PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 726–728 (1974)

Properties of Rat Liver Alkaline Phosphatase Before and After Bile Duct Ligation (37883)

ADRIANA BIANCHI-BOSISIO RIGHETTI AND MARSHALL M. KAPLAN
(Introduced by M. S. Raben)

Gastroenterology Service, Department of Medicine, New England Medical Center Hospital, Boston, Massachusetts 02111

We have previously reported that bile duct ligation causes a 5–10-fold increase in the activity of rat-liver alkaline phosphatase and that this increase can be prevented by the inhibition of either protein or RNA synthesis (1, 2). These results suggested that the rise in enzyme activity was due to enzyme induction. In similar studies, Griffin and Cox demonstrated that prednisolone increased the activity of HeLa cell alkaline phosphatase and that puromycin prevented this increase (3). Although these data also suggested enzyme induction as the mechanism, further studies showed that the increase was due to activation of a preformed enzyme (4). The more-active form of the enzyme differed in several respects from the base-level enzyme. The more-active or "induced" enzyme was more labile at 64.5° and eluted earlier from a Sephadex G-200 column (4).

This report compares the properties of rat-liver alkaline phosphatase before and after "induction" by bile-duct ligation. The data indicate that there is no difference in any of the properties examined and suggest that the increased activity is not due to activation of preformed enzyme.

Methods. Enzyme preparation and assays. Male Charles River CD strain rats weighing 100 g were used in all experiments. They were fed standard chow and tap water and fasted overnight prior to sacrifice. Bile-duct ligations were performed under light ether anesthesia as previously described (1). Animals were sacrificed 24 hr after bile-duct ligation. After sacrifice, livers were excised, weighed, minced with scissors, and washed for 30 min at 2° in three changes of 0.25 *M* sucrose. The tissue was then homogenized in a Waring Blender for 1 min at 2° in 5 vol of 0.25 *M* sucrose. The homogenate was extracted with *n*-butanol and dialyzed extensively as previously described (1).

Alkaline phosphatase activity was determined with *p*-nitrophenyl phosphate at 30° and pH 10.2 in a Gilford thermoregulated recording spectrophotometer (5). The method was modified slightly for sucrose-gradient experiments. After ultracentrifugation, 0.11-ml fractions of the sucrose gradient were collected into 12-ml conical centrifuge tubes; 0.9 ml of a solution containing 600 μmoles 2-amino-2-methyl-1-

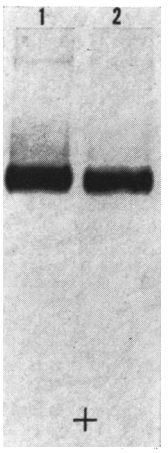

FIG. 1. Electrophoresis of base-level and "induced" rat-liver alkaline phosphatase. Slot 1 contains base level enzyme and slot 2 "induced" enzyme.

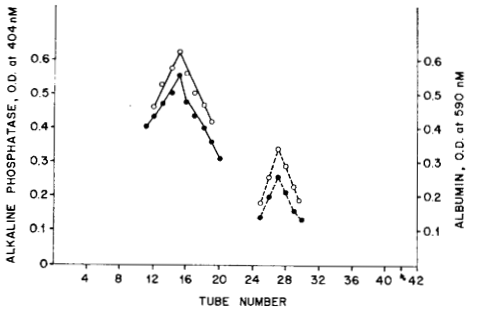

FIG. 2. Sucrose gradient sedimentation of base-level and "induced" rat-liver alkaline phosphatase. Bovine serum albumin, tagged with bromphenol blue, served as an internal marker. Sedimentation was at 65,000 rpm at 2° for 7 hr in a Beckman Spinco Model L2-65B ultracentrifuge. (○) Bile-duct-ligated liver; (●) base-level livers, (—) alkaline phosphatase activity, (- - -) bovine serum albumin.

propanol (pH 10.2), 4 μmoles *p*-nitrophenyl phosphate, and 0.2 μmoles $MgCl_2$ were then added to each tube with a Cornwall syringe pipet, and each tube was vigorously stirred. The tubes were then placed in a 30° water bath and observed at 5-min intervals until a yellow color appeared, usually between 45 and 60 min. The contents were then transferred into 1-ml cuvets and optical density read at 404 nm in a Gilford spectrophotometer.

Determination of isoenzyme properties. Heat-inactivation studies, urea-denaturation studies, and determination of pH optima, Michaelis constants, and sedimentation coefficients were done as previously described (5). Electrophoreses were done on 3-mm-thick 7.5% polyacrylamide gel slabs in a tap-water-cooled vertical gel electrophoresis cell and alkaline phosphatase bands developed as described by Kaplan and Rogers (6).

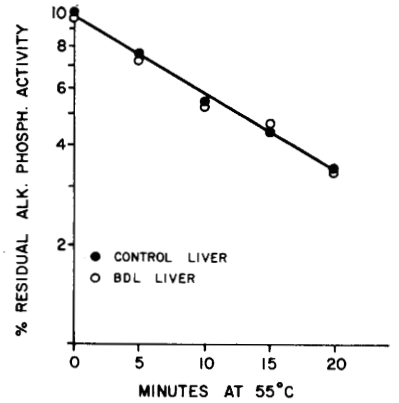

FIG. 3. Heat inactivation of alkaline phosphatase at 55°.

Results. The "induced" alkaline phosphatase, that from rats whose bile ducts had been ligated for 24 hr, migrated identically on polyacrylamide gel electrophoresis with hepatic alkaline phosphatase from nonoperated control rats (Fig. 1). Both enzymes had identical sedimentation coefficients (7.9) as determined by sucrose gradient ultracentrifugation (Fig. 2). There

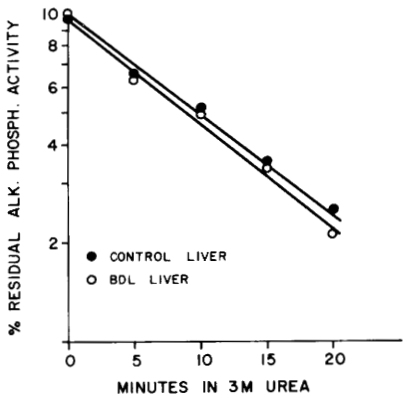

FIG. 4. Denaturation of alkaline phosphatase in 3 *M* urea.

was only one alkaline-phosphatase peak when both enzymes were mixed, and an aliquot of the mixture was subjected to sucrose gradient sedimentation. The "induced" and base-level hepatic alkaline phosphatases were inactivated at identical rates at 55° (Fig. 3) and denatured at identical rates in 3 *M* urea (Fig. 4). Both had the same pH optimum, pH 10.2 (Fig. 5), and similar Michaelis constants (Fig. 6). That of the induced enzyme was 1 m*M* and that of the base-line enzyme 1.2 m*M*.

Discussion. The base-level rat-liver alkaline phosphatase was identical to the "induced" enzyme with respect to all six properties studied—electrophoretic mobility, sedimentation coefficient in a sucrose gradient, Michaelis constants, pH optima, and rates of heat and urea denaturation. These

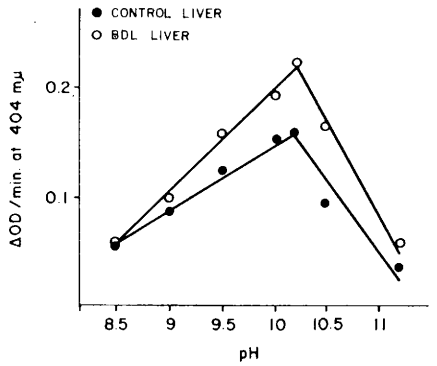

FIG. 5. pH optimum of alkaline phosphatase. The substrate was *p*-nitrophenyl phosphate, and the buffer was 2-amino-2-methyl-1-propanol, 0.6 *M*.

results indicate that base-level and induced alkaline phosphatase are the same enzyme and suggest that the increased enzyme activity found in rat liver after bile-duct ligation is due to enzyme induction rather than to activation of a preformed enzyme. The data of Griffin and Cox indicated that the activation of HeLa cell alkaline phosphatase was due to a conformational change in alkaline phosphatase which was, in turn, reflected in altered physical properties. The methods employed in this study examined properties that were dependent on diverse factors such as molecular weight, electrical charge, charge density ratio, enzyme stability, and affinity of enzyme for substrate. Since the base-level and induced rat-liver alkaline phosphatase were identical by all criteria, it seems unlikely that any conformational change occurred. Our results suggest that enzyme induction accounts for the increases in rat-liver alkaline phosphatase activity after bile-duct ligation.

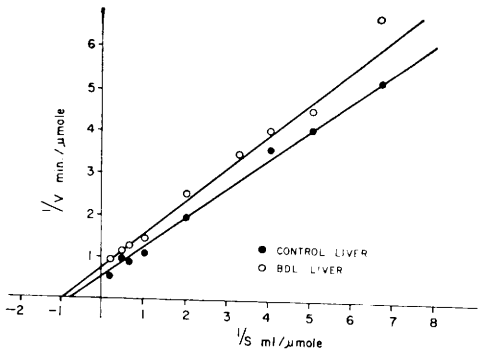

FIG. 6. Michaelis constants for alkaline phosphatase. Assays were run at pH 10.2 in 2-amino-2-methyl-1-propanol, 0.6 *M*.

Summary. Bile-duct ligation causes a 5–10-fold increase in rat-liver alkaline phosphatase activity. The alkaline phosphatase in bile-duct-ligated rat liver is identical to electrophoretic mobility on polyacrylamide gel, rate of sedimentation in a sucrose gradient, rates of heat and urea denaturation, pH optima, and Michaelis constants. Since the "induced" and base-level enzyme are identical by all criteria studied, it is unlikely that the increase in alkaline phosphatase activity is due to activation of a preformed enzyme. Enzyme induction appears a more likely mechanism.

This work was supported by Research Training Grant AM-5424 and Research Grant AM-10571 from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, and by a grant from the John A. Hartford Foundation, Inc. Dr. Kaplan is the recipient of Research Career Development Award AM-31760 from the National Institutes of Health.

1. Kaplan, M. M., and Righetti, A. B.-B., J. Clin. Invest. **49**, 508 (1970).

2. Righetti, A. B.-B., and Kaplan, M. M., Proc. Soc. Exp. Biol. Med. **136**, 491 (1971).

3. Griffin, M. J., and Cox, R. P., J. Cell Biol. **29**, 1 (1966).

4. Griffin, M. J., and Cox, R. P., Proc. Nat. Acad. Sci. USA **56**, 946 (1966).

5. Righetti, A. B.-B., and Kaplan, M. M., Biochim. Biophys. Acta **230**, 504 (1971).

6. Kaplan, M. M., and Rogers, L., Lancet **2**, 1029 (1969).

Received Oct. 9, 1973. P.S.E.B.M., 1974, Vol. 145.