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A Study of Acid Phosphatase Heterogeneity in the Rat Liver by means of a Modified Disc Electrophoretic Method (A Preliminary Note)[1)2)]

By

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(*With 1 Text-figure and 1 Table*)

Following the identification of lysosomes in hepatic cells of the rat by de Duve (1955), much evidence has been accumulated with basic information of their biological significance. It has been shown that many types of hydrolytic enzymes occur in lysosomes, and that the integrity condition of the lysosomal membrane affects release of the enzyme from these particles. Lysosomal function seems to be involved in many vital processes including certain diseases (Brachet, 1960; Carranza and Cabarini, 1962; Scheib, 1963; Tappel, 1963; Weber, 1963; Weissmann, 1965, 1967). To study the property of lysosomal enzymes is, therefore, very significant for elucidation of the lysosomal function. Acid phosphatases represent a group of hydrolases occurring in the lysosomes of a variety of animal cell types (Weissmann, 1965). Earlier investigators have reported that more than one enzyme with acid phosphatase activity are present in various tissues from different species (Goodlad, 1957; MacDonald, 1962; Neil, 1961, 1962, 1964). The heterogeneity of these enzymes have been investigated by means of electrophoretic or chromatographic techniques. The literature refers to earlier reports on the separation of acid phosphatases in some materials (Barka, 1961a, 1961b; Moore and Angeletti, 1961; Allen *et al.*, 1963b; Allen and Gockerman, 1964; Lundin and Allison, 1966). The present report deals with a modified disc electrophoretic technique for the separation of acid phosphatases, with some accounts on the property of the separated acid phosphatases.

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Materials and Methods

Animals used were male rats of Wistar-King A strain weighing 150 to 200 gm. Immediately after sacrifice by decapitation, the liver was perfused with 0.25 M sucrose, and homogenized in 0.5% Triton X-100 in 0.25 M sucrose at 0°C. The homogenate was centrifuged for 20 min at 15,000 ×g and the supernatant was used for electrophoresis.

In the present experiment, the method of Allen and Gockerman (1964) was modified considerably as follows:

Small pore gel:*1

A.	0.25% TEMED in 0.3M Tris-borate buffer, pH 7.5*2	3.0 ml
B.	Acrylamide 20 gm, BIS 0.4 gm, H_2O to 100 ml	6.0 ml
C.	0.03% $K_3Fe(CN)_6$ in H_2O	1.5 ml
D.	0.56% Ammonium persulfate in H_2O	1.5 ml

Large pore gel:*3

E.	0.25% TEMED in Tris-HCl (Tris 5.7 gm added HCl to pH 6.9, total volume 100 ml with H_2O)	0.6 ml
F.	Acrylamide 10 gm, BIS 2.5 gm, H_2O to 100 ml	1.6 ml
G.	Riboflavin (4 mg/100 ml H_2O)	0.6 ml
H.	40% Sucrose	2.0 ml

The gels were cast into plastic columns measuring 3×12×100 mm. Layered above 2 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 the mixture of 0.02 to 0.05 ml of tissue sample and 0.4 ml large pore gel. The spacer and sample gels were polymerized by ultraviolet light emitted from a fluorescent lamp tube. The polymerization time was about 40, 15 and 30 min at 20°C in small, large and sample gels, respectively. Electrophoresis in Tris-glycine buffer, pH 8.3 (Ornstein and Davis, 1962) was carried out for about 2 hours at room temperature with a constant current of 10 mA. Initial potential was about 50 volts.

After electrophoresis, the gel was rinsed in water, and stained in an incubating medium consisting of Na-α-naphthyl acid phosphate 10 mg, Naphthanyl Diazo Red Rc 10 mg and 0.05 M acetate buffer, pH 5.0, 10 ml.

Effects of some chemicals on electrophoretically separated forms of the acid phosphatase were investigated by incubating the gels in the medium containing PCMB, 0.001M; formaldehyde, 0.12 M; $MnCl_2$, 0.001M; Na-tartrate, 0.001M; NaF, 0.001M; $MgCl_2$, 0.001M: and KCN, 0.001M.

Results

After electrophoresis, homogenates of rat livers exhibited at least nine defined bands with acid phosphatase activity. They were designated as A-I in the order of increasing anodal mobility (Fig. 1). The results of this experiment are summarized in Table 1. Bands A, B, C, G and H showed no activity when the tissues were homogenized with 0.25 M sucrose. The homogenization with 0.5% Triton X-100 in 0.25M sucrose showed that bands A and B were very strong in activity, while bands C, G and H showed a weak activity. On this basis, those bands are regarded as bound type enzymes. The other bands seem to be soluble enzymes.

*1 This is a modification of procedure of Allen and Gockerman (1964).

*2 See Allen *et al.* (1963b). *3 This is the same procedure as that of Ornstein and Davis (1962), except the replacement of Tris-phosphate buffer to Tris-HCl buffer.

$MnCl_2$ (0.001M) enhanced the activity of band B, C, D, E, F and I enzymes but not other bands. PCMB inhibited the bound enzymes, while formaldehyde inhibited completely band I enzyme. Na-tartrate and NaF gave a considerable influence on the electrophoretically separated forms of acid phosphatase. Both of them exerted similar inhibitory effects on almost all isoenzymes, except band D which was resistant at a concentration of 0.001M.

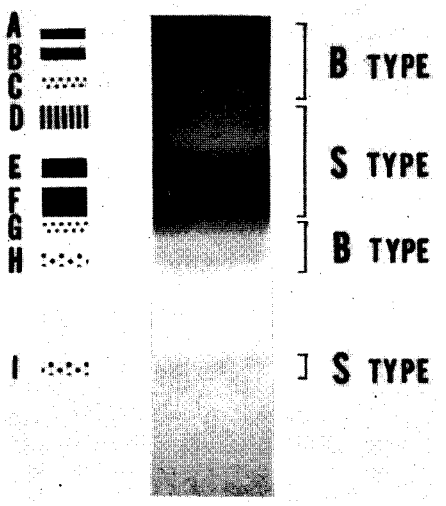

Fig. 1. Electrophoretic pattern of acid phosphatase from a normal rat liver. B type and S type mean bound type and soluble type of acid phosphatase, respectively.

Table 1. Effects of chemicals on electrophoretically separated forms of acid phosphatase from rat livers

Chemicals	Concentration	Band A	B	C	D	E	F	G	H	I
None		++	+++	+	+	++	++++	+	+	+
PCMB	0.001 M	+	++	+	+	++	++++	+	±	±
Formaldehyde	0.12 M	++	+++	+	+	++	++++	±	±	−
$MnCl_2$	0.001 M	++	+++	++	++	+++	++++	+	+	++
Na-tartrate	0.001 M	+	+	−	+	−	++	+	−	±
NaF	0.001 M	±	±	−	+	−	+	−	−	±

The following chemicals had no marked effect: $MgCl_2$, KCN.

Discussion

Many electrophoretic techniques have been tested for the separation of acid phosphatases of rat livers. However, most techniques gave unsatisfactory

results in our hands. In the present experiment, therefore, the procedure of Allen and Gockerman (1964) was modified considerably. The electrophoretic technique finally adopted in this study gave satisfactory results to a considerable extent. It may be allowed to conclude that this technique is beneficial for further investigations on the property of acid phosphatase and the lysosomal function.

Electrophoretic patterns of acid phosphatase obtained in this experiment seem to correspond fundamentally to the results of Moore and Angeletti (1961). They separated the acid phosphatase of rat livers chromatographically into 3 major and 1 minor peaks of activity. Three major peaks, which were designated as peak I, II, and III, may correspond to bands A and B, E, and F, respectively obtained in the present experiment. The minor peak may correspond to bands C and D, which were the bound type enzymes. In present study, three minor satellite bands were present ahead of the main acid phosphatase bands.

Allen and Gockerman (1964) reported that two major groups of acid phosphatase were present in rat liver cells. In accordance with their findings, the present study also suggested the occurrence of 2 types of acid phosphatases in rat livers: bound type enzymes released from lysosomes by treatment with Triton X-100 and soluble type enzymes. The bound type enzymes and soluble enzymes obtained in the present experiment seem to correspond to those described by Allen and Gockerman (1964). Although they demonstrated only one band for each bound and soluble enzyme, the present experiment has shown that five of nine bands are of a bound type, while the other bands of a soluble type. It is uncertain which bands obtained in this study correspond to which bands reported by them.

Summary

The acid phosphatases of rat livers were separated into nine defined bands by a modified electrophoretic technique. There were two groups of molecular form in the acid phosphatase, the bound type and soluble type. Five molecular forms were of a soluble type. Studies on effects of some chemicals on acid phosphatase showed that the molecular forms of soluble type were activated by $MnCl_2$, but those of bound type showed no specificity to chemicals.

References

Allen, S.L., M.S. Misch, and B.M. Morrison 1963b. Variations in the electrophoretically separated acid phosphatases of Tetrahymena. J. Histochem. Cytochem. **11**: 706-719.

Allen, J.M., and J. Gockerman 1964. Electrophoretic separation of multiple forms of particle associated acid phosphatase. Ann. N.Y. Acad. Sci. **121**: 616–633.

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

——— 1961b. Studies of acid phosphatase. II. Chromatographic separation of acid phosphatases of rat liver. J. Histochem. Cytochem. **9**: 564–571.

Brachet, J. 1960. The biochemistry of development, Pergamon Press, New York.

Carranza, F.A. Jr., and R.L. Cabarini 1962. Histochemical distribution of acid phosphatase in healing wounds. Science **135**: 672.

de Duve, C., B.C. Pressman, R. Gianetto, R. Wattiaux, and F. Appelmans 1955. Tissue fractionation studies 6. Intracellular distribution patterns of enzymes in rat-liver tissue. Biochem. J. **60**: 604–617.

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

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

MacDonald, K. 1962. Some comparisons of the hydrolysis of phosphomonoesters by homogenates of rat liver and of rat spleen. Biochim. Biophys. Acta **58**: 356–358.

Moore, B.W., and P.U. Angeletti 1961. Chromatographic heterogeneity of some enzymes in normal tissues and tumors. Ann. N.Y. Acad. Sci. **94**: 659–667.

Neil, M.W. 1961. Phosphomonoesterase activity of mouse liver preparations at pH 5.9. Biochem. J. **81**: 41P.

———, and M.W. Horne 1962. The acid-p-nitrophenyl phosphatase activity of adult guinea-pig liver subcellular fractions. Biochem. J. **84**: 32P.

———, and ——— 1964. Studies on acid hydrolases in adult and foetal tissues. 2. Acid phenyl phosphomonoesterases of adult mouse liver. Biochem. J. **93**: 220–224.

Ornstein, L., and B. Davis 1962. Disk electrophoresis. Parts I and II. Distillation Products Industries, Rochester, N.Y.

Scheib, D. 1963. Properties and role of acid hydrolases of the Müllerian ducts during sexual differentiation of the chick embryo, in "Lysosomes". (ed. by A.V.S. de Reuck and M.P. Cameron), J. and A. Churchill, London.

Tappel, A.L. 1963. Distribution in animals and hydrolytic capacity and other properties of isolated lysosomes, in "Lysosomes" (ed. by A.V.S. de Reuck and M.P. Cameron), J. and A. Churchill, London.

Weissmann, G. 1965. Lysosomes. New Eng. J. Med. **273**: 1084–1090.

——— 1965. Lysosomes (Concluded). New Engl. J. Med. **273**: 1143–1149.

——— 1967. The role of lysosomes in inflammation and disease. Ann. Rev. Med. **18**: 97–112.

Weber, R. 1963. Behaviour and properties of acid hydrolases in regressing tail tissue of tadpoles during spontaneous and induced metamorphosis in vitro, in "Lysosomes" (ed. by A.V.S. de Reuck and M.P. Cameron), J. & A. Churchill, London.