4.1 General

Genetic and Molecular Aspects of the Human Red Cell Acid Phosphatase Polymorphism

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The red cell acid phosphatase polymorphism is a classical enzyme marker in paternity testing and blood stain analysis. It was discovered on the basis of its electrophoretic patterns (Hopkinson et al. 1963), but subsequently it was found to exhibit several other phenotypic characteristics such as differences in enzyme activity levels (Spencer et al. 1964), activity modulation by purines and pteridines (Mansfield and Sensabaugh 1978; Sensabaugh and Golden 1978; Mohrenweiser and Novotny 1982), phosphotransferase activity (Golden and Sensabaugh 1986), and stability (Luffman and Harris 1967). Another property is that each allele encodes two isozymes, **f** and **s** (Hopkinson et al. 1964). Recent results have provided new knowledge of the genetic and biochemical basis of this polymorphic system, and the objective of the present paper is to summarize these results.

Quantitation of the acid phosphatase isozymes using specific antibodies has shown that the allelic differences in the red cell enzyme activity levels (ACP1*C > ACP1*B > ACP1*A) is mainly the consequence of a similar allele dependent difference in enzyme protein level (Dissing 1987). It was further found that **f** and **s** isozymes are generated in allele specific ratios (**f**:**s** = 2:1, 4:1 and 1:4 for the ACP1*A, B and C alleles, respectively). The order of this allelic effect (B > A > C) is the same as that observed for activity modulation (Mansfield and Sensabaugh 1978; Sensabaugh and Golden 1978; Mohrenweiser and Novotny 1982). The three **f** and **s** isozyme pairs (**Af**/**As**, **Bf**/**Bs** and **Cf**/**Cs**) encoded by the ACP1*A, B and C alleles have been characterized and significant differences between **f** and **s** isozymes were observed in both enzymatic properties (Km, Ki, activity modulation, specific activity) and molecular properties (immunochemical properties, molecular size, stability) (Dissing 1986; Dissing and Svensmark 1990). In contrast the three genetically different **f** isozymes (**Af**, **Bf**, **Cf**) showed identical properties as did the three **s** isozymes. For example, **f** isozymes are inhibited with adenine and activated with hypoxanthine, whereas **s** isozymes are activated with adenine and insensitive towards hypoxanthine; this and the different **f**/**s** ratios account for the allelic differences in activity modulation. Similarly the higher stability of **s** isozymes relative to **f** isozymes explains earlier observations of a higher stability of the phenotypes BC and AC as compared to the phenotypes A, AB and B (Luffman and Harris 1967), the latter phenotypes having a higher **f**/**s** ratio.

Therefore, from a functional point of view the acid phosphatase system consists of only two different isozymes, **f** and **s**, the different proportion of which determine the properties of the various phenotypes. The different properties of **f** and **s** isozymes suggest that they serve different functions in the cell.

The amino acid sequence of the **Af**, **As**, **Bf**, **Bs**, **Cf** and **Cs** isozymes (Dissing et al. 1991; Dissing and Johnsen (unpublished results)) has recently been determined (fig. 1). All 6 isozymes consist of single peptide chains of equal length (157 residues). The most

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interesting finding is, however, that each **f/s** isozyme pair exhibits a substantial sequence difference in a specific region (40-73), despite being encoded by the same allele. This segment of 34 residues is identical for the three **f** isozymes and is also identical for the three **s** isozymes. The remaining four-fifths of the peptide chains of each isozyme pair are identical. Another finding is that the **Bf/Bs** and **Cf/Cs** isozyme pairs are identical, whereas the **Af/As** isozyme pair differs from these at residue 105, the neutral glutamine residue in the **B** and **C** isozymes being substituted with the basic arginine residue in the **A** isozymes. This explains the higher isoelectric points of the **A** isozymes relative to those of the **B** and **C** isozymes (Divall 1981).

The specific **f** and **s** sequence segments must necessarily account for the differences in catalytic and molecular properties of the **f** and **s** isozymes. The **s** specific sequence contains an extra basic residue (histidine 69) which explains the lesser anodal mobility of the **s** isozymes at acid pH values. The same histidine residue may also account for the higher Km values of **s** isozymes at acid pH values (Dissing and Svensmark 1990). The **f** specific sequence exhibits a lesser capacity for hydrophobic and a higher capacity for hydrophilic interaction than the **s** specific sequence. This may account for the lower stability of the **f** isozymes relative to the **s** isozymes.

As previously hyphotesized (Dissing and Sensabaugh 1987) it seems likely that the ACP1 locus is composed of at least 4 coding sequences, exons, interspersed with noncoding sequences, introns. These exons code for the N-terminal region (N), the **f** specific region (F), the **s** specific region (S) and the C-terminal region (C) of the isozymes, respectively. By alternative splicing of the primary RNA transcript two different mRNA molecules are generated from each allele, a **f**-mRNA and a **s**-mRNA, consisting of the N,F,C and the N,S,C exons, respectively.

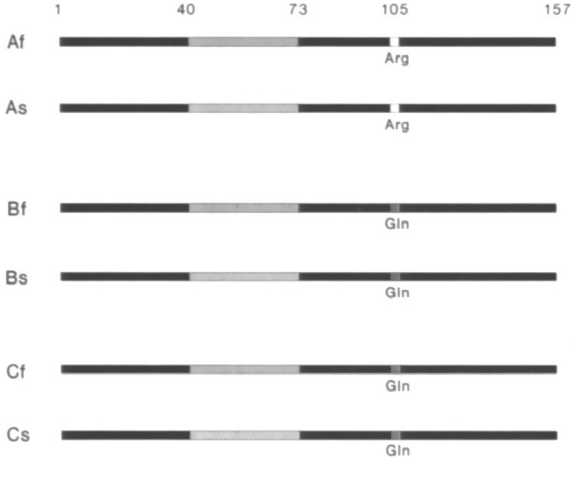

Fig. 1. Human red cell acid phosphatase: Schematic representation of the structure of the 6 common isozymes

The glutamine-arginine substitution at residue 105, which distinguish **B** and **C** isozymes from **A** isozymes, may be accounted for by a single base substitution in the respective codon. The ACP1*B and ACP1*C alleles encode, however, exactly the same isozymes, the only difference at the protein level being the **f/s** ratio. These two alleles may differ in the DNA sequence in a way that influences the splicing mechanism. It is apparent that an important question is how the allelic control of the **f/s** ratio is accompliced.

To further explore the ACP1 gene, the ACP1 locus is currently being sequenced, and preliminary results do indicate the existence of an intron between the N-coding region and the F-coding region (Dissing and Sensabaugh (unpublished results)).

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