Isoforms of Alkaline Phosphatase from Mouse Internal Organs after Bilateral Adrenalectomy

N. I. Tsirel'nikov and N. P. Voronina

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 121, № 3, pp. 262-264, March, 1996
Original article submitted March 21, 1995

The activity of alkaline phosphatase of female CBA and BALB/c mice is studied after bilateral adrenalectomy. Interstrain differences in enzyme activity are revealed in some organs of the control and experimental animals. The expression of new isoforms of alkaline phosphatase in hypocorticoidism is demonstrated.

Key Words: *alkaline phosphatase; isoforms; regulation; adrenalectomy*

Alkaline phosphomonoesterases (EC 3.1.3.1) have been extensively studied in experimental and clinical medicine, since these enzymes play an important role in adaptive and pathological responses [7,10]. The fact that alkaline phosphatase (AP) activity is high in actively absorbing or secreting tissues indicates how important this enzyme is for the development and function of living systems. By dephosphorylating NADP and NADPH, alkaline phosphatase affects numerous oxidation-reduction processes in the cell [3]; dephosphorylation of histones and glycogen synthetase testifies to the important role of AP in the regulation of glucose metabolism and the expression of the mammalian genome [2,12].

According to data in the literature data [6,9], adrenal hormones are involved in the expression of AP. In view of the considerable variations of steroid hormone levels during ontogenesis and the role of these hormones in adaptive, stress, and pathological mechanisms, it is important to study the activity of various isoforms of alkaline phosphomonoesterase under conditions of decreased concentrations of corticosteroids in the body after experimental adrenalectomy.

Laboratory of Fetoplacental Pathology, Institute of General Pathology and Human Ecology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk (Presented by V. P. Kaznacheev, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Experiments were performed on pubertal female CBA and BALB/c mice weighing 18-20 g. A deficiency of glucocorticoid hormones was achieved by bilateral adrenalectomy performed under light ether anesthesia [4]. The "cleanness" of adrenalectomy was checked visually during the surgery and by measuring the plasma concentration of 11-hydroxycorticosteroids [5]. Animals with sham adrenalectomy (without removal of the adrenals) were taken for comparison. The activity and spectrum of AP from various organs and tissues were studied seven days after the surgery, when the corticosteroid levels were minimal. Homogenates of the liver, kidneys, lungs, and bone (caudal vertebrae) were prepared in 0.05 M Tris-HCl buffer (pH 7.2) containing Triton X-100. The AP activity was assayed in the supernatants obtained by centrifugation of the homogenates at 6000 rpm for 20 min, the assay [13] being based on measurements of the intensity of fluorescence of α-naphthol formed after the hydrolysis of α-naphthylphosphate at pH 9.6. The AP activity was expressed in μg α-naphthol per mg wet weight of tissue during 15 min.

Isoforms of AP were studied by vertical slab electrophoresis at room temperature in 225□×130×2 mm gel as described elsewhere [8].

0007-4888/96/0003-0241$15.00©1996 Plenum Publishing Corporation

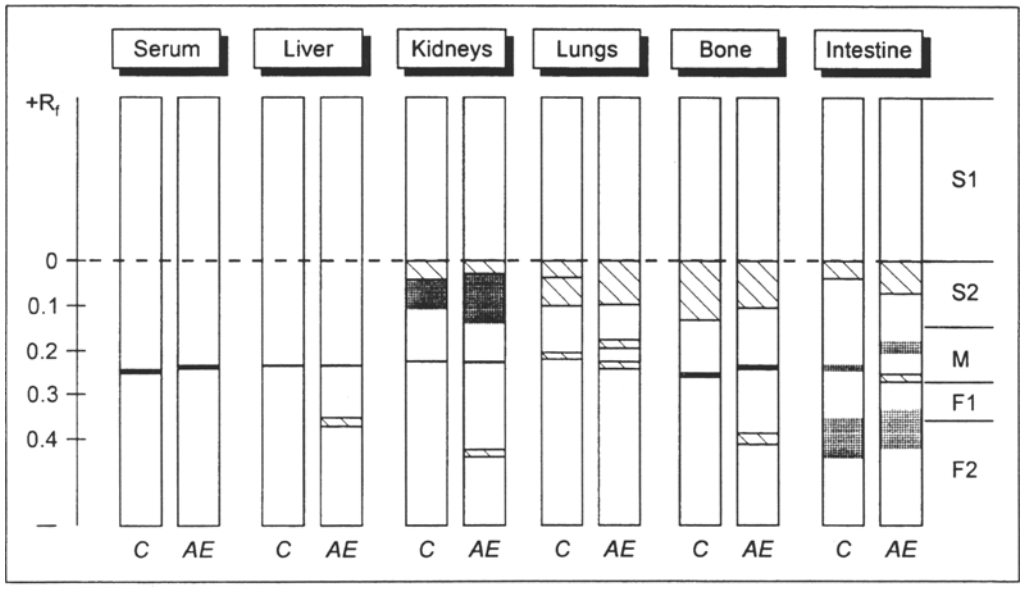

Fig. 1. Major isoforms of alkaline phosphatase from the internal organs and serum of CBA mice 7 days after adrenalectomy (*AE*) (polyacrylamide gel electrophoresis). *C*: control group; 0: borderline between stacking and separating gels; S1 and S2: "slow" isoforms of AP (R_f=0-0.15); M: intermediate isoforms (R_f=0.15-0.25); F1: "fast" isoforms (R_f=0.25-0.35); F2: "very rapid" isoforms (R_f>0.35).

The characteristics of the polyacrylamide gel were calculated from the following formulas:

$$T = \frac{(a+b)}{m} \times 100\%, \quad C = \frac{b}{(a+b)} \times 100\%,$$

where *a* is the amount of acrylamide, g; *b* is the amount of bis-acrylamide, g; and *m* is the volume of buffer, ml.

The parameters of the stacking gel were: *T*=3.9%, *C*=2.6%, and riboflavin 5 μg/ml. The parameters of the separating gel were: *T*=10.4%, *C*=2.6%, and ammonium persulfate 0.175 mg/ml. The working buffer contained 0.07 M Tris-HCl (pH 7.5) and 0.3 μl/ml tetramethylethylenediamine. The electrode buffer contained 0.008 M Tris titrated with boric acid to pH 7.0. The height of the separating gel was 9 cm and the height of stacking gel was 2.5 cm. Bromphenol Blue (1.5 ml of a 0.001% aqueous solution per liter) was added to the upper electrode buffer. The homogenates and sera were mixed with an equal volume of 0.5 M sucrose in the working buffer diluted 5-fold and applied to the gel in a volume of 20 μl. Electrophoresis was carried out at 12 mA for 1 h and then at 25 mA until the band of Bromphenol Blue reached a distance of 7 cm from the top of the separating gel. After electrophoresis, the activities of AP isoforms were determined by the method of nitrogen coupling [1]. The gel was incubated in a mixture containing 3.7 mM naphthol-AS-TR-phosphate, 5 vol.% dimethylformamide, 1 mg/ml Fast Blue, and 0.1 mM $MgCl_2$ in 0.1 M Tris-HCl buffer (pH 9.0) for 30 min at 37°C in the dark in a water bath with shaking, washed with distilled water, incubated in fresh medium overnight, and fixed in an ethanol:distilled water:glacial acetic acid (5:10.5:1.5) mixture [11]. The electrophoretic mobility of proteins (R_f) was calculated relative to Bromphenol Blue. The distance traveled by the proteins and stain was measured from the top of the separating gel.

RESULTS

It was found that the activity of AP from the same organs and tissues is different in CBA and BALB/c mice. In CBA mice, the enzyme activity was maximal

TABLE 1. Activity of Alkaline Phosphatase (μg α-naphthol/mg tissue during 15 min) in Serum and Extracts of Internal Organs in CBA and BALB/c Mice 7 Days after Bilateral Adrenalectomy

Parameter	CBA		BALB/c	
	control (*n*=7)	adrenalectomy (*n*=5)	control (*n*=5)	adrenalectomy (*n*=5)
Liver	0.5±0.08	0.5±0.09	15.8±1.07	3.5±0.26*
Kidneys	39.6±1.43	43.4±2.19	28.3±1.21	25.8±0.37
Lungs	1.6±1.43	3.7±1.84	5.7±0.48	7.6±1.86
Bone	26.5±5.75	34.1±1.07	16.1±6.95	31.8±2.70*
Intestine	29.9±4.33	36.0±6.25	42.3±1.52	22.0±9.37
Serum	17.9±5.31	23.2±6.39	7.9±0.72	5.7±0.37*

Note. *p<0.05 compared with the control group.

in the kidneys, whereas in BALB/c mice it was maximal in the intestine (Table 1).

Seven days after bilateral adrenalectomy, plasma 11-hydroxycorticosteroids in CBA and BALB/c mice had decreased 1.5- and 1.7-fold, respectively. In hypocorticoidism, the activity of AP expressed by a tissue-nonspecific gene in the liver, kidneys, lungs, and bone varies considerably. In the liver of CBA mice, the enzyme activity was the same as in the control, while in BALB/c it was 4.5-fold lower than in sham-adrenalectomized mice. The activity of AP in the kidneys of experimental BALB/c mice did not differ from the control, while in CBA mice it was increased. In both CBA and BALB/c mice, the enzyme activity increased in the lungs and, especially, in the bone (Table 1). The difference between the activity of intestinal AP (which is suppressed by the intestinal AP gene) in CBA and BALB/c mice on the one hand and control mice on the other was statistically insignificant, since the enzyme activity varied considerably from animal to animal. The activity of serum AP in BALB/c mice was lower than in the control due to a decrease in the activity of the hepatic and intestinal isoforms of AP.

Electrophoresis of liver extracts from adrenalectomized and control mice revealed an activity and variability of AP isoforms in the region of "intermediate" fractions (Fig. 1). On electrophoregrams from experimental animals, a faint band was seen in the region of "fast and very fast" forms (R_f=0.28-0.37). On electrophoregrams of kidney extracts, one band was located at the top of the stacking gel and two bands at the top of the separating gel: a fainter band was confined to the R_f=0-0.048 region and more distinct bands were located in the R_f=0.05-0.15 and R_f=0.22-0.24 regions. However, in 2 out of 5 electrophoregrams from experimental mice an additional band was seen in the "very fast" region (R_f>0.35). In lung extracts, two enzyme-positive zones were revealed in the region of "slow" forms (R_f=0-0.048 and 0.048-0.12) and one band was seen in the region of "intermediate" forms (R_f=0.2-0.24). A wide band in the region of S2 forms (R_f=0-0.13) and a narrow band in the region of "intermediate" forms (R_f=0.22-0.25) were present in the electrophoregrams of bone tissue extracts. A faint additional band appeared in the region of "very fast" forms (R_f= =0.37-0.395) after adrenalectomy. Intestinal extracts from experimental and control mice were characterized by substantial individual variability of the activity and number of AP isoforms. Despite marked changes in the activity and spectrum of AP in the internal organs and tissues of adrenalectomized rats, no other isoforms of AP were identified in the serum by electrophoresis.

Thus, we have demonstrated differences in the activity of alkaline phosphatase from the internal organs of CBA and BALB/c mice. Seven days after bilateral adrenalectomy (the period of a pronounced drop of corticosteroid levels) the activity of AP in the organs and tissues of BALB/c and CBA mice was found to be changed, the maximum changes occurring in the organs of BALB/c mice, which are more sensitive to hormones; in other words, there are strain-related differences in the role of corticosteroids in the regulation of the expression (or activity) of various forms of AP. An opposite response of AP expression was recorded in the same organs of CBA and BALB/c mice. The cellular and tissue regulatory mechanisms of the genes expressing the same type of AP in the kidneys and intestine were found to be nonuniformly sensitive to a decrease in the corticosteroid concentration.

We are grateful to T. A. Tsymbalova for technical help in the electrophoretic identification of AP isoforms.

REFERENCES

1. L. I. Korochkin *et al.* (Eds.), *Genetics of Isoenzymes* [in Russian], Moscow (1977).
2. L. A. Gromashevskaya, in: *Isoenzymes in Medicine* [in Russian], Kiev (1982), pp. 166-185.
3. L. A. Gromashevskaya and I. L. Radzevich, *Lab. Delo,* № 1, 3-10 (1991).
4. *Methods of Developmental Biology* [in Russian], Moscow (1974), pp. 246-254.
5. Yu. A. Pankov and A. I. Usvatova, in: *Methods of Studying Some Hormones and Transmitters* [in Russian], Moscow (1966), pp. 137-147.
6. Yu. I. Savchenkov and K. S. Lobyntsev, *Reviews of the Physiology and Morphology of the Mother-Fetus System* [in Russian], Moscow (1980).
7. M. G. Tvorogova and V. N. Titov, *Lab. Delo,* № 6, 10-17 (1991).
8. T. A. Tsymbalova and N. I. Tsirel'nikov, *Byull. Eksp. Biol. Med.,* **114**, № 9, 236-238 (1992).
9. W. H. Fishman, *Am. J. Med.,* **56**, 617-650 (1974).
10. W. H. Fishman, *Clin. Biochem.,* **23**, № 2, 99-104 (1990).
11. K. D. Kirek-Jaszesuk and H. Gelderman, *Anim. Blood Groups Biochem. Genet.,* **16**, № 3, 205-216 (1985).
12. W. Meyer-Saballek, P. Sinha, and E. Kottegen, *J. Chromatogr.,* **420**, 419-444 (1989).
13. D. Moss, *Enzymologia,* **31**, 193-202 (1966).