USE OF AMP (2-AMINO-2-METHYL-1-PROPANOL) BUFFER FOR HISTOCHEMICAL DEMONSTRATION OF ALKALINE PHOSPHATASE ACTIVITY

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AMP (2-amino-2-methyl-1-propanol) buffer was tried for the histochemical detection of alkaline phosphatase (ALPase) activity by the lead citrate method. The incubation medium tested was as follows: 170–350 mM AMP, 20 mM sodium β -glycerophosphate, 3.9 mM magnesium sulfate, 2.0 mM lead citrate and 8% sucrose; pH was adjusted to 9.2. By using this medium, ALPase activity in the liver was found in the sinusoidal and lateral surface of hepatocytes as well as in the bile canalicular surface, which was the only positive site by the original lead citrate method using Tris buffer. Moreover, the reaction product (lead phosphate) appeared finer than when using the Tris method. By quantitating ALPase activity biochemically, higher activity was found with AMP than with Tris. Apparently, this new incubation medium with AMP is suitable for detecting weak ALPase activity.

In the previous report (4), we tested glycine-KOH buffer to improve the lead citrate method for the histochemical detection of ALPase activity; the final reaction product was found finer as the concentration of glycine became lower. Obviously, changing buffers in the incubation medium could affect the size of the lead phosphate. In an attempt to further improve the lead citrate method, we tried another buffer, AMP (2-amino-2-methyl-1-propanol) buffer as detailed in the present paper. This buffer has been used when measuring ALPase activity biochemically in clinical tests (1). As stated below, by replacing Tris with AMP, we could obtain higher enzyme activity and moreover, a finer reaction product.

MATERIALS AND METHODS

Wistar rats, weighing 150–200 g, were perfusion fixed with 0.5% glutaraldehyde in 0.1 M sodium cacodylate buffer containing 8% sucrose, pH 7.4, at 4°C for 10 min. The liver and kidney were excised, rinsed and sectioned to 40 μ m in thickness by a Microslicer (Dosaka EM, Kyoto). The tissue slices were incubated in 6 different media as indicated in Table 1 for 40 min at 37°C. After rinsing, slices

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TABLE 1. *Histochemical incubation media tested in the present study*

Buffer	Concentration of β -glycerophosphate
350 mM AMP	20 mM
350 mM AMP	5 mM
175 mM AMP	20 mM
175 mM AMP	5 mM
28 mM Tris-HCl	20 mM
50 mM glycine-KOH	20 mM

All media contain 3.9 mM magnesium sulfate, 20 mM lead citrate and 8% sucrose. pH was adjusted to 9.2 in media with AMP, and to 9.0 in others.

were osmicated, dehydrated by an ethanol series and embedded in Spurr's resin. Ultrathin sections lightly counterstained with uranyl acetate was observed by a JEOL 100CX electron microscope at 100 KV.

For the biochemical quantitation of ALPase activity, homogenates of liver and kidney were incubated with the six media without lead citrate for 40 min at 37°C. After centrifugation, free phosphate in the supernatant was quantitated by the Fiske-Subbarow method (2).

RESULTS

By the original lead citrate method using Tris-HCl buffer (3), ALPase activity was found in the bile canaliculi of the liver (Fig. 1) and in the brush border of the renal proximal tubule. Usage of glycine-KOH buffer changed the size of the lead phosphate precipitate, but gave the reaction product at the same location (4). In contrast, AMP buffer brought about ALPase activity in additional sites in the liver; the lead phosphate deposit was found not only in the bile canaliculi, but in the sinusoid and along the lateral surface of hepatocytes (Fig. 2). Occasionally, the reaction product was also seen along the membrane of intracellular organelles including mitochondria and lysosomes (Fig. 3). In the kidney, the lead deposits were seen only in the brush border even with AMPase (data not shown). Either the two concentrations of AMP, 175 and 350 mM, or β -glycerophosphate, 5 and 20 mM, did not make any visible difference to the above results.

The biochemical quantitation of ALPase showed that both in the liver (Fig. 4a) and the kidney (Fig. 4b) higher ALPase activity was detected with AMP buffer than when using either Tris or glycine. In both organs, 350 mM of AMP gave higher activity than 175 mM, as did 20 mM of the substrate than 5 mM.

FIG. 1. ALPase activity detected in the rat liver by the original Mayahara method with Tris-HCl buffer. Reaction products are seen only in the bile canaliculus of hepatocytes. $\times 27,000$

FIG. 2. ALPase activity of the liver detected by the medium containing 175 mM AMP as a buffer. Reaction products are distributed in the sinusoidal (arrow) and the lateral surface of hepatocytes. $\times 27,000$

FIG. 3. ALPase activity of the liver detected by the medium with 350 mM AMP. The lead precipitate is seen not only along the plasma membrane but in the intracellular organelles including mitochondria (arrow) and lysosomes (arrowhead). $\times 27,000$

DISCUSSION

Although ALPase is principally an enzyme which hydrolyzes organic phosphoric monoester, it also has a transphosphorylation effect which transfers phosphate to its acceptor in the reaction mixture. It is known that amino-alcohols such as AMP and diethanolamine greatly enhances the latter transphosphorylation activity of ALPase (1). The use of AMP buffer in the histochemical incubation medium is thus a logical attempt. In fact, as shown in electron micrographs, ALPase activity was found all around the hepatocyte surface, in contrast to the original method, which gave the lead precipitate only in the bile canaliculi. It was shown previously that hepatocytes exhibit ALPase activity when fixed in PIPES-NaOH buffer (5) which preserves the enzyme activity better than cacodylate buffer. It is most likely that the residual ALPase activity in the sinusoidal and lateral hepatocyte surface was enhanced by AMP and made detectable histochemically.

The biochemical measurement showed that liberated phosphorus from tissue homogenates also increased with AMP buffer. As there is no acceptor for phosphate in the reaction mixture (lead citrate is omitted), the hydrolase activity of ALPase is also increased with AMP. Apparently it also helped to increase the chance of detec-

FIG. 4a, b. Biochemical quantitation of ALPase activity. Liver (a) and kidney (b) homogenates were incubated with various media without lead citrate for 40 min at 37°C, and liberated phosphorus was quantitated by the Fiske-Subbarow method (2).

ting weak ALPase activity histochemically.

As shown in Fig. 3, intracellular organelles showed some lead precipitate with the incubation medium containing AMP. Probably phosphatase activity residing in the organelles is also enhanced by AMP and became detectable by the medium. It may be of interest to study the characteristics of the intracellular activity in comparison to that on the cell surface.

The present study presented a new histochemical incubation medium for ALPase; the medium has a better chance of detecting weak enzyme activity. By applying this new medium in various cells and tissues, we expect to see a newer version of ALPase localization in the future.

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