Enzyme Activity in Two Red Cell Adenylate Kinase Phenotypes

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Hereditary variation of enzymatic activities has interesting biochemical, physiological, pharmacological, and evolutionary implications. Several examples are now known of electrophoretically distinguishable genetic polymorphisms associated with minor-to-moderate variations of red cell enzymatic activities. Among these are acid phosphatase (Spencer et al. 1964), uridine diphosphate (UDP)-galactose-transferase (reviewed by Beutler and Mathai 1968), glucose-6-phosphate dehydrogenase (Long, quoted in Motulsky 1968), and 6-phosphogluconate dehydrogenase (Parr 1966; Carter et al. 1968; Gordon et al. 1969). This quantitative variability may be ascribed to a variability of the structural genes specifying these enzymes.

Red cell adenylate kinase (AK) is an enzyme which presents an electrophoretically detectable genetic polymorphism (Fildes and Harris 1966). Preliminary quantitative studies of this enzyme have shown no marked differences between the AK 1 and the AK 2-1 phenotypes (Harris et al. 1968). Recently, Szeinberg et al. (1969) have described a case of AK deficiency. The present paper reports the results of studies of the distribution of the enzymatic activities in two different AK electrophoretic phenotypes.

MATERIALS AND METHODS

The red cell electrophoretic AK phenotypes have been determined by the technique described by Fildes and Harris (1966).

Solutions

The following solutions were used: (1) triethanolamine-HCl buffer (5×10^{-2} M, *p*H 7.55); $MgCl_2$ (5×10^{-3} M); (2) substrate solution (ADP) (10^{-2} M in solution 1); (3) cysteine (as an activator, used within $\frac{1}{2}$ hr from moment of preparation) (10^{-2} M in solution 1); (4) solution of TCA (15%); (5) tris-HCl buffer (10^{-1} M, *p*H 9.00); (6) glucose (4×10^{-2} M, in solution 5); (7) $MgCl_2$ (3×10^{-2} M, in solution 5); AMP (as an inhibitor of AK activity usually present in commercial lots of HK) (5×10^{-4} M, in solution 5); (8) NADP (10^{-3} M in solution 7); (9) G6PD (Boehringer; 1 mg per ml: 1 mg $\simeq$ 140 U); HK (reasonably free of AK activity) (Fluka: $\simeq$1400 U per ml) (10 μl and 1 μl per ml, respectively, both in solution 8); (10) standard solutions of ATP in triethanolamine buffer (solution 1).

Received July 23, 1969; revised September 29, 1969.

This work was supported by a grant of the Consiglio Nazionale delle Ricerche (CNR).

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Method

Fresh hemolysates prepared by freezing and thawing packed washed red cells are diluted approximately 35 times with solution 1 and, after 30–60 min, further diluted 101 times with solution 3. Soon after, they are mixed with 1 volume of substrate solution (time 0). The ATP production is terminated when two different aliquots taken from the incubation mixture at 5 min* and 25 min are transferred into 0.5 volumes of TCA solution. The TCA is then removed by ether extraction until neutrality is attained. Finally, the ATP content of the resulting solutions is determined by the following method: tubes are filled with 0.5 ml of solution 1 (blanks), ATP standard solution, or the solutions to be tested. Then 0.5 ml glucose solution (solution 6) is added. All solutions and a freshly prepared solution 8 are incubated in a 37° C waterbath for about 20 min. Then 1.0 ml of solution 9 (dosing solution) is added to all tubes (final volume, 2 ml), and incubation is continued for 12 min. The E_{3400A} of each resulting solution is measured using a Beckman DU spectrophotometer.

Determinations were always performed within 6 hr after bleeding, and ATP assays were carried out in duplicate.

Hemoglobin concentrations of the hemolysates diluted 35 times were determined by mixing them with 10 volumes of Drabkin solution (Van Kampen and Zijlstra 1961). The $\bar{E}_{5400A}$† of the resulting solutions were determined ($\bar{E}_{Hb}$).

The red cell AK activity (expressed as mmoles of ATP produced per g Hb per hr) is calculated by this formula:

$$\text{AK activity} = \frac{\bar{E}_{25\,\text{min}} - \bar{E}_{5\,\text{min}}}{\bar{E}_{Hb}} \times 35.206\,,$$

where $\bar{E}_{25\,\text{min}}$ and $\bar{E}_{5\,\text{min}}$ are the $\bar{E}_{3400A}$ obtained with the aliquots of the reaction mixture in which the ATP production was arrested after 25 min and 5 min of incubation, respectively.

The molar ratio between ATP and the corresponding NADPH was found always to be unity; it was not affected by the presence of the TCA-treated solution of ADP and hemolysate.

The method is accurate and reproducible. It involves only one time-consuming step, the removal of TCA. Attempts using a TCA-substitute to arrest the ATP production were unsuccessful. In particular, heating could not be used because of a tremendous increase of the blank E_{3400A}.

Determinations were carried out on uncentrifuged hemolysates. The present data are in rather good agreement with those of Levin and Beutler (1967) who used stromata-free hemolysates at 30° C without adding cysteine.

RESULTS

The AK activity was measured in 119 hemolysates of known AK phenotype using a slightly more laborious method than described, but one with identical results.‡

* For the first 2–3 min, the ATP production is nonlinear.

† Bar indicates mean of two or more replicated determinations.

‡ When performing AK determinations, it was not realized why the E_{3400A} of the solution used for the ATP determination (solution 9) increased linearly with time. Our solution 9 contained glucose in addition to G6PD, HK, and NADP and this resulted in an increase of the E_{3400A} owing to the

Results obtained with AK 1 and with AK 2-1 phenotypes are shown separately in figure 1. Levin and Beutler (1967) report a mean AK activity of about 23 mmoles of ADP produced per g Hb per hour, which practically agrees with the present data. Levin and Beutler's method (measuring the ADP produced by the reaction ATP + AMP $\xrightarrow{AK}$ 2ADP) yields double the product of the AK-catalyzed reaction (2ADP $\xrightarrow{AK}$ AMP + ATP) which this study used.

The mean AK activity of the AK 1 phenotype is significantly higher than that found in the AK 2-1 phenotype ($t = 3.89$; $P < .001$).

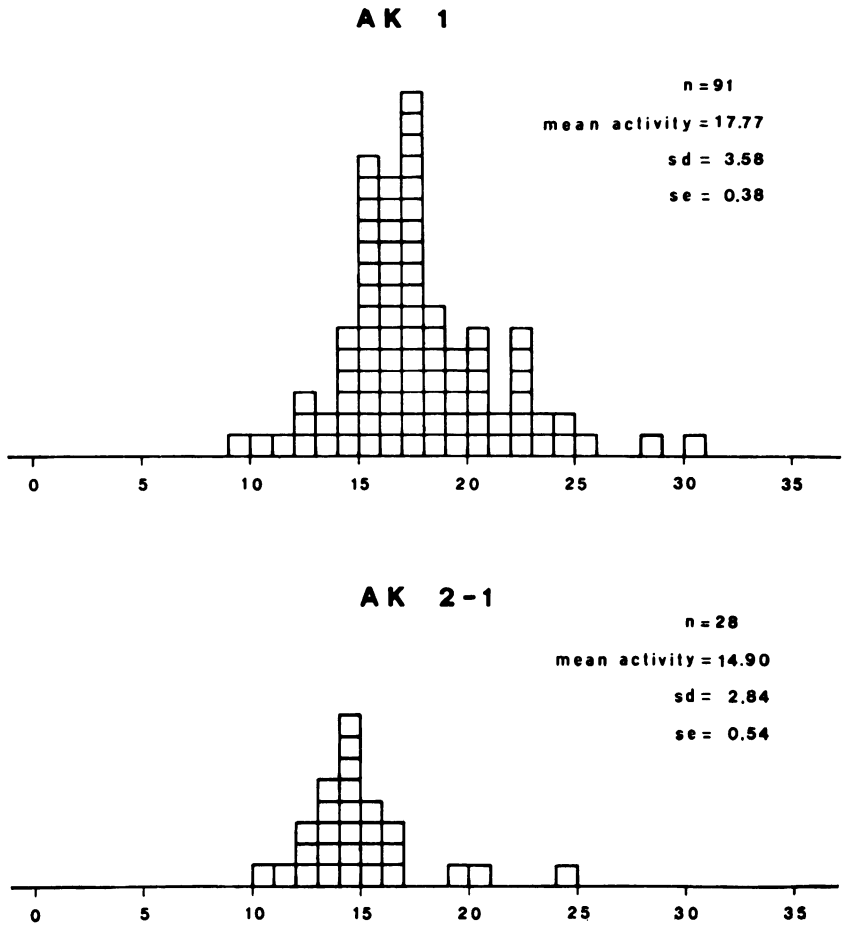

FIG. 1.—Distribution of the red cell AK activity, expressed as millimoles of ATP produced per g Hb per hour at 37° C, in AK 1 and AK 2-1 phenotypes. Each square represents one individual. *sd* = standard deviation; *se* = standard error.

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glucose dehydrogenase activity of the G6PD (Anderson and Nordlie 1968; Anderson et al. 1968). Compensation was made for the increase.

DISCUSSION

Figure 1 shows a rather large variation among individuals with the same electrophoretic AK phenotype (SD approximately 20% of the mean). Still, the difference between the mean red cell AK activities of the two AK phenotypes which have been examined appears to be beyond doubt. The mean activity of one AK^1 gene is estimated to be 8.89, calculated from the mean activity found in the AK 1 phenotype ($[\overline{1\text{-}1}]/2$).

Data on the AK 2 homozygote are unfortunately lacking, but it appears reasonable to infer, by analogy with the acid phosphatase system studied by Spencer et al. (1964), that the mean activity associated with one AK^2 gene is equal to 6.01

TABLE 1

ENZYMATIC ACTIVITIES ASSOCIATED WITH ELECTROPHORETICALLY DIFFERENT ALLELES OF FIVE RED CELL ENZYME POLYMORPHISMS

Polymorphisms	Alleles Common in at Least One Population Whose Corresponding Activities Have Been Compared Because They Were Known to Be Electrophoretically Different	No. of Alleles Found to Be Associated with Minor-to-Moderate Variations of Enzymatic Activities	Approximate Measurable Relative Enzyme Activity Associated with Different Alleles	References
Acid phosphatase (AP)	P^A, P^B, P^C	3	100, 150, 200	Spencer et al. 1964
6-phosphogluconate dehydrogenase (6PGD)	Pgd^B, Pgd^A	2	100, 115	Parr 1966 Carter et al. 1968 Gordon et al. 1969
Glucose-6-phosphate dehydrogenase (G6PD)	Gd^A, Gd^B	2	100, 125	Long, quoted in Motulsky 1968
Phosphoglucomutase (PGM_1)	PGM_1^1, PGM_1^2	None	100, 100	Modiano et al. 1970 Terrenato et al. 1970
Adenylate kinase (AK)	AK^2, AK^1	2	100, 150	This paper

(calculated by $[\overline{2\text{-}1}] - [\overline{1\text{-}1}]/2$). The predicted mean activity of the AK^2 homozygote is therefore 12.02. Difference between the activities of the two AK alleles is rather large, the AK^1 activity being fifty percent higher than AK^2.

With the present data, it is not possible to speculate on the nature of the difference between the mean red cell AK activities observed in AK 1 and AK 2-1 phenotypes. Any factor or combination of factors, such as differences in conformation, stability, or rate of synthesis of the AK^1 and AK^2 enzymes, could be responsible.

The nature of such differences is unknown, except for the Gd^A and Gd^B alleles of the Gd locus, which differ for a single amino acid substitution (Yoshida 1967). This paper does show that, whatever the mechanism, a difference does exist between the enzymatic activities associated with the AK^1 and AK^2 alleles.

Table 1 shows that such differences have been found in four of the five genes

with common alleles known to be electrophoretically different. It is generally true, therefore, that electrophoretically distinguishable enzymes do show different enzyme activities. This is not surprising, considering the many different factors in different genotypes which might affect the measurable enzyme activities.

Very little is known about the relative contributions of the environmental and the genetic component (structural, modifier, and regulator genes, etc.) to the total variability of activity of every enzyme. Variability of the structural gene(s), which is not electrophoretically demonstrable, is not likely to be identified at the present time. However, during the last few years, a number of enzymes have been found with electrophoretic alleles associated with minor-to-moderate variations of enzymatic activities.

The quantitative variation due to an enzyme's electrophoretic alleles depends not only upon the magnitude of the quantitative differences existing between these alleles, which appears quite large (see table 1), but also upon their frequencies. For example, although the differences between the enzymatic activities associated with different electrophoretic alleles are similar for red cell acid phosphatase and for the red cell adenylate kinase, the contribution to the total variation of the enzyme activity is fairly large in the first case (accounting for more than half of the variance found in the general population; see Harris 1966, fig. 66), and negligible for the AK because of the low frequency of its less common allele.

It is of interest to estimate the proportion of the enzymes whose variability is at least partly due to electrophoretically demonstrable variations of structural genes. A crude estimate of the genes involved is 0.25, the product of the proportion of the enzymes randomly selected for examination and found to have electrophoretic alleles (0.3), and of the frequency among these genes, of those whose electrophoretic alleles have been found to be quantitatively different (0.8). The first figure has been given by Harris (1966, 1969); the second comes from table 1.

For many enzymes, then, some of the variability in enzymatic activitity could be explained by the existence of genetic polymorphisms involving their structural genes. Virtually nothing is known of the evolutionary mechanisms which led to the presently existing pattern of enzymatic polymorphisms. It appears unlikely, however, that this general phenomenon (see Lewontin 1967, for a review concerning the *Drosophila*) could be explained in terms of genetic drift and migration. The continual observation of biochemical differences between enzymes determined by common alleles (those less likely to be selectively neutral) gives support to the hypothesis that some of them have a selective value.

SUMMARY

A quantitative assay of the red cell AK activity is described. The red cell AK activities found in a group of 91 AK 1 and 28 AK 2-1 individuals have been compared. The average activities of these two groups were 17.77 and 14.90 mmoles of ATP produced at 37° C per hour per g Hb, respectively. Therefore, the activities associated with a single AK^1 or AK^2 gene are estimated to be 8.89 and 6.01.

ACKNOWLEDGMENTS

The authors are deeply indebted to Dr. A. Yoshida for his extremely helpful suggestions concerning method. The stimulating discussions and criticism by Dr. A. G. Motulsky have been very much appreciated.

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