CHARACTERIZATION OF THE PHOSPHOTRANSFERASE ACTIVITY OF RED CELL ACID PHOSPHATASE (ACP_1).

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Red cell acid phosphatase (ACP_1) is well established as a useful genetic marker in population studies, in paternity testing, and in the forensic analysis of bloodstains. One interesting biochemical property of this marker is its striking activity increase in the presence of certain alcohols (1-3). This property has been exploited to enhance the sensitivity of ACP_1 detection in electrophoretic typing systems (4). We describe here important features of this enhancement phenomenon.

MECHANISTIC CONSIDERATIONS. There is good evidence that the reaction mechanism of ACP_1 involves two steps. Step 1 is the reaction of enzyme with substrate and results in the formation of a phospho-enzyme intermediate with the concomitant release of the donor group from the substrate.

(1) $E + ROP \longrightarrow E\text{-}P + ROH$

The second step involves the transfer of the phosphate from the intermediate to water yielding inorganic phosphate (2a) or to an acceptor alcohol yielding a new phosphate ester (2b).

(2a) $E\text{-}P + H_2O \longrightarrow E + Pi$

(2b) $E\text{-}P + ROH \longrightarrow E + R'OP$

The overall reaction rate is determined by the slower step in this two step sequence. In the presence of certain alcohols, the apparent reaction rate (as measured by the release of the phosphate donor) is greatly increased; this is illustrated with glycerol as the acceptor in table I. These results indicate that the second step of the reaction sequence is the rate limiting step. The rate increase is suppressed by phosphate; this is important to note since many ACP_1 electrophoresis buffers contain phosphate.

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TABLE I

INCREASE IN p-NITROPHENYL PHOSPHATASE ACTIVITY IN THE PRESENCE OF GLYCEROL

Glycerol % (v/v)	0	5	10	20	30	40	50
Relative Activity	1.0	2.5	3.8	5.2	5.6	5.2	4.2

Activity was determined spectrophotometrically in an assay mixture containing hemolysate, 2 mM p-nitrophenyl phosphate, and 100 mM acetate buffer pH 5.5.

Chemical analysis of the reaction products shows that phosphate production from step 2a is unchanged in the presence of alcohols. Thus the increase in the overall reaction rate is due entirely to the transferase reaction 2b. This also indicates that although 2b is faster than 2a, the two pathways are not competitive.

ACCEPTOR SPECIFICITY. Table II shows the effect of several alcohols on ACP_1 activity. Polyols such as glycerol are better acceptors than the corresponding simple alcohols; of the polyols, ribitol is clearly the best acceptor. There is a requirement for linear chain conformation; despite their polyol structure, sugars in ring conformation are not good acceptors. Comparison of the several 5 carbon polyols gives evidence of configuration and stereo specificity.

TABLE II

ACCEPTOR ACTIVITIES OF ALCOHOLS, POLYOLS, AND SUGARS

Compound (0.5 M)	Relative Activity
Methanol	1.42
Ethylene Glycol	1.74
1-Propanol	1.43
2-Propanol	1.05
Glycerol	2.40
Erythritol	2.80
D-Arabitol	2.38
L-Arabitol	1.32
Ribitol	4.11
Mannitol	2.83
D-Ribose	1.52
D-Arabinose	1.55

ISOZYME DIFFERENCES. Each ACP_1 allele product is represented electrophoretically by two isozyme bands. Analysis of the chromatographically separated isozymes from a B homozygote indicated that the anodal isozyme had more than twice the phosphotransferase activity of the cathodal isozyme. Comparison of staining patterns from replicate gels stained in the absence and presence of glycerol indicates that glycerol enhances the intensity of the anodal band relative to the cathodal band with all the phenotypes. This effect is illustrated for the B and CB types in figure 1. It is obvious that this effect must be recognized if typing problems are to be avoided.

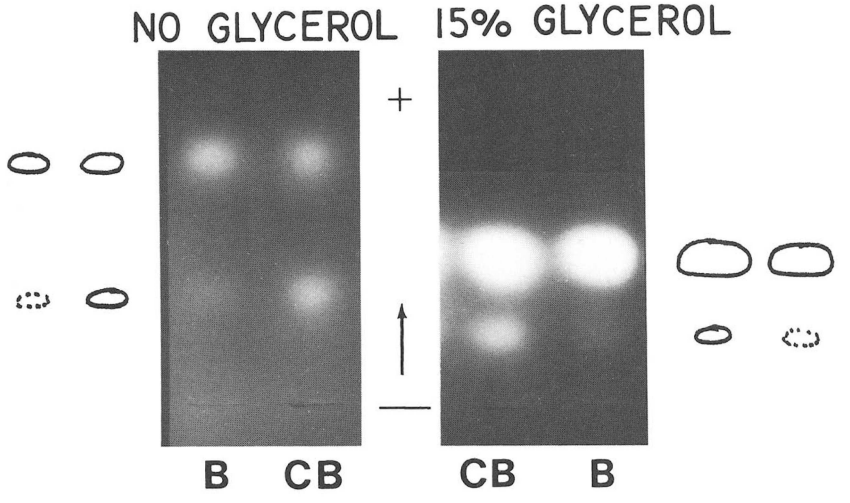

Figure 1. Electrophoretic patterns of ACP_1 in the presence and absence of glycerol. Electrophoresis was done on starch gels using the phosphate-citrate buffer system of Swallow, et. al. (5); run time was 17 hrs at 11 ma/gel. The gel shown on the right contained 15% glycerol; in this solvent, mobility is retarded. The stain on both gels was 2 mM methylumbelliferone phosphate and both were photographed after 10 minutes of staining.

PHENOTYPE DIFFERENCES. Assay of dialysed hemolysates of different types in the presence of 2 M (ca. 15%) glycerol showed a distinct phenotype dependence in phosphotransferase activity. The distributions of activities are summarized in table III. The phenotypes show an ordering B > BA > A > CB > CA > C; the differences between the means

are statistically significant ($p < 0.001$). The lower value for the C type may be accounted for in part by the preponderance of the cathodal, low transferase activity, isozyme in this type.

TABLE III

PHENOTYPIC VARIATION IN PHOSPHOTRANSFERASE ACTIVITY IN 2 M GLYCEROL

Phenotype (n)		Relative Activity Mean	S.D.
B	(23)	4.74	0.25
BA	(22)	4.49	0.46
A	(25)	4.38	0.22
CB	(8)	3.94	0.33
CA	(10)	3.74	0.34
C	(1)	3.43	

CONCLUSIONS. The addition of glycerol or other acceptor polyols to gel staining solutions or to gel media can enhance significantly the sensitivity of the staining reaction. The effect is greatest in gel buffers not containing phosphate. The differential effect on isozyme enhancement must be taken into account when typing but the phenotype dependent differences in transferase activity levels should not affect typing.

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