SOME PHYSICAL PROPERTIES OF ALKALINE PHOSPHATASES FOUND IN VARIOUS TISSUES OF AKR AND C57BL/6 MICE, NORMAL AND LEUKEMIC

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1. Introduction

The appearance and levels of alkaline phosphatases in the thymus of AKR and C57BL/6 leukemic and normal mice have been investigated mainly histochemically [1–3]. As far as we are aware no systematic study of the quantity and kinetic behavior of the alkaline phosphatases of the thymus, the mesenteric nodes, the spleen, the liver, and the kidney derived from normal and leukemic tissues of mice has been reported.

In this publication we describe the enzymatic hydrolysis of the substrates *p*-nitrophenyl phosphate (*p*-NPP) and cysteamine *S*-phosphate (CASP) by alkaline phosphatases extracted from various tissues of normal and leukemic mice. Quan itative determinations of the rate of hydrolyses of the above substrates were studied under different experimental conditions such as pH and the concentration dependence on $MgCl_2$, NaCl, in tris, and in sodium barbital buffers. The data obtained were compared with the corresponding data using alkaline phosphatases from the chicken intestine and *Escherichia coli* under similar conditions either described in the literature [4–6] or obtained in this study.

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2. Materials and methods

Male and female mice of the AKR and C57BL/6 strains from our inbred colony were used in these experiments. The different normal tissues were collected from two month old mice. The inbred AKR strain from our colony developed an 80–90% incidence of spontaneous lymphoid leukemia. The disease is first manifested in the thymus and subsequently spreads to other lymphoid and non-lymphoid organs. The spontaneous tumors used in the present studies were removed from 6–8 month old mice. The C57BL/6 strain of mice have a low spontaneous incidence (0.5%) of lymphoid leukemia, but are highly susceptible to radiation leukemogenesis, or to lymphoid leukemia induction by the radiation leukemia virus [7]. The radiation leukemia virus used in the present investigation was obtained from irradiated non-leukemic tissues of the C57BL/6 strain of mice [8]. The virus (0.02 ml) was injected directly into one thymus lobe of two month old male mice, and two days after virus inoculation these mice were exposed to 400 R whole body irradiation. Ninety percent of these treated mice developed lymphoid leukemia within an average latent period of 80 days. The lymphomas observed were usually generalized: the thymus extremely enlarged, leukemic and leukemic infiltrations were also noted in liver, spleen, kidney and lymph nodes.

The tissues investigated were homogenized in 0.1 M

tris buffer, pH 8.0, with a Sorvall omni-mixer at a ratio of 2 g tissue per 10 ml of buffer. The insoluble material was then separated by centrifugation at 100,000 *g* for 1 hr in a Spinco model L50 ultracentrifuge. The supernatant yielded by this procedure was employed in the present study.

The kinetic studies of alkaline phosphatase activities were conducted using *p*-NPP as a substrate. In this procedure [5] the *p*-nitrophenol (*p*-NP) liberated was estimated spectrophotometrically as a function of time. When CASP served as the substrate the cysteamine (CA) formed was determined by the method of Ellman [9].

All spectrophotometric measurements were carried out at room temperature with a Zeiss model PMQ spectrophotometer using cuvettes with a light path of 10 mm. The pH measurements were carried out with a pH meter, model 22, Radiometer, with a type G-202B glass electrode and a type K4312 calomel electrode.

3. Chemicals

The alkaline phosphatases, chromatographically purified from *E. coli* and chicken intestine, were purchased from Worthington. *p*-NPP was purchased from Sigma, DTNB from Aldrich, and CASP was prepared according to the literature [10]. All other compounds used were of analytical grade.

4. Results and discussion

Alkaline phosphatase activities in the supernatants of various tissues after homogenization were routinely measured in 0.5 M tris-HCl buffer, pH 9.0, at room temperature. A preliminary study of the rate of hydrolysis of *p*-NPP was found to be tris-HCl concentration dependent. A concentration of 0.5 M tris-HCl buffer was found to be convenient for the assay system employed. Table 1 shows the results of a representative set of experiments. These results indicate an enhancement of the initial rate of enzymatic hydrolysis in tissues derived from leukemic animals as compared to tissues from normal mice. The differences in the initial velocities of the enzymatic hydrolyses between normal and leukemic tissues can not be directly related to the amount of alkaline phosphatase present because the influence of other factors such as an inhibitor, the lack of cofactors, etc. can not be excluded.

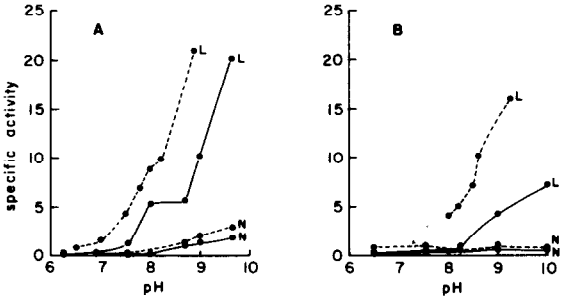

Fig. 1. The pH dependency of enzymatic hydrolysis of *p*-NPP. A, in 0.5 M tris-HCl; B, in 0.025 M sodium barbital. L and N represent enzymatic activities from leukemic and normal tissues, respectively. ——, the AKR strain of mice; - - - - -, the C57BL/6 strain of mice.

It is pertinent to note that the same apparent enhancement of alkaline phosphatase activities was found associated with the insoluble precipitate after the initial extraction. Due to inaccuracies inherent to spectrophotometrically followed kinetic studies with insoluble materials we are reporting only the results where the experiments were conducted with the supernatant material.

The hydrolysis of *p*-NPP both in tris-HCl and in sodium barbital buffers was found to be pH dependent for all tissues studied (figs. 1A, B). The variation with pH of the rate of enzymatic hydrolysis of the extracts investigated was found to be similar to the variation in the rates of other alkaline phosphatases reported in the literature [4–6, 11]. However, it is worthwhile to mention that there was a noticeable shift in the pH dependency when tris-HCl was used instead of sodium barbital. This observation is currently under further investigation.

The hydrolysis rate of *p*-NPP catalyzed by alkaline phosphatases from leukemic and normal tissues in tris-HCl and sodium barbital buffers showed only a slight dependency on the $MgCl_2$ concentration (0.001–0.06 M). Concentrations of $MgCl_2$ higher than 0.06 M could not be used because of the occurrence of precipitation during the enzymatic assay. A further study on the $MgCl_2$ dependence after dialysis against 0.1 M tris-HCl, pH 8.0, also resulted in only a slight enhancement of

Table 1
Alkaline phosphatase levels in tissues of normal and leukemic mice.

Tissue	AKR		C57BL/6	
	Normal*	Spontaneous lymphoid leukemia*	Normal*	Virus induced lymphoid leukemia*
Thymus	2.20	9.26	1.33	76.90
Mesenteric nodes	0.76	27.60	1.00	35.50
Spleen	3.90	20.65	4.00	39.40
Kidney	1.85	7.40	2.67	96.50
Liver	21.60	41.90	8.75	16.90

* Enzymatic activities expressed as the amount of *p*-NP ($\times 10^{-5}$ moles) liberated per min per ml per g wet weight tissue.

Table 2
Alkaline phosphatase activities toward the substrates *p*-NPP and CASP with normal and virus-induced leukemic tissues.

Condition	Tissue				
	Thymus	Mesenteric nodes	Spleen	Liver	Kidney
Normal	~ 1	–**	–**	–**	~1
Virus-induced lymphoid leukemia*	28	3.5	3.6	17.2	2.8

* Activities expressed as the initial velocity of *p*-NP formation per initial velocity of CA formation.
** In these cases the activity toward the two substrates was minimal.

the enzymatic activity when $MgCl_2$ (0.001–0.06 M) was included in the assay mixture. These findings indicate a much smaller dependency on $MgCl_2$ concentartions than alkaline phosphatases previously studied [4] and suggested that we were dealing with a different kind of alkaline phosphatase.

The effect of NaCl on the alkaline phosphatase activity extracted from various tissues was also investigated in both tris-HCl and sodium barbital buffers. In these experiments the NaCl concentrations were varied from 0.05 to 1.2 M; no enhancement of the enzymatic hydrolysis of *p*-NPP could be detected.

Investigations have shown that alkaline phosphatases from *E. coli* [5] and chicken intestine [6] hydrolyze both the monoesters of ortho-phosphoric acid and thiophosphoric acid at a similar rate. It was therefore of interest to see if the alkaline phosphatases under study exhibited similar characteristics. The results in table 2 indicate that there exists a definite difference between the relative ability of the alkaline phosphatases of the normal and virus-induced leukemic tissues toward the hydrolysis of the substrates *p*-NPP and CASP. Identical studies on the alkaline phosphatases from AKR tissues were also performed. No activity toward the substrate CASP could be detected. In the cases where no activity towards CASP could be found the possibility of 5,5′-dithiobis(2-nitrobenzoic acid) inhibition could not be excluded. Therefore, incubation mixtures of the enzyme and the substrate were prepared, aliquots removed at appropriate time intervals, and assayed for the amount of cysteamine present. Also through this procedure no enzymatic hydrolysis of CASP occurred with the soluble preparations from AKR tissues.

5. Conclusions

The increased activity of the alkaline phosphatases from leukemic tissues toward the substrate *p*-NPP substantiate earlier histochemical observations [1–3]. Because our study was conducted with the soluble enzyme we were able to quantitate the alkaline phosphatase activities present in various tissues, and to measure the dependency of alkaline phosphatase activities on such parameters as pH, buffer, and $MgCl_2$ and NaCl concentrations. From the $MgCl_2$ and NaCl studies we can conclude that the enzymes investigated were different in their overall behavior than alkaline phosphatases from *E. coli* [5] and chicken intestine [6]. The differences in the ratios of the activities toward the substrates *p*-NPP and CASP may be due to the presence of two or more different types of alkaline phosphatases dependent upon the physiological condition of the animals. Conversely, the presence or absence of unknown cofactors interfering with the measured activities cannot be excluded. Isolation and further characterization of kinetic parameters of the enzymes used in this study could, hopefully, elucidate between either the accumulation of different types of alkaline phosphatases or the presence or absence of other cofactors during the development of the leukemic condition.

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