Evidence for a single butyrylcholinesterase gene in individuals carrying the C_5 plasma cholinesterase variant (CHE2)

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DNA of 3 unrelated individuals carrying the human plasma butyrylcholinesterase C_5 variant (CHE2) was isolated from white blood cells. Southern blot patterns of DNA restriction fragments probed with each of the 4 butyrylcholinesterase exons provided evidence that the production of C_5 is not directed by a second butyrylcholinesterase gene. This finding supports the suggestion that the C_5 variant is a hybrid enzyme resulting from the association of butyrylcholinesterase subunits with a non-cholinesterase protein.

Butyrylcholinesterase; Southern blot analysis; CHE2; (Human plasma)

1. INTRODUCTION

Butyrylcholinesterase (pseudocholinesterase; acyl-cho-line acylhydrolase: EC 3.1.1.8) is present in various human tissues as well as in plasma and in other biological fluids. So far, no physiological function has been assigned to this enzyme but it is of pharmacological and toxicological importance because it is involved in the metabolism of ester containing drugs [1] and because it is inhibited by organophosphates and carbamates like the related enzyme acetylcholinesterase [2].

The plasma BChE shows a complex molecular polymorphism. Numerous molecular forms can be detected by gel electrophoresis. Four main forms named C_1, C_2, C_3 and C_4 according to their electrophoretic mobility have long been identified [3]. C_1, C_3 and C_4 are monomer, dimer and tetramer, respectively [4,5]. The C_2 component is a BChE monomer-albumin conjugate [6]. Other components are often observed depending on storage [3], partial proteolysis [7] and/or electrophoresis conditions [8].

Two genetic loci, E_1 and E_2 (designated CHE1 and CHE2 before 1990; and renamed BChE and CHE2 in 1990 by the Human Gene Nomenclature Committee), directing the production of plasma BChE have been identified from genetic population studies. The E_1 locus shows a high polyallelism [1]; it encodes for the 'normal' enzyme which exhibits the above-mentioned molecular forms. The E_2 locus is not allelic to the first

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one [9]; this gene has been presumed to direct the production of an additional plasma form called C_5 because it is a slow-migrating component. This form is present in 8–10% of Caucasians but is found with lower frequency in most other populations [1]. Individuals carrying the C_5 form have an elevated plasma BChE activity (up to 30% higher than normal level). The C_5 variant appears to be a hybrid enzyme composed of BChE subunits coded by the E_1 gene [9] associated with another protein whose nature is still unknown [10]. Other slow-migrating variants have been reported [1]; some of them have been observed in malignant diseases [11,12], but little is known about these forms, and their relationship with C_5 is uncertain.

Genetic linkage analysis [13] and an in situ hybridization study [14] assigned the E_1 locus to chromosome 3. As regards the E_2 locus, linkage studies have shown loose linkage between haptoglobin (HP) on chromosome 16 and C_5 (CHE2), Lovrien et al. [15] reporting a lod score of 2.347 at a recombination distance of 0.25 (males) and 0.32 (females) and Marazita et al. [17] reporting a lod score of 2.51 at a recombination distance of 0.32. Lod scores below 3.0 are considered statistically insignificant and do not favor linkage [15]. In contrast, Eiberg et al. [16] found a tight linkage between the γ-crystallin gene cluster (CRYG) on chromosome 2 and C_5 (CHE2), reporting a highly significant lod score of 4.21 at a recombination distance of 0.00. It should be noted that in situ hybridization of chromosomes with cloned BChE cDNA as probe suggested a homologous site on chromosome 16 [14]. This hybridization site might correspond to the E_2 locus. However, the recent deter-
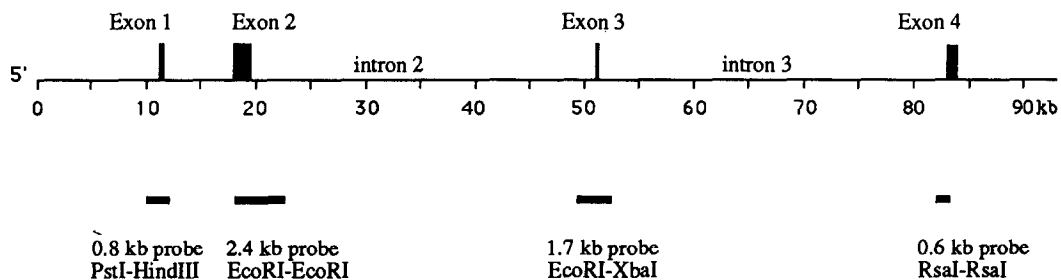
Fig.1. Organization of the BChE gene and position of the exon probes (adapted from Arpagaus et al. [18]). The diagram indicates the organization of the human BChE gene and the size of introns. 83% of the coding sequence is in exon 2. The diagram exaggerates the size of exon probes to show the presence of flanking intronic sequences. Exon 1 probe is a 0.8 kb PstI/HindIII fragment containing 563 bp of 5' untranslated sequence, 120 bp of exon 1 and 129 bp of intron 1; exon 2 probe is a 2.4 kb EcoRI/EcoRI fragment containing 1343 bp of exon 2 (exon 2 has 1525 bp total) and 1018 bp of intron 2; exon 3 probe is a 1.7 kb EcoRI/XbaI fragment containing 0.7 kb of intron 2, 167 bp of exon 3 and 0.8 kb of intron 3; exon 4 probe is a 0.6 kb RsaI/RsaI fragment containing 101 bp of intron 3 and 518 bp of exon 4 (exon 4 has 604 bp total).

mination of the structure of the BChE gene established that BChE is encoded by a single gene corresponding to the E_1 locus [18].

To investigate the question of the multiplicity of BChE genes we decided to probe restriction length fragments of genomic DNA from individuals carrying the C_5 variant. For this purpose we prepared 4 ^{32}P-labeled genomic probes covering the 4 exons of the BChE gene and containing adjacent intronic sequences (fig.1). Southern blot analysis of hybridized DNA fragments indicates unambiguously that the DNA of individuals of C_5^+ phenotype contains only one BChE gene.

2. MATERIALS AND METHODS

2.1. Blood samples

Blood samples were taken from the Lyon Blood Transfusion Center (CRTS de Lyon, 01704 Miribel, France). After venepuncture plasma was immediately separated from the blood cells for C_5 phenotyping. Blood bags of 450 ml from donors of phenotype C_5^+ were collected. Buffy coats were obtained from the blood bags according to the procedure of Cantell et al. [19]. After adding 5 ml of dimethylsulfoxide, buffy coat bags were stored at −20°C until DNA extraction.

2.2. C_5 phenotyping

C_5 was phenotyped by unidimensional polyacrylamide gel electrophoresis using the discontinuous buffer system of Juul [8]. We operated in a Hoefer vertical slab gel unit thermostated at 10°C. Gels were 1 mm thick and 6% acrylamide gels were used as separating gels. 3 μl plasma samples were loaded and electrophoreses were run for 3 h at constant current (25 mA/plate). After electrophoresis gels were stained for cholinesterase activity by the method of Karnovsky and Roots [20] with butyrylthiocholine iodide (1 mM) as the substrate.

2.3. DNA extraction from white blood cells

High molecular weight DNA was isolated from white blood cells by the classical phenol-chloroform procedure [21] as modified by R.D. Larsen (Howard Hughes Medical Institute, University of Michigan; personal communication).

2.4. Southern blot analyses

DNA was solubilized in 5 mM Tris-HCl, pH 7.4, containing

0.1 mM EDTA. DNA samples (~1 μg/μl) were digested with 7 highly concentrated restriction endonucleases (EcoRI, HindIII, PvuII, TaqI, HindII, XbaI and PstI) purchased from Boehringer Mannheim. 300 units of each enzyme were added to 40 μg of DNA in specific buffers. The digestions were allowed to proceed for 3 h at 37°C except for TaqI that operated at 65°C.

Electrophoreses of the restriction fragments (10 μg/lane) were performed on 1% agarose gel in Hoefer Minnie Horizontal Agarose Submarine Units for 90 min at 70 V in 40 mM Tris/1 mM Na₂EDTA-acetate, pH 8.0, containing 0.2 μg/ml of ethidium bromide. For each DNA sample, 4 gels for probing the 4 BChE exons (fig.1) [18] were run. After electrophoresis the DNA fragments were blot transferred onto nylon membranes (GeneScreen, NEN Research Products) according to the standard protocol.

The 4 BChE exon probes were ^{32}P-labeled by the random oligolabeling method of Feinberg and Vogelstein [22]. Labeling of probes was achieved in 6 h at room temperature; 10 μl of [α-^{32}P]dCTP (111 TBq/mmol) from Amersham and 2 units of labeling grade Klenow enzyme (Boehringer Mannheim) were used for each labeling (44−105 ng of probe in 100 μl of reaction medium).
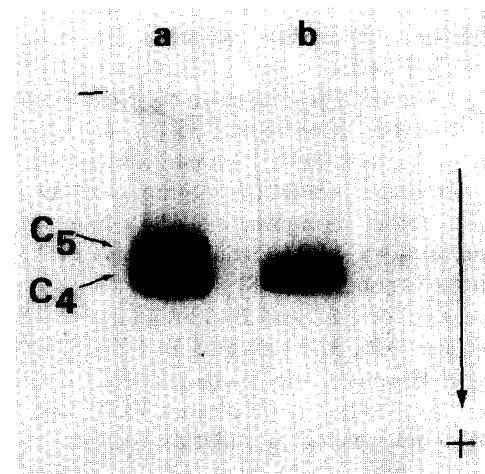

Fig.2. Polyacrylamide gel electrophoresis patterns of human plasma cholinesterase. (a) Staining for BChE activity of a plasma sample of C_5^+ phenotype. (b) Staining for BChE activity of a plasma sample of C_5^- phenotype. (Because the staining process was stopped when the major components of BChE C_4 and C_5 were revealed, the minor forms are not perceptible.)
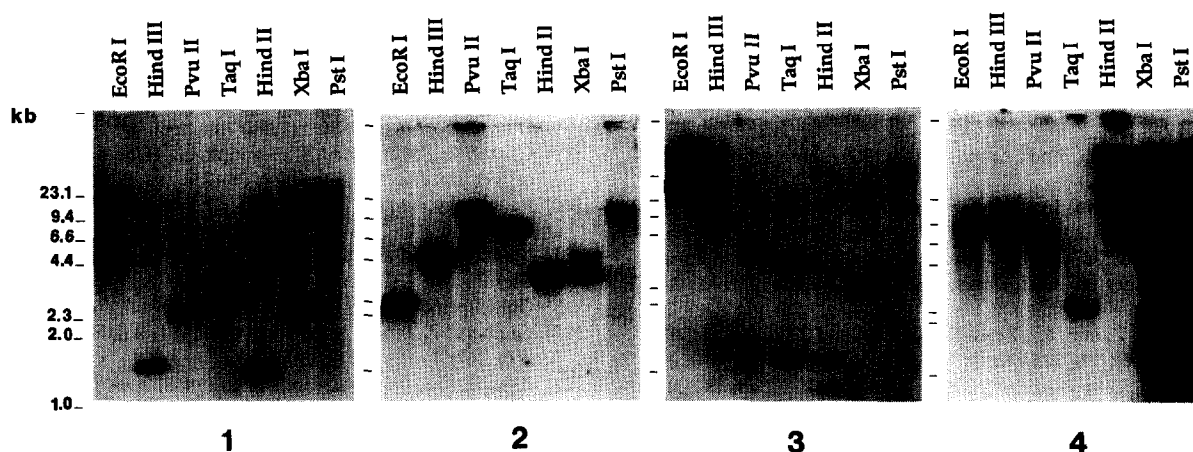
Fig.3. Hybridization of genomic blots with four ^{32}P-labeled exon probes. DNA was extracted from white blood cells of an individual of phenotype C$_5^+$. Blot 1 was hybridized with exon 1 probe; 2 with exon 2 probe; 3 with exon 3 probe; 4 with exon 4 probe.

Prehybridization of membranes was for 6 h at 61°C in 6 × SSC buffer (SSC is 0.15 M NaCl/0.015 M sodium citrate, pH 7.0) containing 0.25% Carnation instant non-fat milk. Hybridization with 100 μl of each probe was performed for 20 h at 61°C in 25 ml of 6 × SSC/0.25% dry milk (probe concentration: 1.75 ng/ml for exon 1 probe; 4 ng/ml for the other probes). Then the blots were washed in 2 × SSC/0.1% sodium dodecylsulfate at 60°C (3 times, 1 h each). Autoradiographic exposures were for 3 days at 20°C with Kodak X-Omat films.

3. RESULTS AND DISCUSSION

Among a population of 100 regular blood donors, 6 unrelated individuals were found to be of C$_5^+$ phenotype (fig.2). In order to eliminate false positives, phenotyping was repeated several times over a period of 6 months. Three donors, named A, B and C, exhibited a high intensity C$_5$ band. Interestingly, the individual C suddenly turned as C$_5^-$ and became again C$_5^+$ two months later. This confirms previous observations of several investigators who noted that expression of C$_5$ is variable and non-mendelian [16,23]. The individuals A, B and C were selected for DNA isolation from their white blood cells.

Fig.3 are genomic blots of restriction fragments hybridized with the 4 ^{32}P-labeled BChE exon probes. As shown, radioactive bands range in size from 0.7 to 23 kb. For samples A, B and C, whatever the endonucleases used, the number and the size of these fragments are identical to those generated from genomic DNA from individuals in which the C$_5$ variant is not phenotypically expressed [18]. These data are in agreement with the BChE gene structure as determined from restriction map of genomic clones [18]. In addition, direct sequencing of DNA after amplification by the polymerase chain reaction used for identification of BChE allelic variants never showed band heterogeneity that could mean the existence of a second BChE gene ([24,25], La Du et al., submitted). All these results support the conclusion that only one BChE gene encodes

the catalytic BChE subunits of C$_5$ as well as other BChE forms.

Following the example of the human plasma C$_2$ component [6], C$_5$ has to be considered as a conjugate. The non-BChE protein still has to be identified. Work is in progress in this direction. So far it is certain that this protein is neither a degradation product of BChE nor acetylcholinesterase, nor albumin nor a collagenous tail fragment (P. Masson, unpublished results). The observed linkage between CHE2 and the γ-crystallin gene cluster [16] does not imply that γ-crystallin is conjugated with BChE subunits to form C$_5$; but this hypothesis has still to be tested. We conclude that a second gene, previously called E$_2$, directs the production of the non-BChE component of C$_5$ and/or the enzymic system that controls the assembly of the BChE subunits with this protein.

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