Effect of Pyridoxine-Deficiency on Degradation of Cytosolic Aspartate Aminotransferase in Rat Liver Lysosomes

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Summary Highly purified lysosomes were isolated from the livers of control and pyridoxine-deficient rats. The calculation of the lysosomal protein contents indicated that the livers of both groups of rats contain virtually the same amounts of the lysosomal proteins (12.0 and 13.0 mg lysosomal proteins/g liver proteins for the control and pyridoxine-deficient rats, respectively). The immunoblotting of the lysosomal proteins with anti-cytosolic aspartate aminotransferase (cAspAT) showed 46 kDa band, corresponding to the subunit molecular weight of cAspAT, as well as the bands representing degradative intermediates of cAspAT. The relative amounts of the immuno-reactive substances were estimated by scanning the immuno-stained bands and measuring the densitometric tracings. It was found that the lysosomes in the pyridoxine-deficient rat liver contain almost twice as much cAspAT and its degradative intermediates as those in the control rat liver. On the basis of these observations, it was concluded that the increased rate of degradation of cAspAT in the liver of the pyridoxine-deficient rats is brought about by the increased rate of sequestration of cAspAT into lysosomes.

Key Words pyridoxine-deficiency, degradation of cytosolic aspartate aminotransferase, lysosomes, immunoblotting

We have previously observed that severe pyridoxine-deficiency develops in rats receiving a high-protein pyridoxine-deficient diet (1). In the liver of the deficient rats, the enzymatic activity of the cytosolic aspartate aminotransferase (cAspAT) [EC 2.6.1.1] is decreased but the antigenic activity of the enzyme remains at the

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control level (2). Studies on the metabolic turnover of cAspAT indicated that both the rate of synthesis and the rate of degradation of the enzyme were increased in the liver of the pyridoxine-deficient rats (3). However, the mechanism of the accelerated degradation of the enzyme in pyridoxine-deficiency has remained unknown.

We have recently shown that cAspAT is degraded in the lysosomal system of rat liver (4). The present report provides evidence that the higher rate of degradation of cAspAT in the liver of the pyridoxine-deficient rats is ascribable to the higher rate of sequestration of the enzyme into lysosomes.

MATERIALS AND METHODS

Animals. Male Wistar-strain rats weighing about 50 g were fed ad libitum on 70% casein diet with or without pyridoxine for 4 weeks. The composition of the diet was described previously (5).

Preparation of lysosomes from rat liver. The livers were homogenized in 4 volumes of cold 0.25 M sucrose with a Potter-Elvehjem-type homogenizer. Highly purified lysosomes were isolated by the method developed in our laboratory (6) with some modifications (4).

Immunoblot analysis. Electrophoresis of the lysosomal proteins and immunoblot assay of cAspAT were carried out as described previously (4) using the antibody against rat liver cAspAT (2). The relative intensities of the immunoreactive bands were measured by scanning the filter at 600 nm in a Shimadzu CS-900 Scanning Spectrophotometer. The relative amounts of the immunoreactive substances were estimated by measuring the areas of densitometric tracings.

Enzyme and protein assays. As a marker enzyme of lysosomes, arylsulfatase [EC 3. 1. 6. 1] (combined activities of A and B) was assayed by the method of Milsom et al. (7). Units of the enzymatic activity are defined as the amount of enzyme causing the transformation of 1 μmol of substrate per min under the conditions of the assay. Protein was determined by the method of Lowry et al. (8) with bovine serum albumin as the standard.

RESULTS

Contents of lysosomal proteins in rat liver

Lysosomes were isolated from the livers of the control and pyridoxine-deficient rats as described under MATERIALS AND METHODS. As shown in Table 1, the specific activities of arylsulfatase, a lysosomal marker enzyme, for the lysosomes isolated from both groups of rats were twice as high as the values obtained for the lysosomes isolated from the liver of adult rats maintained on a commercial diet (6). The relative specific activities of arylsulfatase (specific activity found in the purified lysosomes/specific activity measured in the homogenate) showed approximately 80-fold purification of lysosomes from both groups. Since the lysosomal fractions were not contaminated with mitochondria, as evidenced by the absence of the activity of

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Table 1. Estimation of the lysosomal protein contents in the livers of the control and pyridoxine-deficient rats.

|                     | Specific activity of arylsulfatase (units/g protein) | Relative specific activity (lysosome/homogenate) | Lysosomal protein content (mg/g liver protein) |
|---------------------|----------------------------------------------------|-------------------------------------------------|-----------------------------------------------|
|                     | Homogenate  | Lysosome                                        |                                                |                                               |
| Control             | 31.6±2.9    | 2,640±124                                       | 83.7±7.3                                       | 12.0±1.0                                      |
| Deficient           | 37.1±4.2    | 2,850±271                                       | 76.9±8.5                                       | 13.0±1.3                                      |

Values are mean±SD of four animals.

Assuming a mitochondrial marker enzyme (succinic-INT reductase), the lysosomes isolated in the present study may be considered as essentially pure.

Assuming that arylsulfatase is exclusively localized in lysosomes and that the isolated lysosomes are 100% pure, we can estimate the lysosomal protein content from the specific activities of arylsulfatase in the total liver homogenate and in the isolated lysosomes as follows:

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\text{mg lysosomal protein/g total liver protein} = \frac{\text{enzyme units/g total liver homogenate protein}}{\text{enzyme units/mg lysosomal protein}}
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The result of such calculations is also presented in Table 1. The lysosomal protein contents in the livers of the control and pyridoxine-deficient rats are virtually the same; 12.0 and 13.0 mg lysosomal proteins/g liver protein, respectively.

**Immunoblot analysis of aspartate aminotransferase in rat liver lysosomes**

The lysosomal proteins from the control and pyridoxine-deficient rat livers were subjected to immunoblot analysis using the antibody against cAspAT (Fig. 1). The immunoblotting showed a major 46 kDa band and minor bands of lower molecular weights. The major 46 kDa band had the same electrophoretic mobility as the authentic cAspAT (lane 9) whose subunit molecular weight is 46,295 (9). The lower-molecular-weight bands include 41 and 36 kDa bands, which represent relatively stable intralysosomal degradative intermediates of cAspAT (4). Furthermore, particularly in the cases of the pyridoxine-deficient rats (lanes 5–8), immuno-reactive bands of higher molecular weights are clearly seen. The nature of these bands is unknown; these may represent aggregated products of cAspAT which arose during the electrophoresis.

Comparison of the immunoblotting pictures between the control (lanes 1–4) and pyridoxine-deficient (lanes 5–8) groups immediately gives an impression that the lysosomes prepared from the pyridoxine-deficient rat livers contain greater amounts of cAspAT and its degradative intermediates than those from the control rat livers. In order to express this impression in quantitative terms, the immuno-stained bands were scanned and the relative amounts of the immuno-reactive
Fig. 1. Immunoblotting of lysosomal proteins with anti-cAspAT antibody. Lysosomal proteins (2.0 μg) from the livers of the control (lanes 1–4) and the pyridoxine-deficient (lanes 5–8) rats were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotted with anti-cAspAT antibody. Lane 9 represents the authentic cAspAT.

Fig. 2. Densitometric tracings of the immunoblotted lysosomal proteins. The ordinate shows the density of the bands, seen in Fig. 1, and the abscissa shows the direction of migration in the gel (from right to left). ----, control (lane 3 in Fig. 1); ---, pyridoxine-deficient (lane 7 in Fig. 1).
substances were estimated as described under MATERIALS AND METHODS. Typical densitometric tracings of the control and pyridoxine-deficient samples are shown in Fig. 2. Measurements of the areas of such tracings, taking the mean of the control samples as arbitrary 100, gave the relative values of 100 ± 38 for the control samples (lanes 1–4 of Fig. 1) and 167 ± 6 for the pyridoxine-deficient samples (lanes 5–8 of Fig. 1). The areas that were higher than the 46 kDa peak were not included in these calculations.

DISCUSSION

Our previous study (3) showed that the metabolic half-life of cAspAT in the liver of the control rats was 4.78 days. The turnover rate of cAspAT in the pyridoxine-deficient rat liver could not be determined unambiguously because the decay curve was biphasic. However, the increased rate of incorporation of a labeled amino acid into cAspAT in pyridoxine-deficiency indicated that at least one component of cAspAT in the pyridoxine-deficient rat liver had a very short half-life.

Lysosomes are considered to be the main site of intracellular protein degradation (10). We now found that the lysosomal contents in the livers of the control and pyridoxine-deficient rats are essentially the same, but the lysosomes in the pyridoxine-deficient rat liver contain almost twice as much cAspAT and its degradative intermediates as those in the control rat liver. Theoretically, an increased steady-state concentration of any cytosolic proteins and their degradative intermediates in the lysosomes is the result of an increased rate of sequestration of cytosolic proteins into lysosomes and/or a decreased rate of intralysosomal degradation. However, in view of the increased rate of degradation in vivo of cAspAT in the liver of the pyridoxine-deficient rats, the latter possibility is unlikely. We therefore conclude that the increased rate of degradation of cAspAT in the liver of the pyridoxine-deficient rats is brought about by the increased rate of sequestration of cAspAT into lysosomes.

Why then is cAspAT sequestered into lysosomes at an accelerated rate in the pyridoxine-deficiency? Dice and Goldberg (11) found a correlation between protein charge and degradation rates; proteins with low isoelectric points tend to be degraded faster than those with neutral or basic isoelectric points. It is also well known that abnormal proteins, such as amino acid analog-containing proteins, are more rapidly degraded than native normal proteins (12). We have indeed found that proteins with lower isoelectric points and abnormal proteins are preferentially sequestered into rat liver lysosomes in vivo (10). We previously observed that the pyridoxine-deficiency induced some conformational change in rat-liver cAspAT as judged by alterations in circular and fluorescent spectra, and that the pyridoxine-deficiency caused a shift of isoelectric points of cAspAT subforms toward an acidic side (13). It is quite possible that these structural modifications of cAspAT render the enzyme molecule “abnormal” and more liable to facilitated degradation in the
lyrosomal system.

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