Electron-Histochemical Data on the Localization of Aminopeptidase M in the Liver

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The activity of aminopeptidase M in rat liver is studied electron histochemically. The enzyme is shown to be localized in the lysosomes of Kupffer's cells and endotheliocytes, and extracellularly on hepatocyte microvilli.

Key Words: *aminopeptidase M; liver; electron histochemistry*

Aminopeptidase M (APM, EC 3.4.11.2, corpuscle-bound membranous aminopeptidase 1) is a metalloenzyme relatively firmly bound to cell structures. APM cleaves amino acids from peptide chains from the side of the terminal amino group [1,2,4]. Despite some species and organ-specific differences in the pH optimum of the enzyme, it retains its activity within a wide range of pH values (6 to 9). Its localization in various organs and tissues has been little studied, and its physiological significance is still unknown [2]. Aminopeptidases associated with membranes are believed to play an important role in the degradation of physiologically active oligopeptides [5]. It is possible that aminopeptidases contribute to protein and peptide processing, performing various biological functions [3].

This research was aimed at detecting the localization of APM activity in rat liver at the ultrastructural level.

MATERIALS AND METHODS

The liver of male albino rats weighing 180 to 200 g was examined. The material was treated in accordance with the method described for light histochemistry [2], which we modified for electron histochemical detection of APM activity. Tissue fragments of 0.5 mm^3 were fixed in 1.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, for 3 h, and then incubated at 37°C for 45 min in an incubation medium of the following composition: 10 mg Leu-MBNA substrate (Sigma) dissolved in dimethylformamide (DMF, 100 μl/10 ml final volume), 10 ml 0.05 M citrate-phosphate buffer at pH 6.5, and hexanitrided pararosaniline (50 μl/ml). The material was then treated with 2% OsO_4 for 90 min. The control reaction was carried out in medium without substrate. Ultrathin slices were not contrast-stained.

RESULTS

The reaction product was represented as individual granules or as more or less homogeneous conglomerations of various electron density, depending on the degree of enzyme activity.

An intensive reaction to APM was observed in the lysosomes of Kupffer's cells (Fig. 1, *a*) and endotheliocytes (Fig. 1, *b*). No reaction product was detected in hepatocytes, but we observed a slight extracellular activity of APM. Granular reaction product was seen on the microvilli of some hepatocytes (Fig. 1, *c*).

Reaction product was not detected in control preparations (Fig. 1, *d*).

Hence, in the liver APM activity is localized in the lysosomes of sinusoidal cells (Kupffer's cells

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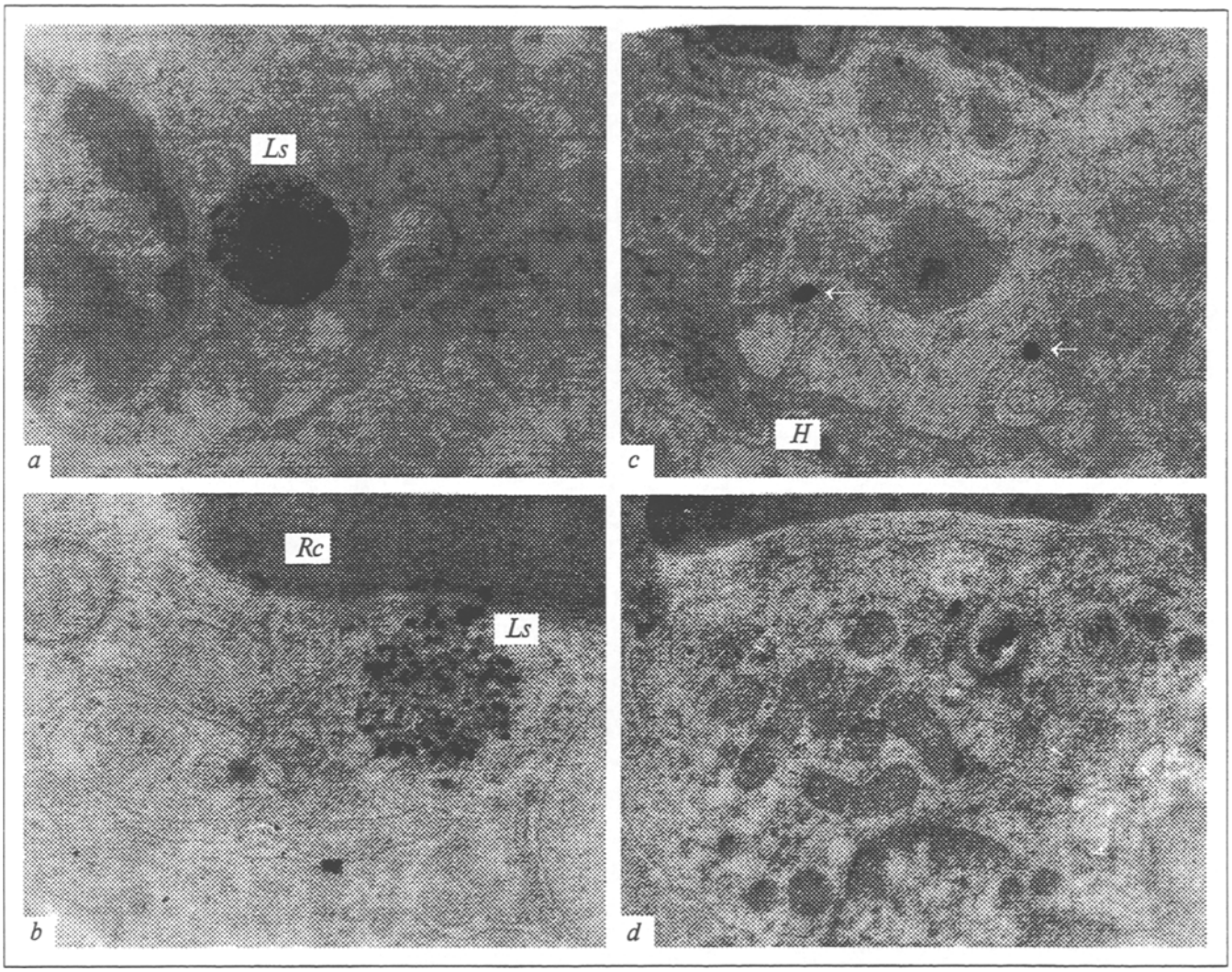

Fig. 1. APM localization in the liver. *a*) intensive reaction to APM in Kupffer's cell lysosome (*Ls*); *b*) reaction to APM in endotheliocyte lysosome (*Rc* — red cell); *c*) reaction product (shown with arrows) on hepatocyte (*H*) microvilli; *d*) control, no reaction product. *a*—*c*: ×30,000; *d*: ×20,000.

and endotheliocytes) and extracellularly on hepatocyte microvilli.

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