The Heterogeneity of Acid Phosphatases in Different Organs of the Rat [1)]

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(With 1 Text-figure)

The heterogeneity of acid phosphatases was investigated by means of the electrophoretical method in various organs of animals, mostly, in liver, spleen and prostate (Barka, 1961; Allen *et al.*, 1964a, b; Allison *et al.*, 1966a, b; Smith *et al.*, 1967). Recently, nine electrophoretical forms of acid phosphatases were noted in rat liver by Yamamoto (1968), with the demonstration of the sensitivity of acid phosphatase isozymes to some chemicals.

It was shown by Adul-Fadl and King (1949), and Goodlad and Mills (1957) that the reactivity of acid phosphatases to some chemicals was wholly dissimilar among different organs, such as liver, prostate, erythrocyte and adrenal cortex as revealed by biochemical method.

The present study deals with the heterogeneity of acid phosphatases in different tissues of the rat, based on acrylamide gel electrophoresis, with an additional study of effects of certain chemicals on electrophoretically separated forms of acid phosphatases.

Materials and Methods: Male and female rats of Wistar King A were used in the present experiments. Animals were starved for 12 to 24 hours and sacrificed by cervical dislocation. The liver, spleen, kidney and prostate were homogenized with 0.5% Triton X-100 and centrifuged for 30 min at 15,000×g. Plasma and red blood cells centrifuged as mentioned above. The supernatants thus obtained were used for electrophoresis.

Disc electrophoresis in acrylamide gels followed the method reported by Yamamoto (1968): the small pore gel contained 1.6% N, N'-methylenebisacrylamide, 1.5 ml; 0.25% tetramethylene diamine (TEMED) in 0.3 M Tris-borate buffer (pH 7.5), 3 ml (0.3 M boric acid-Tris buffer containing 0.245 M boric acid and 0.0546 M Tris (hydroxymethylaminomethane)); 0.03% potassium ferricyanide, 1.5 ml; 0.56% ammonium persulfate, 1.5ml; 10% acrylamide 4.5 ml. The large pore gel contained 0.25% TEMED in Tris-HCl buffer (pH 6.9), 0.6 ml; 4.1% acrylamide 1.6 ml; 0.4% riboflavine, 0.6 ml; 40% sucrose, 2.0 ml.

Gels were cast in plastic columns (3×12×100 mm). Layered above 2.0 ml of small pore gel were 0.1 ml of large pore gel and 0.4 ml of sample gel. The sample gel consisted of a mixture of 0.03-0.05 ml of tissue samples. Electrophoresis was carried out for about

1) Contribution No. 849 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan 060

2 hours at room temperature with an initial current of 10 mA. Initial potential was about 50 volts with final potential at about 200 volts. After electrophoresis in Tris-glycine buffer, pH 8.3, gels were rinsed in distilled water and incubated bout 2 hours at room temperature in the medium containing 10 mg each of sodium alpha-naphthyl-phosphate and Diazo Red RC in 10 ml of 0.05 M acetate buffer, pH 5.0.

By treating the gel with the incubating medium containing one of the following chemicals, $CoCl_2$, $MnCl_2$, $CuSO_4$, 1–100 mM; NaF, Na-tartrate, 1 mM; and 0.5% formalin, the effects of those chemicals on acid phosphatases were examined.

Results and Discussion

The results are summarized diagramatically in Figure 1. It was shown that band IX of the liver was activated by Co and Mn at a concentration of 0.1 M. Similarly, Co and Mn activated band V of the spleen, band IV of the kidney, and

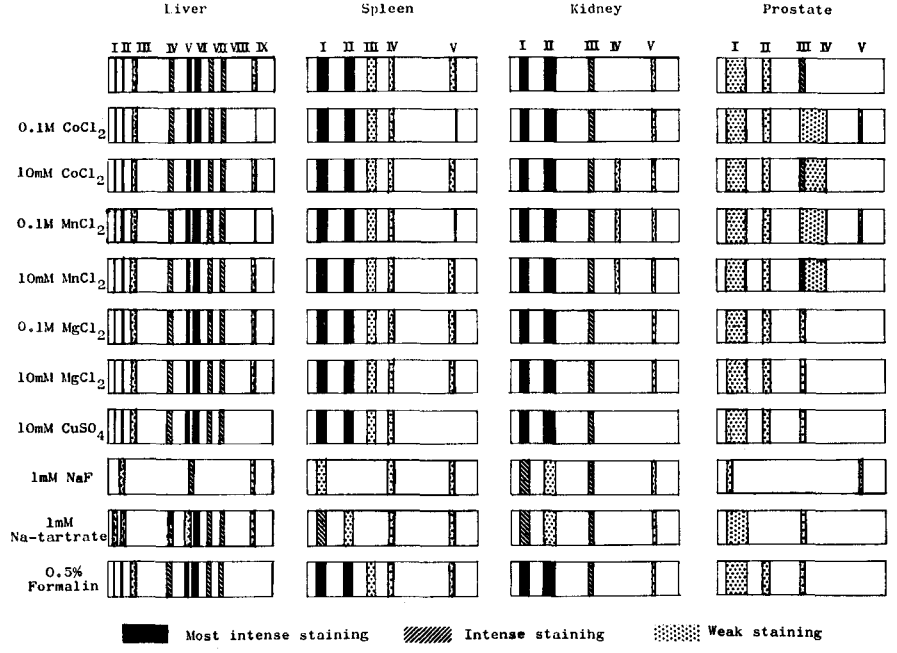

Fig. 1. Diagramatic representation of electrophoretically separated bands of rat acid phosphatases and effects of various chemicals in different organs.

band IV and V of the prostate. On the other hand, $MgCl_2$ was found to show no effect on any band of the liver, spleen, kidney and prostate. Co and Mn had no effect on plasma and red blood cells. Walker *et al.*, (1954) reported that Mn ions exerted a distinct activating effect on the acid phosphatases when incorporated

in azo-dye substrate solution, while Mg ions showed no such effect with the exception of phosphomonoesterases IV. Barka (1961) showed that Co, Zn, Mn, Mg and Ni markedly inhibited the acid phosphatases of rat liver at concentrations of 1 to 10 mM. On the other hand, Sable *et al.*, (1965) demonstrated that the acid phosphatases were inactivated by Ba, Mn and Cu which were prevented by excess substrate, whereas Ni, and Co showed some protective effect.

Cu ions exerted a very slightly inhibitory effect on the bands other than band IX of the liver, and band V of the spleen, and band V of the kidney, and band IV and V of the prostate, and band IV of the plasma. According to Adul-Fadl and King (1949), red blood cells acid phosphatases were notably inhibited by even low concentrations of Cu, whereas the prostatic enzyme was slightly activated. Goodlad and Mills (1957) reported 85% inhibition of liver acid phosphatases by 0.2 mM Cu, in striking contrast to the present finding. Such discrepant results obtained by the present study and by others may probably be attributable to the difference of the methods employed. Diazo Red RC used in the present study in order to visualize the enzyme location has been known to form a chelate compound with Cu ions; so that the addition of Cu to the incubation medium would have nullified the effect of Cu ions on acid phosphatases.

NaF was strongly inhibitory to the enzymes of liver, spleen, and prostate, but it was much less or almost non-inhibitory to the enzymes of kidney, plasma and red blood cells. At the same time, tartrate strongly inhibited the enzymes of liver and spleen, especially those on bands I and II, and had no or little effect on the enzymes of plasma, red blood cells and kidney. Adul-Fadl and King (1949) noted that fluoride and tartrate inhibited human prostate enzymes almost completely, whereas Pearce (1952) found only 40% inhibition of rat enzymes by fluoride. A similar situation was obtained by Goodlad and Mills (1957) on the liver enzymes; 53% by fluoride and 41% by tartrate.

Formalin was almost non-effective on the enzymes of liver, spleen, kidney, plasma and red blood cells, while it showed an inhibitory effect on band V in prostate. Goodlad and Mills (1957) and Adul-Fadl and King (1949) noted 68% inhibition of liver enzymes by 0.5% formalin, but no inhibition of the prostate enzymes.

Summary

The heterogeneity of acid phosphatases was investigated in liver, spleen, kidney, prostate, red blood cells and plasma of rats by means of acrylamide gel electrophoresis. Among several bands separated, the fast migrating bands, such as band IX of the liver, band V of the spleen, bands IV and V of the prostate and bands IV and V of the kidney, were activated by Co or Mn, but not by Mg. $CuSO_4$ and 0.5% formalin exerted no or little effect on the enzymes, while NaF and Na-tartrate were strongly inhibitory to acid phosphatases derived from all the organs here examined.

Acknowledgements: The author is much obliged to Professor Sajiro Makino for his keen interest in this subject. Expert guidance by Dr. Samuel H. Hori and helpful advice by Dr. Kohtaro Yamamoto are cordially acknowledged here.

References

Allen, J.M. and J. Gockerman 1964a. Electrophoretic separation of multiple forms of rat liver on polyacrylamide gels. J. Histochem. Cytochem. **9**: 542–547.

———— 1964b. Electrophoretic separation of multiple forms of particle associated acid phosphatase. Ann. N. Y. Acad. Sci. **121**: 616–633.

Allison, A.C. and L.-G. Lundin 1966a. Acid phosphatases from different organs and animal forms compared by starch-gel electrophoresis. Biochim. Biophys. Acta **127**: 527–529.

Barka, T. 1961. Studies of acid phosphatase. I. Electrophoretic separation of acid phosphatases of rat liver on polyacrylamide gels. J. Histochem. Cytochem. **9**: 542–547.

Goodlad, G.A.J. and G.T. Mills 1957. The acid phosphatases of rat liver. Biochem. J. **66**: 346-354.

Lundin, L.-G. and A.C. Allison 1966b. Acid phosphatases from different organs and animal forms compared by starch-gel electrophoresis. Acta Chem. Scand. **20**: 2579–2592.

Pearce, A.G.E. 1960. Histochemistry. (Little, Brown and Company-Boston) pp. 431-433.

Sable, K.G., J. Glomset, and J. Porath 1961. Studies on the reductive activation of spleen phosphoprotein phosphatase. Biochim. Biophys. Acta **50**: 135–140.

Smith, J.K. and L.G. Whitby 1968. The heterogeneity of prostatic acid phosphatase. Biochim. Biophys. Acta **151**: 607–618.

Walker, B.S., H.M. Lemon, M.M. Davidson and M.K. Schwartz 1954. Acid phosphatase review. Ameri, J. Clin. Path. **24**: 807–837.

Yamamoto, K. 1968. A study of acid phosphatase heterogeneity in the rat liver by means of a modified disc electrophoretic method (a preliminary note). Jour. Fac. Sci. Hokkaido Univ. Ser. IV, Zool. **16**: 548–552.