Erythrocyte Acid Phosphatase, Esterase and Lactate Dehydrogenase Isozyme ; Comparison with Serum Isozymes

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We previously reported a method by which serum enzyme isozymes (isoenzymes) could be easily separated by agar gel electrophoresis and observed on the zymogram [1-3]. Among the enzyme found in erythrocytes, this method was applied to the observation of acid phosphatase (Ac. P.) esterase and lactic dehydrogenase (LDH). The pourpose of this paper is as follows.

1) Differentiation between serum Ac.P. prostatic origin and that of non-prostatic origin, especially of erythrocytic origin has been done using inhibitors, but what is the result on the zymogram ?

2) Cholinesterase in serum pseudo-cholinesterase and that in erythrocytes is said to bo true-cholinesterase, but can such a definite separation be made on the zymogram ?

3) In measuring serum LDH, it is said that it is of special importance to prevent hemolysis but does LDH isozyme in erythrocytes resemble serum LDH isozyme pattern ?

These points have been studied.

MATERIAL AND METHOD

The material was erythrocyte hemolysate[5] taken from 20 apparently normal adults (male 10, femal 10). Ac.P. and esterase zymograms were prepared by the method reported previously for serum[2][3] while separation of LDH isozyme was done by agar gel electrophoresis[5] (150 volt, 40 mA, for 40 minutes). The agar plates were immersed in the following staining mixture[6] (37°C) for one hour and after reaction were taken out and washed several times in running water. Their optic densities were measured with a densitometer (filter 540 mμ) and the electrophoretic pattern obtained.

LDH staining mixture : 1 M sodium lactate 1.5 ml ; DPN 4.5 mg ; phenazine-methosulfate (Sigma) 4 mg ; nitroblue tetrazolium (Sigma) 5 mg ; 0.1 M sodium cyanide 1.5 ml and 0.1 M phosphate buffer (pH 7.6) 2.5 ml. Add distilled water until the total volume becomes 15 ml and the pH becomes about 7.2 to 7.3.

RESULT AND DISCUSSION

THE areas having Ac.P., esterase and LDH activities always appear on agar as pink or purplish red bands. When the activity is low, pink stripes appear and when it is high, purplish red bands. The hemoglobin portion appears to be reddish brown to yellowish brown and can be definitely descriminated from the area where enzyme exists. Erythrocytic Ac.P. isozyme appears as only 1 band between the α_1 and α_2 fractions of the serum globulin, nearer to the α_2 fraction (provisionally called isozyme $\alpha_1\alpha_2$). On the other hand, serum Ac.P. shows 3 kinds of isozymes, namely, $\alpha_1\alpha_2$, β and γ. Half of the total serum Ac.P. activity is concentrated in isozyme $\alpha_1\alpha_2$ where the activity is the highest[3]. Erythrocytic Ac.P. ($\alpha_1\alpha_2$) is not affected by formaldehyde (10^{-4} M and 10^{-2} M) but is completely suppressed by L-tartrate, NaF, ethanol. Serum Ac.P. isozyme $\alpha_1\alpha_2$ is relatively sensitive not only to L-tartarate, NaF and ethanol but also to formaldehyde and only a rather small amount of activity remains. Therefore, even if serum Ac.P. partially originates from erythrocytic Ac.P., its proportion in normal serum apparently is not very high. Erythrocytic esterase isozyme (α-naphthylacetate) is noted in 3 bands corresponding to α_1 fraction (α_1), α_2 fraction (α_2) and γ fraction (γ). Its content is the largest in α_1 (55%) followed by γ (30%) and α_2 (15%). These percentages are almost constant. When hemolysed blood is preserved for a long time, the activity of α_1 rapidly deteriorates so that it cannot be detected in old material. Andersen[7] extracted erythrocytic protein with n-butanol and after agar gel electrophoresis, esterase staining was conducted and 3 bands (β-naphthylactate) were noted corresponding to protein fractions, α_1, α_2, and α_2 β. However, no mention had been made of the relative quantitative ratio of each isozyme. Three to 6 serum esterase isozymes were noted which showed relatively large variability in activity according to the sera.[3] Isozyme prealbumin (PA_1, PA_2) noted in serum cannot be detected in erythrocytes while the activity of α_1 is the largest in erythrocytes. Thus, there are relatively large differences in the pattern between these two. As serum esterase isozyme α_1 is inhibited by 10^{-3} mol eserine sulphate solution, it was thought that it perhaps may be cholinesterase. From the fact that erythrocytic esterase isozyme is likewise inactivated by eserine, it seems that this α_1 stripe also can be regarded as cholinesterase as in the case of serum. Erythrocytic cholinesterase is considered to be true cholinesterase which specifically decomposes acethylcholine and has similar electrophoretic mobility to that of serum cholinesterase. Erythrocytic and serum cholinesterase hydrolyze the common substrate α-naphthylacetate and the activity of both is inhibited with eserine. From these points, these two are considered to have characteristics which closely resemble each other. This problem must be further studied.

Erythrocytic LDH isozyme is noted in sites corresponding to albumin (isozyme

Alb), α_1-globulin, α_2-globulin and β-globulin (isozyme α_1, α_2 and β, respectively) of serum protein fractions in the following proportions. Alb (35%), α, (37%), α_2 (22%) and β (8%). These percentages remain almost unchanged. Serum LDH isozyme was usually noted to be 5, namely isozyme Alb, α_1, α_2, β and γ. Serum isozyme Alb, α_1, α_2 and β were at the same sites as those of erythrocytic isozyme but no serum isozyme γ was detected within erythrocytes. Excluding this point, erythrocytic and serum LDH isozymes show similar pattern. For instance, when hemolyzed blood is added to serum and this solution is electrophoresed, activity bands of isozyme Alb, α_1, α_2 and β become darker but the ratio relatively remain unchanged. LDH isozyme patterns of the stomach, small intestine, pancreas, spleen and kidney by this method resemble the serum isozyme patterns.

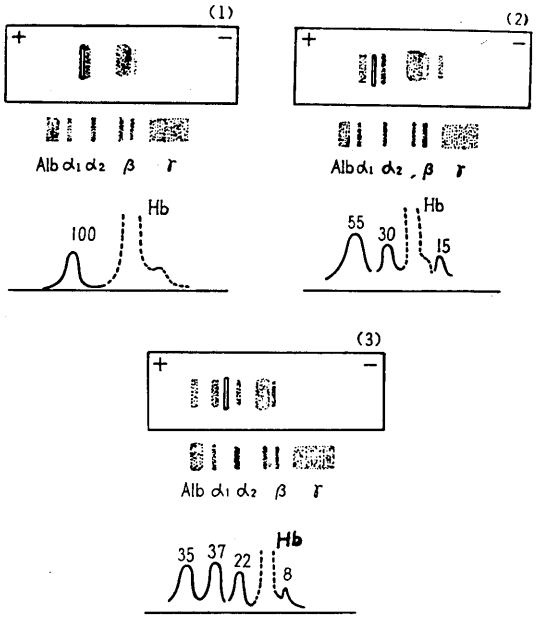

Fig. 1. Erythrocyte isozyme patterns.
(1) Acid phosphatase (2) Esterase (3) Lactate dehydrogenase.
The upper row is isozyme patterns. The middle row is serum protein fractions by amidoblack 10 B stain and the lowest is scanning curves by densitometry; attached numbers indicate of percentage of activity.

Resseler and Joseph[8] separated erythrocytic LDH by agar gel electrophoresis into 3 isozymes and said that the ratio of relative activity were 51.4%, 40.6% and 8.0%. Vesell[9] reported similar results. Kvamme[10] separated erythrocytic LDH by DEAE-column chromatography into 4 fractions. The number of LDH isozymes thus separated differs and the reason for this difference is considered to be in histochemical technique[11] used in demonstrating LDH isozyme and the method for preparation of erythrocytic hemolyzed blood.

CONCLUSION AND SUMMARY

The method of serum isozyme determination using agar-gel electrophoresis and azo dye method was applied to the determination of acid phosphatase (Ac.P.), esterase and lactate dehydrogenase (LDH) of erythrocytes and the isozyme pattern of the erythrocyt was compared with the serum. An Ac.P. isozyme was present in erythrocyte which demonstrated migration corresponding to serum Ac.P. isozyme (between α_1-globulin and α_2-globulin) and there was no inhibitory effect of formaldehyde. Three esterase isozymes were present which corresponded to serum protein α_1-globulin, α_2-globulin and β-globulin and their percentages of activity are 55%, 15% and 30% respectively. Isozyme α_1 is inhibited by eserine sulfate.

Four LDH isozymes are noted in the position corresponding to serum albumin, α_1, α_2 and β-globulin. Percentage of activity for each is almost constant being 35%, 37%, 22% and 8%. This pattern is resemble with that of serum LDH isozyme.

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REFERENCES

1) Takahashi, H., Wajima, T. and Mizushima, A.: Alakline phosphatase isozymes of normal human blood serum as examined by a combined procedure of agar gel electrophoresis and diazo-coupling staining. *Med. & Biol.*, **66** (5): 224-229,1963.
2) Takahashi, H., Wajima, T. and Mizushima, A.: Acid phosphatase isozymes of normal human blood serum as examined by a combined procedure of agar gel electrophoresis and diazo-coupling staining. *Med. & Biol.*, **66** (6): 306-311, 1963.
3) Wajima, T., Mizushima, A. and Takahashi, H.: A simple method for the demonstration of esterase zymogram of human serum by means of agar gel electrophoresis and azo coupling procedure. *Med. & Biol.*, **67** (1): 39-43, 1963.
4) Hill, B.R.: Some properties of serum lactic dehydrogenase. Cancer Reserch 16: 460-467, 1965.
5) Shibata, S. and Iuchi, I.: Abnormal hemoglobin. *Saishin Igaku.*, **15**: 2567-2581, 1960.
6) Yakulis, V.J., Coultas, W., Gibson, B.S. and Heller, P.: Agar-gel electrophoresis for the determination of isozymes of lactic and malic dehydrogenase. *Am. J. Clin. Path.*, **38**: 278-382, 1962.
7) Andersen, V.: Extraction of erythrocyte proteins with n-butanol. *Clin. Chim. Acta.*, **8**: 454-456, 1963.
8) Resseler, N. and Joseph, R.: Simple method for electrophoretic analysis of serum lactic dehydrogenase. *J. Lab. Clin. Med.*, **60**: 349-353, 1962.
9) Vesell, E.S.: Significance of the heterogeneity of lactic dehydrogenase activity in human tissues. *Ann. New York Acad. Sci.*, **94**: 877-889, 1963.
10) Kvamme, E., Knudsen, K. and Sund, L.: Chromatpgraphic fractionation of lactic dehydrogenase from human tissues. Scand. *J. Clin. Lab. Invest.*, **14**: 453-460, 1960.
11) Pearse, A.G.E.: *Histochemistry*. 2nd Ed. Churehill, London, 1960.