asn 322. We found no differences in the isoelectric point, mobility on non-denaturing gel electrophoresis, affinity for acetylthiocholine, activity per milligram of RBC ghost protein, substrate inhibition constants, binding to the peripheral site ligand (propidium), and binding to active site ligands (tetrahydroaminoacridine and edrophonium). Thus, although the point mutation elicits antibody production in nonmatching blood transfusion recipients, it has no effect on the enzymatic properties of acetylcholinesterase.

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YT*1/1 and YT*2/2 acetylcholinesterases. pl ranged from 4.10 to 4.40 and smears were always present from pH 4.8 to 5.10. The absence of a detectable pH difference can be attributed to the mixture of fragment lengths produced by proteolysis as well as to the heterogeneity of charge in the three carbohydrate chains attached to acetylcholinesterase. These mask the charge difference that must be present in a peptide containing asparagine in place of histidine.

Non-denaturing gel electrophoresis patterns (Fig 1) showed a single band whose mobility was identical for both enzymes whatever the pH of the electrophoresis buffer (5.3 or 8.9). These results indicate that ionization of the presumed solvent-exposed His 322 (expected pKa ≈ 6) does not have a significant effect on the electric charge of the enzyme. Because both enzymes have an apparently identical charge to mass ratio, this implies that the mutation of histidine 322 to asparagine does not induce electrophoretically detectable enzyme conformation changes. Thus, the YT blood group polymorphism does not explain the electrophoretic variant reported by Coates and Simpson.7

Kinetic analysis did not show any differences between YT*1/1 and YT*2/2 enzymes. As shown in Table 1, Km, Vmax, and Ks were similar, indicating that the mutation has no effect on the catalytic mechanism of acetylthiocholine hydrolysis. No decrease in specific activity was associated with the Asn 322 mutation, suggesting that the His322Asn variant does not correspond to previously reported hereditary deficiencies of erythrocyte acetylcholinesterase.5,6

Inhibition of acetylthiocholine hydrolysis by propidium, an inhibitor that interacts with the peripheral site, was found to be of a linear mixed type for both enzymes. Competitive inhibition constants (Kc) and noncompetitive inhibition constants (Kc') were slightly higher (Table 1) for red blood cell (RBC) acetylcholinesterase than the values for recombinant human acetylcholinesterase (Kc = 1.4 μmol/L, Kc' = 1.8 μmol/L in 50 mmol/L sodium phosphate, pH 8.0).7 The explanation for the higher values is that the assays for RBC acetylcholinesterase were performed in a buffer with a higher ionic strength. It is known that ligand binding at the peripheral site is very sensitive to changes in ionic strength, with slight increases causing a weakening in affinity.10 Edrophonium and tacrine, two inhibitors that interact with amino acids inside the active site gorge,11 inhibited both YT1 and YT2 enzymes to the same extent. Edrophonium at 5 μmol/L inhibited YT*1/1 by 47% and YT*2/2 by 50%; tacrine at 0.125 μmol/L inhibited YT*1/1 by 40% and YT*2/2 by 41%. These results provide evidence that binding to the peripheral site and to the active site gorge was not affected by the His322Asn mutation.

In addition to the His322Asn mutation, the ACHE gene is polymorphic at codon 561 of exon 5.5 Our YT*1/1 sample was homozygous for Arg 561, whereas our YT*2/2 sample was homozygous for Pro 561. This second polymorphic site is within a peptide that is removed before attachment of the glycolipid anchor to the carboxy terminus. The peptide containing this mutation has never been observed in the acetylcholinesterase protein and is only known to exist from mRNA and cDNA studies. The mutation at codon 561 of exon 5 is unrelated to YT genotype.7 If processing of the precursor form of acetylcholinesterase to the glycolipid anchored form were strongly affected by the Pro561Arg mutation, then the amount of mature glycolipid-anchored acetylcholinesterase in the RBC membrane might have been different in our samples. This was not the case; both enzymes had the same activity per milligram of ghost protein for acetylthiocholine iodide (3.2 μmol/min/mg), acetyl(β-methyl)thiocholine iodide (2.6 μmol/min/mg), propionylthiocholine iodide (1.8 μmol/min/mg), and propionyl(β-methyl)thiocholine iodide (1.3 μmol/min/mg). This suggests that the Pro561Arg mutation does not reduce the amount of RBC acetylcholinesterase.

Table 1. Kinetic and Inhibition Parameters for YT*1/1 and YT*2/2 Variants of Human Acetylcholinesterase

<table>
<thead>
<tr>
<th>Protein</th>
<th>Acetylthiocholine</th>
<th>Propidium</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Km (μmol/L)</td>
<td>Vmax (pmol/min)</td>
</tr>
<tr>
<td>YT*1/1 (His 322)</td>
<td>0.12 ± 0.01</td>
<td>2.65 ± 0.48</td>
</tr>
<tr>
<td>YT*2/2 (Asn 322)</td>
<td>0.11 ± 0.01</td>
<td>2.79 ± 0.32</td>
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Kinetic and inhibition constants were determined for acetylthiocholine hydrolysis in 0.067 mol/L potassium phosphate buffer, pH 7.0, at 25°C. Vmax is expressed as micromoles of acetylthiocholine hydrolyzed per minute per milliliter of ghost suspension (ghost suspensions were adjusted so that their protein content was identical). Kc and Kc' are the competitive and noncompetitive inhibition constants for propidium, respectively. Mean values ± standard error of triplicate determinations are shown.

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Fig 1. Nondenaturing gel electrophoresis patterns of Triton X-100–solubilized YT*1/1 and YT*2/2 variants of human RBC acetylcholinesterase. Lane 1, glycolipid-anchored acetylcholinesterase from Drosophila melanogaster; lane 2, wild-type human acetylcholinesterase His 322 (YT*I/I); lane 3, Asn 322 variant YT*2/2. Twelve microliters of Triton-treated ghosts was loaded per lane, on a horizontal flat-bed 7% polyacrylamide gel containing 0.5% Triton X-100. Electrophoresis was for 3.3 hours at 250 V. Gels were stained for activity.10
ACETYLCHOLINESTERASE MUTANT

The His322Asn mutation is present not only in RBC acetylcholinesterase but also in muscle and brain acetylcholinesterase, and acetylcholinesterase in all tissues in which it is expressed. The evidence for this is that there is only one gene for human ACHE,18 which means that the RBC enzyme is made from the same ACHE gene as acetylcholinesterase in muscle and brain. Secondly, the exon that encodes histidine 322 is present in all alternatively spliced forms of acetylcholinesterase.19 This is the same exon that encodes the active site serine 203, a component of the catalytic triad.2,19 Our results indicate that the naturally occurring point mutation His322Asn has no effect on the overall structure of human acetylcholinesterase, its catalytic properties, or its affinity for peripheral and active site ligands. We conclude that the cholinergic function of acetylcholinesterase in nervous tissues and the neuromuscular junction is unaltered by the YT blood group polymorphism.

ACKNOWLEDGMENT

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NOTE ADDED IN PROOF

An exit channel for product release has been mapped to a region that includes His 322 (Lys 315 in Torpedo acetylcholinesterase) and contains several additional positively charged amino acids.20 The fact that mutation to a neutral amino acid, Asn 322, has no effect on catalysis suggests that the exit channel functions equally well whether position 322 is positively charged or neutral.

REFERENCES

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