HUMAN RED CELL ACID PHOSPHATASE (ACP1): GENETIC TYPING AT THE DNA LEVEL

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Human red cell acid phosphatase (ACP1) is one of the best known of the forensically useful genetic polymorphisms. Two common alleles - ACP1*A and ACP1*B - are found in all major population groups. A third allele - ACP1*C - is common in Caucasians and a fourth - ACP1*R - is common in Blacks of African origin. A number of rare alleles have also been noted.

Each of the common alleles is expressed as a pair of electrophoretically distinct isozymes, termed fast (**f**) and slow (**s**) according to their anodal mobility. The **f:s** isozyme ratios for the *A, *B, and *C alleles are 2:1, 4:1, and 1:4 respectively [1]. This allelic variation in **f:s** ratio accounts for observed phenotypic variation in activity modulation by purines and folates, and in phosphotransferase activity [2-9]. The allele dependent variation in the amount of enzyme produced in red cells is independently regulated.

Recent protein and DNA sequencing studies show that the **f** and **s** isozymes are identical over the protein sequence regions spanning residues 1-39 and 74-157 but differ significantly (59%) in sequence in the middle region, residues 40-73 [10-12]. The sequence differences in this middle "signature" region presumably account for the differences in the catalytic and molecular properties of the **f** and **s** isozymes. The **f** and **s** isozyme pairs have been postulated to be generated by an alternative splicing mechanism in which the **f** and **s** signature sequences are encoded in mutually exclusive exons [10,13]. The gene structure of the ACP1 locus is consistent with this hypothesis [14].

Gene sequence analysis of the *A, *B, and*C alleles was undertaken to identify base substitutions that distinguish between them. A base substitution at codon position 105 should account for the glu-arg amino acid substitution that distinguishes *A from *B and *C [11,15]. Other base differences should account for the allele dependent variation in **f:s** isozyme ratio and in red cell activity level. Base differences between the alleles can also be used for general typing purposes.

ACP1 SEQUENCE AND GENE STRUCTURE

Genomic sequence spanning approximately 6 Kb of the ACP1 locus has been determined. Six linearly positioned exons have been identified; these account for all the coding sequence except for amino acid residues 1-13 at the N-terminus. The missing coding sequence appears to be located in an exon or exons at least 4 Kb upstream of the first sequenced exon. Genomic sequences of homozygous *A, *B, and *C types show the same gene structure.

As previously predicted, the **f** and **s** signature sequences are encoded in different exons, exons 3F and 3S; the two exons are of equal length. The intervening sequence is 41 bp, too short to serve as a functional intron. This gene structure is consistent with the mutually exclusive alternative splicing hypothesis.

The *A, *B, and *C sequences were found to differ at three sites as indicated in the table below:

	*A	*B	*C
Exon 3F base 15	C	C	T
Exon 3S base 12	T	C	C
Exon 5 base 24	G	A	A

The first two substitutions are silent but may play a role in regulating f:s ratios. The substitution in exon 5 is in the second base of codon 105 and accounts for the Glu->Arg difference that distinguishes the *A type from the *B and *C types.

DETECTION OF *A, *B, AND *C ALLELES

The base substitution in exon 5 creates a TaqI restriction site in the *A allelic sequence not present in the *B and *C allelic sequences; this has been exploited in a typing system to distinguish the *A allele from the *B and *C alleles [15]. Amplification using primers ACP38 (5'GGATGTTTCAGAAGACCCTAGCAG3', sense strand) and ACP18 (5'GCTCCCAAGTAGTTCAATTTTAGC'3) yields a 149 bp product for each of the *A, *B, and *C alleles. Restriction with TaqI cleaves the *A allele product into 106 bp and 43 bp fragments; the *B and *C allele products are not cut.

The base substitution in exon 3F creates a CfoI restriction site in the *A and *B allelic sequences not present in the *C allelic sequence; this can be exploited in a typing system to distinguish the *C allele from the *A and *B alleles. Amplification using primers ACP10 (5'GTCGATCACCCATTGCAGAAGC3', sense strand) and ACP44 (5'CACACAACTTCAAGTCCAAGGACG3') yields a 303 bp product for each of the *A, *B, and *C alleles. Restriction with CfoI cleaves the *A and *B allele products into 177 bp and 126 bp fragments; the *C allele product is not cut.

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