RED CELL ACID PHOSPHATASE: ONLY TWO DIFFERENT ENZYMES - THE "SLOW" AND THE "FAST" ENZYME - DETERMINE DIFFERENT BIOCHEMICAL PROPERTIES OF THE SIX COMMON PHENOTYPES.

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Red cell acid phosphatase is well known as a valuable genetic marker. Another feature, however is its intriguing biochemical properties. Spencer, Hopkinson and Harris (1) found over 20 years ago that each of the three common alleles (P^a, P^b, P^c) give rise to two electrophoretically different enzyme forms: a "slow" (s) form and a "fast" (f) form. The staining intensities of these forms as well as the overall enzyme activity in the red cell depend on the phenotype, the order with respect to increasing activity being A, B and C (fig. 1). Mansfield and Sensabaugh (2) showed subsequently that the enzyme is either activated or inhibited by some purines, that this modulation also depends on the phenotype, but that the order is not A, B, C but B, A, C or C, A, B. Examples for adenine and hypoxanthine are given in fig. 1. Little is known, however, about the molecular basis of these properties and the following question can be raised: Are the phenotype dependent differences in activity and modulation due to 1) catalytic differences between the a, b and c allozymes or 2) differences in the contents of the s and f forms in the various phenotypes?

To answer this question the quantity of enzyme protein in red cells of the different phenotypes were determined by crossed immuno electrophoresis using specific antibodies (3). It was found that the ratio between the s and f forms was dependent on the phenotype as shown for the three homozygous types (table 1). The A-type had three times more f protein than s protein, the B-type 5 times more f protein than s protein, whereas the C-type had 4 times more s protein than f protein. Hence the distribution between s and f protein resembles the well known distribution between s and f activity. The total quantity of enzyme protein varied also; hemolysates of type A contained the least enzyme and those of type C contained the most. Again the protein quantities correspond well with the activity levels observed. Therefore the enzyme activity and electrophoretic patterns seem to be related to the quantities present of the s and f forms.

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The activity of the enzyme forms in the presence of various concentrations of p-nitrophenyl phosphate was determined at pH 6.0, 30°C and the Km values were calculated using a cumputor program (4). Similar Km values were found for all the three s forms (table 2). The three f forms also showed identical Km values, but they were significantly lower than those of the s forms. Also with respect to specific activity the s forms were similar and the f forms were similar, whereas there was a significant difference between the s and f forms. Hence the three s forms seem to be enzymatically similar - in spite of their genetic difference, and the three f forms are similar, whereas the s forms are different from the f forms.

The modulation of the enzyme forms by adenine and hypoxanthine was investigated as sketched for the c_s and c_f forms (fig.2). It will be seen that adenine activated the s form and inhibited the f form. The data fitted a hyperbolic function and the maximum activation or maximum inhibition was calculated using the cumputer program. All the three s forms were activated by adenine to about the same degree (aproximately 4 times the unmodulated activity) and the three f forms were all inhibited by a factor of about 0.6. In contrast with adenine, hypoxanthine showed a 5 fold activation of all the three f forms, whereas the s forms were not affected. Concequently, also with respect to modulation by purines the three s forms seem to be enzymatically similar as well as the three f forms seem to be similar, whereas the s and f forms are different.

From these findings on the separated s and f forms one can predict the modulation characteristics of hemolysates of the various phenotypes as shown for the three homozygous types (table 3). The unmodulated activities of the hemolysates are set to 100 and one gets to the modulated activity from the unmodulated activities of the s and the f forms using the respective modulation factors. For example estimation of the effect of adenine on hemolysate of type B gives: Unmodulated s-activity: 22, modulated s-activity: 22x4 = 88; unmodulated f-activity: 78, modulated f-activity: 78x0.6 = 47; modulated activity of B-hemolysate: s+f= 88+47= 135; hence the result is a slight net activation. The A-type is activated more because of its greater content of s form and the C-type is consequently activated to the highest degree. The reverse situation is found with hypoxanthine, which activates the f

forms. It will be seen that the predicted order of increasing modulation for hemolysates of the three types is either B, A, C or C, A, B, which corresponds to the experimental results on hemolysates (2). Hence differences in modulation of the various phenotypes seem to be due to the different proportions of the s and f forms in these phenotypes.

Preliminary results on immunogenic and stability properties also show a similarity between the s forms and between the f forms, and a difference between the s and f forms.

In conclusion it appears that from a biochemical point of view only two different enzyme species exist: a "s-enzyme" and a "f-enzyme". The different proportion of these within the various phenotypes determines the biochemical differences between the phenotypes, whereas genetically determined structural differences between the a, b and c enzymes may determine how much of the enzyme that will occur as s-enzyme and how much as f-enzyme.

References:

1) Spencer, N., Hopkinson, D.A., Harris, H: quantitative differences and gene dosage in the human red cell acid phosphatase polymorphism. Nature 201 299-300 (1964).

2) Mansfield, E., Sensabaugh, G.F.: Red cell acid phosphatase: modulation of activity by purines, in The Red Cell, ed. Brewer, G.J., New York, Alan R. Liss, 1978, pp 233-247.

3) Dissing, J: human red cell acid phosphatase: differences in concentration of isozyme-protein as cause of phenotypic differences in enzyme activity. Proc. 9th Int.Congr.Soc. Forensic Haemogenet, Bern, sept. 29th Oct. 3, 1981, pp 279-283.

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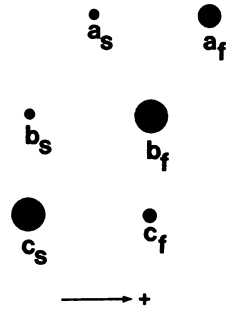
Electrophoretic pattern	Phenotype	Activity (rel) in red cells	Modulation: Adenine	Modulation: Hypoxanthine
	A	50	1.4	2.0
	B	75	1.2	2.1
	C	100	2.3	1.2

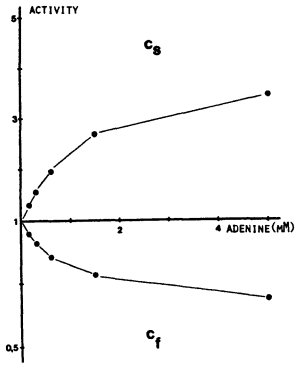
Fig.1. Electrophoretic patterns (in citrate/phosphate buffer, pH 5.9), relative activity levels (1) and modulation characteristics (2) of ACP_1.

Phenotype	Enzyme protein S:F	Enzyme protein (relative quantity)	Activity (relative)
A	1:3	40	45
B	1:5	70	77
C	4:1	100	100

Table 1. Quantitation of ACP_1 allozymes in hemolysates by crossed immuno-electrophoresis (3). Activity levels were determined at pH 6.0, 30°C using p-nitrophenyl phosphate (10 mM) as substrate.

Phenotype	Km (mM) S forms	Km (mM) F forms	Specific activity S forms	Specific activity F forms
A	0.51	0.13	75	53
B	0.48	0.14	70	46
C	0.48	0.14	72	55

Table 2. Comparison of enzymatic properties of purified ACP_1 allozyme forms. Substrate: p-nitrophenyl phosphate at pH 6.0, 30°C. Specific activity: /umoles/(min.x mg).



PHENO-TYPE	MAX. ACTIVATION/INHIBITION ADENINE S FORMS	ADENINE F FORMS	HYPOXANTHINE S FORMS	HYPOXANTHINE F FORMS
A	3,8	0,61	1,0	5,0
B	4,2	0,60	1,0	4,8
C	4,1	0,62	1,0	5,0
MEAN	4,0	0,61	1,0	4,9

Fig. 2. Activation/inhibition of purified ACP_1 allozyme forms by purines. Substrate: p-nitrophenyl phosphate (10 mM) at pH 6.0, 30°C.

PHENO-TYPE	FORM	CONTENT OF S AND F(%)	NORMAL ACTIVITY(%) CONTRIBUTION (SP. ACT.,S:72,F:51)	S+F	MODULATION BY PURINES ADENINE MODUL. FACTOR	ADENINE ACTIVITY(%)	ADENINE S+F	HYPOXANTHINE MODUL. FACTOR	HYPOXANTHINE ACTIVITY(%)	HYPOXANTHINE S+F
B	S	17	22	100	4.0	88	135	1.0	22	404
	F	83	78		0.6	47		4.9	382	
A	S	27	34	100	4.0	136	176	1.0	34	357
	F	73	66		0.6	40		4.9	323	
C	S	80	85	100	4.0	340	349	1.0	85	159
	F	20	15		0.6	9		4.9	74	

Table 3. Prediction of modulation characteristics of hemolysates in relation to the ACP_1 phenotype. Column denoted "Content of s and f (%)" gives the proportion between s and f protein. Column denoted "Normal activity (%)/contribution" gives the proportion between s and f activity as corrected for differences in specific activity.