Human Red Cell Acid Phosphatase (ACP1): Evidence for Differences in the Primary Structure of the two Isozymes Expressed by each Allele.

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One of the notable features of human red cell acid phosphatase (ACP1, E.C. 3.1.3.2) is the generation of 2 isozymes (f and s) by each allele. These isozymes differ with respect to electrophoretic mobility (fig. 1) as well as to catalytic, stability and immunochemical properties (Harris 1980, Dissing 1987). The mechanism responsible for this pair wise production of isozymes has long been a puzzle and is of interest not only to the biochemist and geneticist but also to the forensic scientist.

Fisher and Harris (1971) have reported an apparent inter-conversion of f- and s-isozymes which led to the hypothesis that the isozymes are conformational isomers. Another possibility is that one isozyme is a post genetic modification product of the other, and a third that f- and s-isozymes are synthesized as discrete molecular entities.

The purpose of the present work was to distinguish between these three mechanisms through an investigation on purified Bf

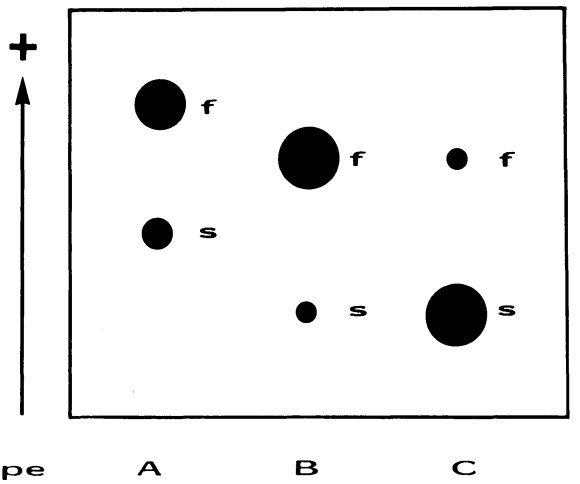

Fig. 1. Diagram of acid phosphatase phenotypes after starch gel electrophoresis in citrate/phosphate buffer, pH 5.9.

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Edited by W. R. Mayr

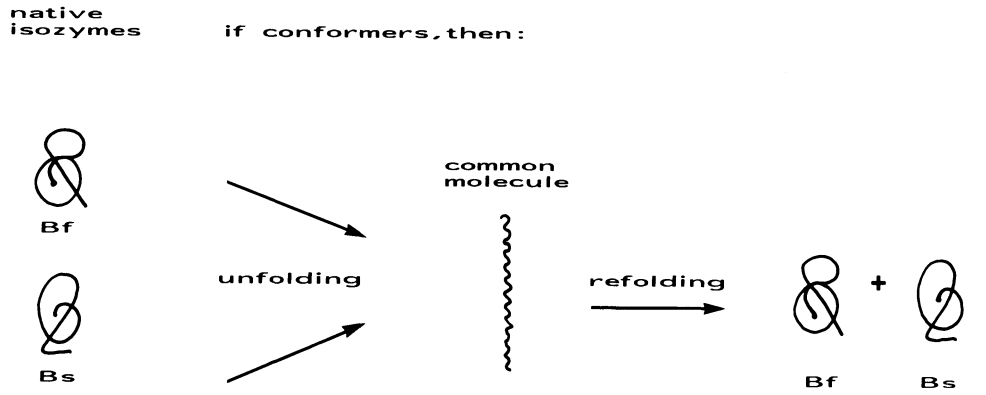

Fig. 2. Considerations about the conformer hypothesis. The example shows the expected behavior of Bf- and Bs-isozymes.

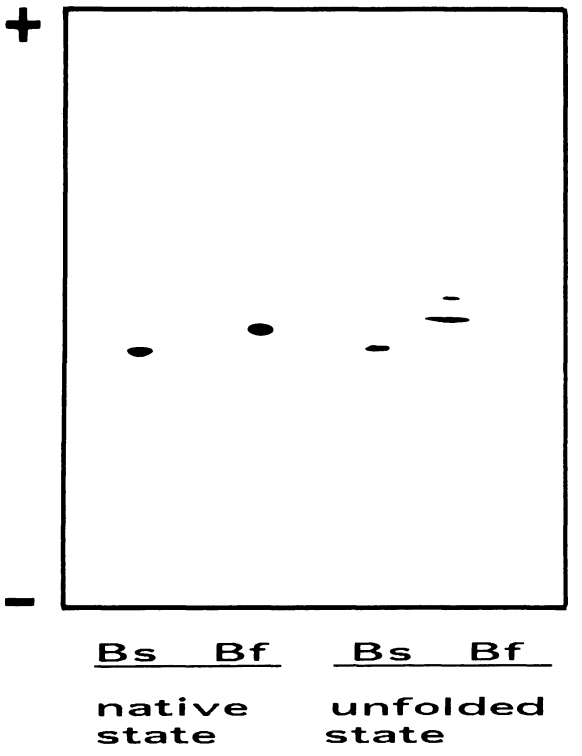

Fig. 3. Agarose gel IEF of purified acid phosphatase isozymes of type B using Ampholine, pH 3-10. Lanes 1 and 2 (from left to right): Isozymes run in the native state. Lanes 3 and 4: Isozymes denatured and run in 8M urea, 10mM dithiothreitol.

and Bs isozymes (Dissing et al. 1979).[1]

If the Bf and Bs isozymes are conformers, unfolding of the molecules should convert them to a common polypeptide chain and refolding would be expected to result in a mixture of the two isozymes (fig. 2). IEF patterns of the native Bf- and Bs-isozymes and of the same isozymes unfolded in 8M urea is shown in fig. 3; it can be seen that the charge difference between the two isozymes persisted under denaturing conditions. Removal of the urea from the denatured isozymes by dialysis resulted in a partial restoration of the enzymatic activity (23% and 17% for the Bf- and Bs-isozyme respectively). Electrophoresis showed that the renatured isozymes had retained their initial mobility -no evidence of interconversion was observed (fig. 4). These findings are clearly incompatible with the conformer hypothesis and they indicate the presence of a chemical difference between the two molecules.

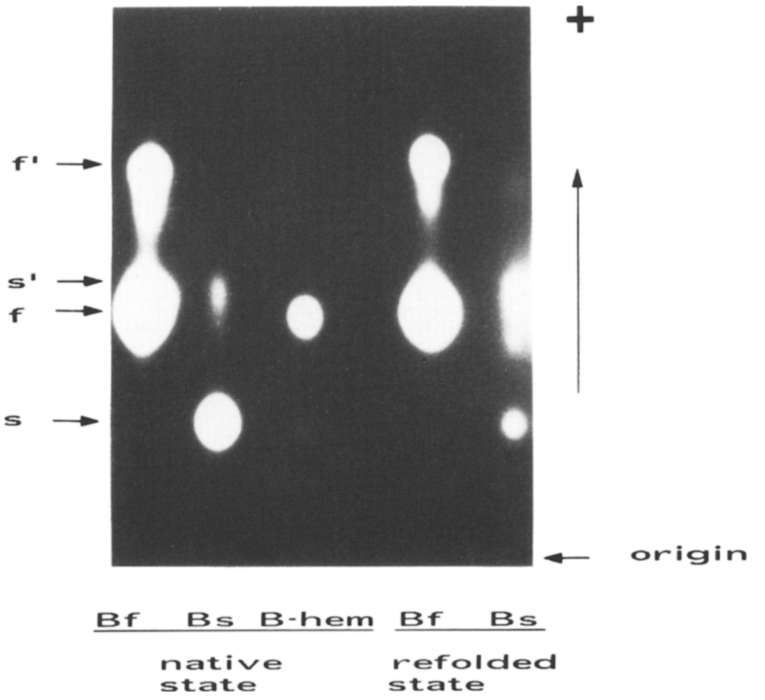

Fig. 4. Starch gel electrophoresis of purified acid phosphatase isozymes of type B in citrate/phosphate buffer, pH 5.9. Lane 1-3 (from left to right): Native isozymes and crude hemolysate (type B). Lane 4 and 5: Isozymes denatured in 8M urea, 10mM dithiothreitol and renatured by removal of the urea. B's and B'f = minor anodal Bs and Bf component respectively.

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[1]A full report on this work will be presented elsewhere (Dissing and Sensabaugh).

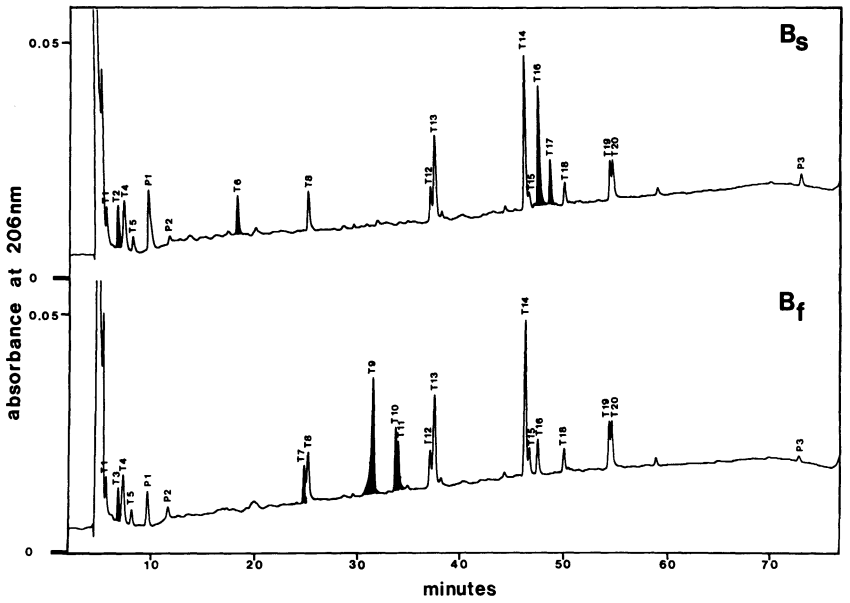

Fig. 5. Reversed phase HPLC analysis of tryptic digests of purified acid phosphatase isozymes of type B using a linear gradient from solvent A (0.1% trifluoroacetic acid in H_2O) to solvent B (0.1% trifluoroacetic acid in 65% acetonitrile (vol/vol). The peptides are numbered T1 through T20. P1, P2 and P3 indicate UV-absorbing peaks contributed by the reagents present in the digestion mixture and by the solvent system.

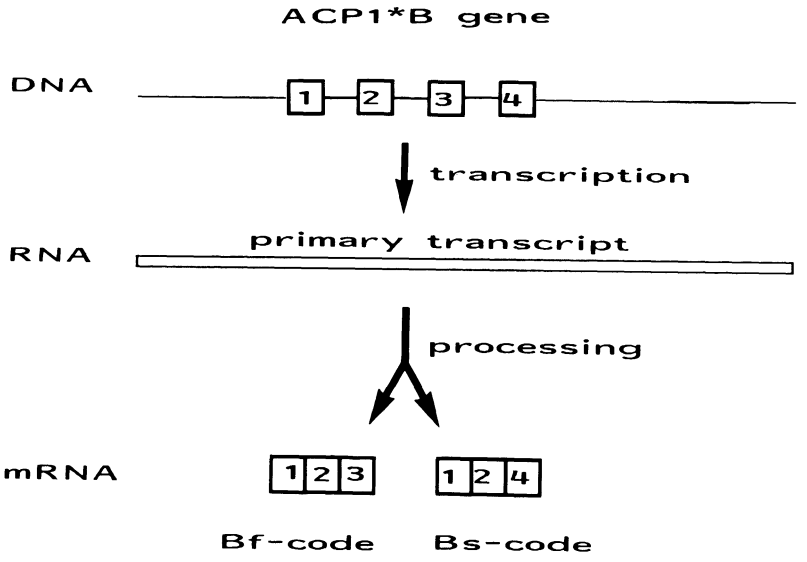

Fig. 6. Hypothetical mechanism generating discrete Bf- and Bs-mRNAs by alternative splicing of the ACP1*B gene.

To investigate the nature of this difference purified Bf- and Bs-isozymes were digested by trypsin (Gracy 1977), which cleaves peptide chains at lysine and arginine residues. A chemical difference between the Bf- and Bs-molecules would then be contained in one or more of the tryptic peptides resulting in one or more unique peptides and a number of peptides common to both molecules.

The tryptic digests were analyzed by reversed phase HPLC (fig. 5). Fifteen major peptide peaks were separated with the Bs digest and 17 with the Bf digest. Twelve peaks were common to both isozymes, whereas three peaks (T2, T6 and T17) were unique to the Bs-isozyme and 5 (T3, T7, T9, T10 and T11) were unique to the Bf-isozyme. One Bs peak, Bs-T16, was consistently at least twice as big as the Bf-T16 peak indicating another peptide difference. This observation of multiple peptide differences between the Bf- and Bs-isozymes indicates a chemical difference more extensive than a charge difference on a single amino acid residue.

It seems unlikely that these differences could arise through simple post-translational modification. Given the constancy in the proportion of the isozymes throughout the lifespan of the red cell (Rogers et al. 1978) the modification mechanism would furthermore have to be regulated such that only a defined proportion of the enzyme molecules are processed.

The molecular differences would be explained postulating that f- and s-isozymes are synthesized as discrete molecules. A mechanism involving two closely linked genes coding for the f- and the s-isozyme, respectively, would account for this, but it implies that the mutations giving rise to the A, B and C allelic variation being duplicated in each pair of linked genes, which seems to be a highly unlikely occurrence. However, a possible mechanism, which has been described for several non polymorphic proteins (Noguchi et al., 1986; Leff et al., 1986), is that the ACP1 alleles each undergo alternative splicing at the RNA level to produce a "f"-RNA and a "s"-RNA messenger (fig. 6).

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