Human Red Cell Acid Phosphatase (ACP1): The Primary Structure of the two Isozymes **Bf** and **Bs** Encoded by the ACP1*B allele

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Being one of the good informative genetic markers, red cell acid phosphatase has served the forensic scientist for a quarter of a century. Over the years this enzyme has been the subject of many studies, yet some important genetic and biochemical questions still remain to be answered.

One of these questions concerns the mechanism by which each allele generates two electrophoretically different isozymes, **f** and **s** (Hopkinson et al. 1964). This isozyme pair is produced in allele specific ratios (Dissing 1987). The **f** isozymes differ from the **s** isozymes with respect to a number of catalytic and molecular properties, while the **f** isozymes are indistinguishable from one another, as are the **s** isozymes (Fenton and Richardson 1971, Dissing 1986, Dissing and Svensmark). The genotypic difference in the relative content of **f** and **s** isozyme may account for the different properties of the various acid phosphatase phenotypes (Dissing 1986).

We have recently detected major chemical differences between the **Bf** and **Bs** isozymes (Dissing and Sensabaugh 1987). Digestion of **Bf** and **Bs** with trypsin yielded 17 and 15 peptides, respectively. Five peptides were unique to the **Bf** isozyme and three to the **Bs** isozyme. This excluded the possibility that the two isozymes are conformational isomers, which has been generally assumed for almost two decades (Harris 1980). It also argued against that one isozyme is the result of a simple post-translational modification of the other. It was suggested that the **f** and **s** isozymes may be the result of alternative splicing of the primary RNA transcript (Dissing and Sensabaugh 1987).

To test this hypothesis the tryptic peptides as well as peptides generated by cleavage by two other proteinases (endoproteinase Glu-C and endoproteinase Asp-N) have been sequenced. Overlapping sequences from the various peptides allowed the construction of the primary structure of the **Bf** and **Bs** isozymes (fig. 1).

Both isozymes consist of a single peptide chain. The **Bf** and **Bs** chains are identical with respect to both the N-terminal and the C-terminal regions representing approximately 4/5 of the molecule. However, starting with amino acid residue 40 each chain contains an internal section of 34 residues that is peculiar either to the **Bf** or to the **Bs** molecule. It is noted that while the two isozymes contain the same number of acidic amino acid residues the **Bs**

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molecule contains one more basic amino acid (histidine) than the **Bf** molecule. This is consistent with the lower anodal mobility of the **Bs** isozyme.

The finding of a **Bf** or **Bs** specific sequence within the acid phosphatase molecule rules out both the conformer and the post translational modification hypotheses. On the other hand it is consistent with the hypothesis of alternative splicing. Assuming that the results obtained with the **B** isozymes are general for ACP1 isozymes, it may be predicted that ACP1 alleles consist of at least 4 exons, one of which codes for the **f** specific region, another for the **s** specific region, yet another for the N-terminal (N) region and the remaining exon(s) for the C-terminal (C) region (fig. 2). A mutational event occurring in a N or a C exon would alter both the **f**-mRNA and the **s**-mRNA, and the effect on the amino acid sequence of the **f** and the **s** isozyme determined by the new allele would be the same. The hypothesis of alternative splicing therefore offers a simple explanation for the pair wise variation in electrophoretic properties observed for most allozymes. The genotypic variation of the **f/s** ratio may be due to allelic differences near to the splice sites e.g. within the introns.

The finding of specific **f** and **s** sequences offers the biochemical basis for the different catalytic and molecular properties of **f** and **s** isozymes, and it suggests that **f** and **s** isozymes may serve different biological functions in the cell.

Bf isozyme (Mw; 17,916):

<u>Ac-Ala^{1}-Glu</u>-Gln-Ala-Thr-Lys-Ser-Val-Leu-Phe-Val-Cys-Leu-Gly-
Asn-Ile-Cys-Arg-Ser-Pro-Ile-Ala-Glu-Ala-Val-Phe-Arg-Lys-Leu-
Val-Thr-Asp-Gln-Asn-Ile-Ser-Glu-Asn-Trp-**Arg^{40}-Val-Asp-Ser-Ala-**
Ala-Thr-Ser-Gly-Tyr-Glu-Ile-Gly-Asn-Pro-Pro-Asp-Tyr-Arg-Gly-
Gln-Ser-Cys-Met-Lys-Arg-His-Gly-Ile-Pro-Met-Ser-His-Val^{73}-
Ala-Arg-Gln-Ile-Thr-Lys-Glu-Asp-Phe-Ala-Thr-Phe-Asp-Tyr-Ile-
Leu-Cys-Met-Asp-Glu-Ser-Asn-Leu-Arg-Asp-Leu-Asn-Arg-Lys-Ser-
Asn-Gln-Val-Lys-Thr-Cys-Lys-Ala-Lys-Ile-Glu-Leu-Leu-Gly-Ser-
Tyr-Asp-Pro-Gln-Lys-Gln-Leu-Ile-Ile-Glu-Asp-Pro-Tyr-Tyr-Gly-
Asn-Asp-Ser-Asp-Phe-Glu-Thr-Val-Tyr-Gln-Gln-Cys-Val-Arg-Cys-
Cys-Arg-Ala-Phe-Leu-Glu-Lys-Ala-His^{157}-(?)

Bs isozyme (Mw; 17,851), specific sequence:

-Val^{40}-Ile-Asp-Ser-Gly-Ala-Val-Ser-Asp-Trp-Asn-Val-Gly-Arg-
Ser-Pro-Asp-Pro-Arg-Ala-Val-Ser-Cys-Leu-Arg-Asn-His-Gly-Ile-
His-Thr-Ala-His-Lys^{73}-

Fig. 1. Amino acid sequence of the **Bf** and **Bs** isozymes encoded by the ACP1*B allele. The sequences printed in boldface are **Bf** or **Bs** specific. The N-terminal alanine residue is blocked (acetylated) and the underlined sequence was deduced from the amino acid composition of an endoproteinase Glu-C peptide and a tryptic peptide containing the 2 first and the 6 first amino acid residues, respectively, and by FAB mass spectrometry[1] of the latter peptide. The C-terminal has yet to be confirmed.

[1]Kindly performed by Dr. P. Højrup, University of Odense

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