Vol. 143, No. 1, 1987
February 27, 1987

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 15-19

THE RABBIT DIFFERS FROM OTHER MAMMALIAN IN THE TISSUE DISTRIBUTION OF ALKALINE PHOSPHATASE ISOENZYMES

Tomoo Noguchi and Yoshihisa Yamashita

Department of Biochemistry, Kyushu Dental College, Kokura, Kitakyushu 803, Japan

Received December 31, 1986

SUMMARY: There are only two gene loci code for alkaline phosphatase of mammalian other than human and great apes: one for the intestinal form and other for the liver/kidney/bone form. The former form is present only in the intestine and the latter form occurs in other tissues such as liver, kidney and bone. In the present study, the rabbit was found to be different from other mammalian in the tissue distribution of alkaline phosphatase isoenzymes: only in the rabbit, most of the enzyme in the kidney and liver was the third form which differs from the liver/kidney/bone form, and this form was enzymatically and immunologically similar to the intestinal form of ALPase.

It has been reported that there are at least three gene loci code for alkaline phosphatase [ALPase; orthophosphoric monoester phosphohydrolase (alkaline optimum) EC 3.1.3.1] of human and great apes (1, 2): one for the placental forms, at least one for the intestinal forms, and at least one for the liver/kidney/bone forms. Evidence for only two loci code, one coding for liver/kidney/bone ALPase and other for intestinal ALPase, has been obtained with various mammalian other than human and great apes (1, 3). The ALPase in placentas of these mammalian differs from human placental ALPase and corresponds to the liver/kidney/bone ALPase (3). Human placental type ALPase occurs in placentas of human and great apes (chimpanzee and orangutan) but not in placentas of other primates, including gibbon (1, 2). These reports suggest that human placental type ALPase in placentas appeared relatively recently in mammalian evolution. As described above, studies on ALPase isoenzymes are of interest from the evolutional point of view. The identification of ALPase isoenzymes has been carried out with various laboratory and domestic animals except for the rabbit. It is miraculous why only the rabbit has not been used. This communication reports that the rabbit differs from other mammalian in the tissue distribution of ALPase iso-

Abbreviation: ALPase, alkaline phosphatase.

0006-291X/87 $1.50

enzymes; only in the rabbit, most of kidney and liver ALPase differs from the liver/kidney/bone form and is immunologically similar to the intestinal form.

MATERIALS AND METHODS

ALPase was assayed at 37°C with 6 mM p-nitrophenylphosphate as the substrate in 0.1 M glycine-NaOH, pH 11.0, containing 1 mM $MgCl_2$ and 0.1 mM $ZnCl_2$ as described (4). One unit of enzyme activity corresponds to 1 μmol of substrate hydrolysed per min at 37°C. Ouchterlony double diffusion analysis and protein determination were carried out as previously described (5).

RESULTS AND DISCUSSION

Fig. 1A shows the elution profile of ALPase from a DEAE-cellulose column with rabbit kidney extract and Fig. 1B shows that with rabbit liver

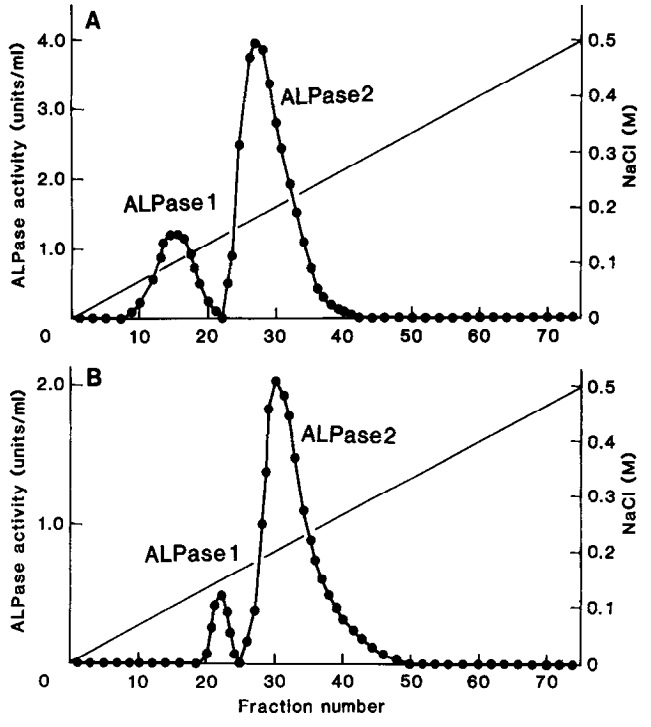

Fig. 1. DEAE-cellulose column chromatography of rabbit kidney and liver extracts. Fig. 1A shows the elution profile of ALPase with the kidney extract and Fig. 1B shows that with the liver extract. Kidney extract (300 ml) and liver extract (300 ml) were prepared from 28 kidneys (wet weight, 200 g) and 2 livers (wet weight, 200 g) of rabbits (body weight, about 2 Kg), respectively, by the butanol method of Morton (8) and dialysed against a large volume of 20 mM Tris-HCl, pH 8.0, to completely remove butanol. The nondiffusible solution was concentrated to about 40 ml by ultrafiltration with a Amicon PM 10 filter and applied to a column (4 x 20 cm) of DEAE-cellulose equilibrated with 20 mM Tris-HCl, pH 8.0, containing 1.0 mM $MgCl_2$ and 0.1 mM $ZnCl_2$. After washing with 500 ml of the same buffer, the enzyme was eluted with a 1,500 ml linear-gradient of 0-500 mM NaCl in the same buffer at a flow rate of 50 ml/h. The effluent was collected in 20 ml fractions.

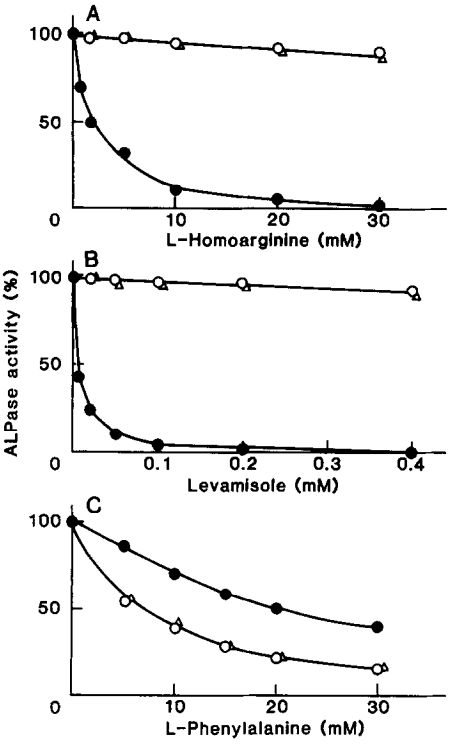

Fig. 2. Inhibition of the kidney ALPase 1 and ALPase 2 and intestinal ALPase of rabbits by L-homoarginine, levamisole and L-phenylalanine. Rabbit kidney extract was prepared and subjected to DEAE-cellulose column chromatography as described in the legend to Fig. 1. ALPase 1 and ALPase 2 fractions were separately pooled from a column of DEAE-cellulose, concentrated by ultrafiltration, and used as the enzyme sources. Rabbit intestinal extract was prepared by the butanol method (8) and used as the enzyme sourse. ● , rabbit kidney ALPase 1; ○ , rabbit kidney ALPase 2; △ , rabbit intestinal extract.

extract. In each case two activity peaks were obtained. One major enzyme (about 80% of the total activity for the kidney extract and about 92% for the liver extract) secondly eluted was hereafter designated ALPase 2, and other minor enzyme (about 20% for the kidney extract and about 8% for the liver extract) first eluted was designated ALPase 1.

Fig. 2 shows the results obtained in the inhibition studies by L-homoarginine, levamisole and L-phenylalanine with ALPase 1 and ALPase 2 fractions after DEAE-cellulose chromatography of rabbit kidney extract. These inhibitors were chosen because they provide a clear discrimination between the intestinal form and the liver/kidney/bone form of ALPase as follows (4): L-homoarginine and levamisole produce a marked inhibition of the liver/kidney/bone form and do only a slight inhibition of an intestinal form, while the intestinal form is inhibited more strongly than the liver/kidney/bone form by L-phenylalanine. As shown in Fig. 2A and B, ALPase 1 was markedly inhibited by both L-homoarginine and levamisole, whereas ALPase 2 was only slightly inhibited by these two inhibitors. In contrast, ALPase

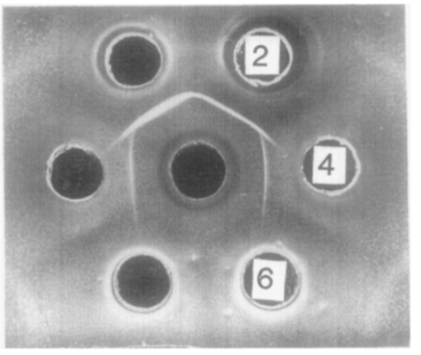

Fig. 3. Ouchterlony double diffusion analysis of ALPase 2 and ALPase 1 from rabbit kidney. The center well contained a guinea-pig antiserum (20 μl) raised against rabbit kidney ALPase 2. 1 and 2 of outer wells contained rabbit kidney ALPase 2 and rabbit liver ALPase 2 respectively. Both 3 and 4 of outer wells contained rabbit intestinal ALPase, and 5 and 6 contained kidney ALPase 1 and liver ALPase 1 of the rabbit respectively. In each case, 0.3 unit of antigen was used. Details of purifications of rabbit kidney ALPase 2 and rabbit intestinal ALPase will be published elswhere. Liver ALPase 2, and liver and kidney ALPase 1 were separately pooled from columns of DEAE-cellulose (Fig. 1), concentrated by ultrafiltration, and subjected to Sephacryl S-200 gel filtration. The pure ALPase 2 (100 μg) from rabbit kidney were intraperitoneally injected to a guinea-pig (about 500 g). One month later, the rabbit was again immunized with the same antigen (100 μg). The serum was collected 1 month later.

2 was inhibited more strongly than ALPase 1 by L-phenylalanine (Fig. 2C). Inhibition profiles of ALPase 2 by these three inhibitors were nearly identical with those of intestinal ALPase of rabbits(Fig. 2A, B and C). When the same inhibition experiments by these three inhibitors were carried out with liver ALPase 1 and ALPase 2 preparations from a column of DEAE-cellulose, ALPase 1 and ALPase 2 showed nearly identical inhibition profiles to those of kidney respectively.

On the basis of the inhibition experiments, it is suggested that most (ALPase 2) of ALPase in the kidney and liver of the rabbit is different from the liver/kidney/bone form and similar to the intestinal form, while minor ALPase 1 is the liver/kidney/bone form. It is surprising that in the kidney and liver, most of ALPase is not the liver/kidney/bone form only in the rabbit.

Therefore, immunological identification was carried out to obtain further evidence. As shown in Fig. 3, on Ouchterlony double diffusion analysis (6), a guinea-pig antibody (center well) raised against rabbit kidney ALPase 2 produced a single connecting band of precipitin between kidney ALPase 2 (1 of outer well) and liver ALPase 2 (2 of outer well) of the rabbit, showing that kidney and liver ALPase 2 are nearly identical in amino acid sequence. In contrast, this antibody did not cross-react with kidney ALPase 1 (5 of outer well) and liver ALPase 1 (6 of outer well) of the rabbit, and also did not cross-react with the liver/kidney/bone forms of

the human (0.3 unit, Sigma) and pig (0.3 unit, Sigma)(not shown). On the other hand, surprisingly the antibody against rabbit kidney ALPase 2 cross-reacted with the intestinal ALPase (3 and 4 of outer wells) of the rabbit. However the precipitin line against the kidney (or liver) ALPase 2 cross with that against intestinal ALPase 2. This antibody also cross-reacted with the intestinal ALPases of the bovine (0.3 unit, Sigma) and dog (0.3 unit, Sigma) (not shown). These results show that there is at least 60% sequence homology between rabbit kidney (or liver) ALPase 2 and mammalian intestinal form of ALPase, because it has been described that immunological cross-reactivity is observed only between proteins that show at least 60% sequence homology (7). The antibody against rabbit kidney ALPase 2 did not cross-react with the human placental form (0.3 unit, Sigma)(not shown).

The present results show that most of ALPase in the kidney and liver of rabbits is the third new form in mammalian other than Hominoideae which is different from the liver/kidney/bone form and is immunologically similar to mammalian intestinal form of ALPase. It has been reported that there are only two loci code for ALPase of mammalian other than Hominoideae; one for the intestinal form and other for the liver/kidney/bone form (1, 3). 1. Why only the rabbit differs from other mammalian in the tissue distributiion of ALPase isoenzymes? 2. Why in the liver and kidney ALPase 2 is present only in the rabbit? 3. Does such an abnormality in the rabbit occur for other isoenzymes? 4. Which is most similar to ALPase 2 in the structure out of the intestinal ALPase of the rabbit and those of other mammalian? These questions are of interest from the animal evolutional point of view. The answer might be in part obtained by the determination of difference in amino acid sequence among rabbit kidney (or liver) ALPase 2, rabbit intestinal ALPase, other mammalian intestinal form of ALPase and rabbit liver/kidney/bone form of ALPase.

REFERENCES

1. Goldstein, D. J., Rogers, C. E. and Harris, H. (1982) Proc. Natl. Acad. Sci. U.S.A. 79, 879-883.
2. Goldstein, D. J. and Harris, H. (1979) Nature 280, 602-605.
3. Goldstein, D. J., Rogers, C. E. and Harris, H. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 2857-2860.
4. Bergmeyer, H. U., Gawehn, K. and Grassl, M. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U. ed) pp. 496-497, Academic press, New York.
5. Noguchi, T., Fujiwara, S. and Hayashi, S. (1986) J. Biol. Chem. 261, 4421-4223.
6. Ouchterlony, O. (1949) Microbiol. Scand. 26, 291-297.
7. Prager, E. M. and Wilson A. C. (1971) J. Biol. Chem. 246, 5978-5989.
8. Morton, R. K. (1954) Biochem. J. 57, 595-603.