Studies on Alkaline Phosphatase Activity in Amphibians III. Effects of Inhibitor and Temperature on Alkaline Phosphatases in Japanese Amphibians

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ABSTRACT

The effects of L-homoarginine, L-phenylalanine, L-phenylalanylglycylglycine and L-leucine and high temperatures on the alkaline phosphatases extracted from the skins, lungs, livers, kidneys, ovaries and small intestines of *Rana nigromaculata*, *Rana japonica*, *Rana rugosa*, *Rana limnocharis*, *Rana catesbeiana*, *Rhacophorus schlegelii*, *Hyla arborea japonica*, *Bufo japonicus* and *Cynops pyrrhogaster* were examined in order to confirm the differences in the stability of alkaline phosphatases to these inhibiting-agents. The alkaline phosphatases of the skins, lungs, livers, kidneys and ovaries from all species, as well as those of the small intestines from *Rana nigromaculata*, *Rana japonica*, *Rana rugosa*, *Rana limnocharis*, *Rana catesbeiana* and *Rhacophorus schlegelii* required nearly the same concentrations of inhibitors for 50% inhibition, while the alkaline phosphatases of the small intestines from *Hyla arborea japonica*, *Bufo japonicus* and *Cynops pyrrhogaster* required remarkably higher concentrations of inhibitors for 50% inhibition than the foregoing alkaline phosphatases. The alkaline phosphatases of all the organs from *Rana limnocharis* other than the small intestine were remarkably temperature-resistant.

INTRODUCTION

Alkaline phosphatase is a group of enzymes that catalyze hydrolysis of various phosphate-containing components at alkalinity. It is ubiquitously distributed in various plants and animals. In vertebrates it is found in various organs, such as the small intestine, kidney and liver. This seems to imply that alkaline phosphatase is in some way integral to certain fundamental functions of these organs. It has been revealed that alkaline phosphatase is concentrated in the brush borders of convoluted tubules of the kidney in rats (Burstone, 1958; Mizutani and Barrnett, 1965) and in the villi of the intestine in mice (Hugon and Borgers, 1966), suggesting a supportive role in membrane transport. The precise physiological function, however, remains unclear. The existence of more than one form in the human alkaline phosphatase has been confirmed by inhibition studies with inhibitors (Fishman, Green and Inglis, 1963; Lin and Fishman, 1972; Doellgast and Fishman, 1977; Mulivor, Plotkin and Harris, 1978). According to Stig-brand (1984), there were three forms in human alkaline phosphatase, while

GOLDSTEIN, ROGERS and HARRIS (1980) recognized two forms in mammalian alkaline phosphatase other than that of humans and primates. GOLDSTEIN, ROGERS and HARRIS (1980) have reported that the two forms of alkaline phosphatase differ from each other in structure of the inhibitor-binding site.

The existence of alkaline phosphatase in the brush borders of the small intestine has been observed in Rana temporaria (BROWN and MILLIGNTON, 1968) and in the kidney of Bombina orientalis (HAH, 1974). SORIMACHI, MIZUNO, KONNO, NIWA, YASUMURA and UCHIYAMA (1983) have investigated the catalytic properties of alkaline phosphatase in the kidney of Rana catesbeiana. YORA and SAKAGISHI (1986) have compared the alkaline phosphatases of the intestine, liver and kidney with one another by two kinds of inhibitors in Rana catesbeiana, Xenopus laevis and Cynops pyrrhogaster. On the whole, however, very little information has yet been obtained as to the analysis of alkaline phosphatase by inhibitors in amphibians.

The main purpose of the present study is to confirm the difference in stability of alkaline phosphatases obtained from six kinds of organs to various kinds of inhibiting-agents or high temperatures in nine different species in order to clarify how many forms of the enzymes can be discriminated from one another.

MATERIALS AND METHODS

Specimens used in this study, their collecting stations and the number of specimens are as follows: Rana nigromaculata HALLOWELL, Bingoochiai, Hiroshima Prefecture, 5; Rana japonica GÜNTHER, Hiroshima City, 5; Rana rugosa SCHLEGEL, Hiroshima City, 7; Rana limnocharis GRAVENHORST, Hiroshima City, 7; Rana catesbeiana SHAW, Hiroshima City, 3; Rhacophorus schlegelii (GÜNTHER), Hiroshima City, 3; Hyla arborea japonica GÜNTHER, Hiroshima City, 10; Bufo japonicus SCHLEGEL, the plateau of Kammuri, Hiroshima Prefecture, 5; Cynops pyrrhogaster (BOIE), Hiroshima City, 6.

The skin, lung, liver, kidney, ovary and small intestine were removed from each frog and immediately frozen. Each organ was minced with scissors, washed in distilled water, and then homogenized in a glass homogenizer by adding nine volumes of distilled water. An extract was made from the homogenates with n-butanol (5 ml/g tissue). Then, temperature-critical alkaline phosphatases were incubated for 15 minutes at 30°C, while the other alkaline phosphatases were incubated in a water bath at 37°C. The butanol mixtures were centrifuged at 4°C at 20,000 g for 30 minutes. Supernatants containing alkaline phosphatases were stored at −20°C.

In preparation for alkaline phosphatase assay, 0.1 ml of alkaline phosphatase solution was incubated at 30°C or 37°C for 30 minutes after adding 0.45 ml of 50 mM Ammediol-HCl (pH 9.5) buffer solution and 0.45 ml of substrate solution containing 10 mM disodium p-nitrophenylphosphate (Sigma), 1 mM HCl and 10 mM $MgCl_2.6H_2O$. The reaction at this time was stopped by adding 5.0 ml of 0.1 N NaOH. The amount of p-nitrophenol liberated was determined at 415 m μ with a Japan Spectroscopic UVIDEC–320H spectrophotometer. Protein content

of the supernatant was measured according to the method of LOWRY, ROSE-BROUGH, FARR and RANDALL (1951), using bovine serum albumin as a standard. The specific activity of alkaline phosphatase in the supernatant was defined as liberated micromole p-nitrophenol (PNP) per milligram protein per hour.

Four inhibitors, L-phenylalanine (*Phe*), L-homoarginine (*Har*), L-phenylalanylglycylglycine (*PheGlyGly*) and L-leucine (*Leu*) (Sigma), were used to inhibit alkaline phosphatase activity in extracts. Inhibitors were used at the concentrations of 1, 2.5, 5, 10, and 20 mM. The concentration of the inhibitor required for 50% inhibition of alkaline phosphatase (I_{50}) was calculated as described by GOLDSTEIN and HARRIS (1979).

Extracts were heated for 30 minutes at various temperatures in a water bath, cooled to 0°C and later assayed for remaining alkaline phosphatase activity. The temperature required for 50% inactivation of alkaline phosphatase activity (T_{50}) was defined according to GOLDSTEIN and HARRIS (1979).

OBSERVATION

I. Inhibition

The sensitivity of alkaline phosphatase to inhibition by inhibitors was investigated in six tissues from nine amphibian species (Tables 1 and 2; Fig. 1).

The I_{50} values of *Har* and *PheGlyGly* were between 2.00 and 3.98 for the alkaline phosphatases of the skins, lungs, livers, kidneys and ovaries from all species examined. However, the alkaline phosphatases of the small intestines from different species displayed differing sensitivity to these inhibitors. The I_{50} values of *Har* for the alkaline phosphatases of the small intestines from *Rana nigromaculata, Rana rugosa, Rana limnocharis, Rana catesbeiana* and *Rhacophorus schlegelii* were between 3.06 and 4.66 and rather similar to the values found in the other tissues. The I_{50} value of *Har* for the alkaline phosphatase of the intestine from *Rana japonica* was 6.34 and was somewhat greater than the values for the other tissues. The I_{50} values of *Har* for the alkaline phosphatases of the small intestines from *Hyla arborea japonica, Bufo japonicus* and *Cynops pyrrhogaster* were far greater than the I_{50} values of *Har* for the other tissues. *Har* was almost invalid in the inhibition of the alkaline phosphatases of the intestines from these three species. The I_{50} values of *PheGlyGly* for the alkaline phosphatases of the small intestines from *Rana rugosa, Rana limnocharis, Rana catesbeiana* and *Rhacophorus schlegelii* were from 2.68 to 3.92 and also similar to the values for the other tissues. In contrast, the I_{50} values for *Rana nigromaculata* and *Rana japonica* were 5.33 and 6.32, and were slightly greater than the I_{50} values for the other tissues. The I_{50} values of *PheGlyGly* for the alkaline phosphatases of the small intestines from *Hyla arborea japonica, Bufo japonicus* and *Cynops pyrrhogaster* were remarkably different from the I_{50} values for the other tissues and at the same time displayed a great variation among these three species. *Hyla arborea japonica* showed the greatest I_{50} value, that is, *PheGlyGly* was almost invalid in the inhibition of alkaline phosphatase of the small intestine from this

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TABLE 1

Inhibition of alkaline phosphatase activities by four kinds of
inhibitors in the skin, lung and liver

Organ	Species	No. of specimens	I_{50} (mM)			
			Har	*Phe*	*PheGlyGly*	*Leu*
Skin	R. nigromaculata	3	2.04	5.43	2.38	15.67
	R. japonica	3	3.05	11.72	3.04	22.75
	R. rugosa	3	3.90	11.98	2.79	25.59
	R. limnocharis	4	3.00	6.73	3.58	17.41
	R. catesbeiana	3	3.88	7.05	3.45	20.62
	Rh. schlegelii	3	2.01	5.81	2.62	17.64
	H. a. japonica	5	2.10	6.46	3.95	18.87
	B. japonicus	3	2.60	8.25	2.46	22.47
	C. pyrrhogaster	4	3.20	5.64	2.04	11.19
Lung	R. nigromaculata	3	2.03	6.17	2.65	17.06
	R. japonica	3	2.17	11.80	3.96	16.29
	R. rugosa	5	2.68	10.45	3.85	16.53
	R. limnocharis	5	2.12	4.48	2.13	10.24
	R. catesbeiana	3	3.03	6.54	3.98	15.04
	Rh. schlegelii	3	2.05	5.43	2.13	11.82
	H. a. japonica	6	2.03	6.19	3.41	16.18
	B. japonicus	3	2.00	7.83	3.05	13.42
Liver	R. nigromaculata	3	2.86	7.76	3.18	20.70
	R. japonica	3	3.27	13.94	3.06	22.12
	R. rugosa	3	3.05	11.66	2.22	23.25
	R. limnocharis	3	3.64	8.97	3.52	22.68
	R. catesbeiana	3	3.48	7.20	2.88	19.31
	Rh. schlegelii	3	2.11	5.71	2.41	12.14
	H. a. japonica	5	2.05	8.96	3.72	18.28
	B. japonicus	3	2.07	8.17	2.46	20.28
	C. pyrrhogaster	3	2.02	6.10	2.14	10.55

I_{50}, Average concentration of the inhibitor required for 50% inhibition.

species. The I_{50} values of *Bufo japonicus* and *Cynops pyrrhogaster* were 25.69 and 11.70 which were six and three times the average I_{50} value 4.23 for small intestinal alkaline phosphatases from the other six species.

The I_{50} values of *Phe* for the skins, lungs, livers, kidneys and ovaries from seven species other than *Rana japonica* and *Rana rugosa* ranged from 4.48 to 8.97. In the latter two species, the I_{50} values were between 10.21 and 13.94, with the exception of the I_{50} value for the alkaline phosphatase of the ovary from *Rana japonica* which was 5.73. The I_{50} values of *Phe* for the alkaline phosphatases of the small intestines from *Rana nigromaculata*, *Rana rugosa*, *Rana limnocharis*, *Rana catesbeiana* and *Rhacophorus schlegelii* were between 3.66 and 6.34 and similar to the I_{50} values of *Phe* for the other tissues which were 4.48 to 8.97. The I_{50} values of *Phe* for the alkaline phosphatases of the small intestines from *Hyla arborea japonica*, *Bufo*

TABLE 2

Inhibition of alkaline phosphatase activities by four kinds of inhibitors
in the kidney, ovary and small intestine

Organ	Species	No. of specimens	I_{50} (mM)			
			Har	*Phe*	*PheGlyGly*	*Leu*
Kidney	*R. nigromaculata*	3	2.22	7.54	2.99	14.20
	R. japonica	3	3.33	12.80	3.10	23.76
	R. rugosa	5	3.94	10.21	2.20	23.03
	R. limnocharis	5	3.42	7.64	2.37	20.92
	R. catesbeiana	3	3.93	7.65	2.96	18.73
	Rh. schlegelii	3	2.14	6.13	2.78	18.51
	H. a. japonica	6	2.03	6.86	3.22	18.80
	B. japonicus	3	2.04	7.65	2.58	17.42
Ovary	*R. nigromaculata*	3	2.18	7.12	2.95	19.88
	R. japonica	3	2.99	5.73	2.02	15.64
	R. rugosa	3	3.06	10.31	2.19	22.99
	R. limnocharis	4	2.70	8.58	2.48	19.65
	R. catesbeiana	3	2.35	7.50	2.08	16.81
	Rh. schlegelii	3	2.44	6.78	2.96	13.59
	H. a. japonica	4	2.48	7.43	2.51	19.96
	B. japonicus	3	2.02	7.63	2.41	15.41
Small intestine	*R. nigromaculata*	3	4.66	6.34	5.33	11.62
	R. japonica	3	6.34	16.46	6.32	43.12
	R. rugosa	4	3.49	5.27	2.68	11.03
	R. limnocharis	4	4.29	5.37	3.92	13.58
	R. catesbeiana	3	3.06	3.66	3.37	11.02
	Rh. schlegelii	3	3.91	5.26	3.78	11.34
	H. a. japonica	5	*	37.28	*	*
	B. japonicus	3	*	32.41	25.69	*
	C. pyrrhogaster	3	121.68	30.13	11.70	52.54

I_{50}, Average concentration of the inhibitor required for 50% inhibition.
* invalid

japonicus and *Cynops pyrrhogaster* were about six or seven times, being from 30.13 to 37.28, and that of *Rana japonica* was about three times, being 16.46, as compared with the average I_{50} value 5.18 of *Phe* for the alkaline phosphatases of the small intestines from the other five species.

The I_{50} values of *Leu* for the alkaline phosphatases of the skin, lung, liver, kidney and ovary somewhat differed from species to species, ranging from 10.24 in the lung of *Rana limnocharis* to 25.59 in the skin of *Rana rugosa*. The alkaline phosphatases of the small intestines from *Rana nigromaculata*, *Rana rugosa*, *Rana limnocharis*, *Rana catesbeiana* and *Rhacophorus schlegelii* showed similar I_{50} values of *Leu* which were between 11.02 and 13.58. The alkaline phosphatases of *Hyla arborea japonica* and *Bufo japonicus* were not almost inhibited by *Leu*, while the I_{50} values of *Leu* for the alkaline phosphatases of the small intestines from *Cynops pyrrhogaster* and *Rana*
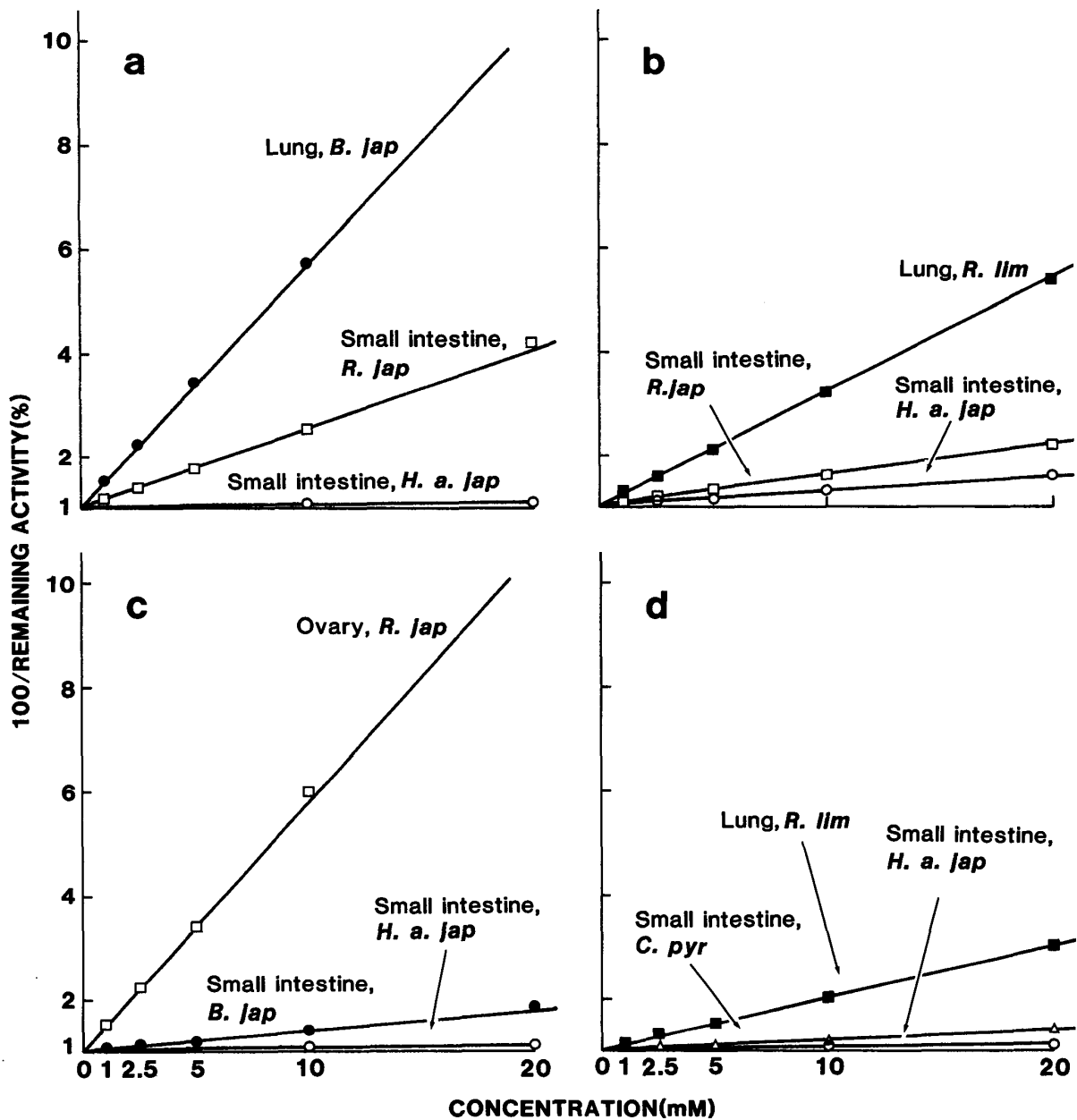
Fig. 1.   Inhibition of tissue alkaline phosphatase activities by four kinds of inhibitors in the lung, small intestine and ovary from five amphibian species.

a.   L-Homoarginine.
b.   L-Phenylalanine.
c.   L-Phenylalanylglycylglycine.
d.   L-Leucine.

R. jap, *Rana japonica*.   *R. lim*, *Rana limnocharis*.   *H. a. jap*, *Hyla arborea japonica*.   *B. jap*, *Bufo japonicus*.   *C. pyr*, *Cynops pyrrhogaster*.

japonica were 52.54 and 43.12, which were about four and three times the average I_{50} value of *Leu* for the small intestines from the other five species which was 11.72.

The foregoing results clearly show that there are two forms of alkaline phosphatases in various organs from the nine species examined.   The first form is common

to the skins, lungs, livers, kidneys and ovaries of all the species as well as to the small intestines of *Rana nigromaculata*, *Rana japonica*, *Rana rugosa*, *Rana limnocharis*, *Rana catesbeiana* and *Rhacophorus schlegelii*. The second form is found in the small intestines of *Hyla arborea japonica*, *Bufo japonicus* and *Cynops pyrrhogaster*.

II. Temperature stability

Inactivation of the alkaline phosphatases extracted from six organs of several amphibian species by high temperatures was examined (Figs. 2 and 3). Fig. 3 plots the average T_{50} value for each organ in two individuals of a given species. Temperature stability of alkaline phosphatase was found to vary from organ to organ in any given species.

The alkaline phosphatases from closely allied species did not always display similar T_{50} values. The T_{50} values of the alkaline phosphatases from the liver, kidney, skin, lung and ovary of *Rana limnocharis* were from 61.6 to 65.0 which were
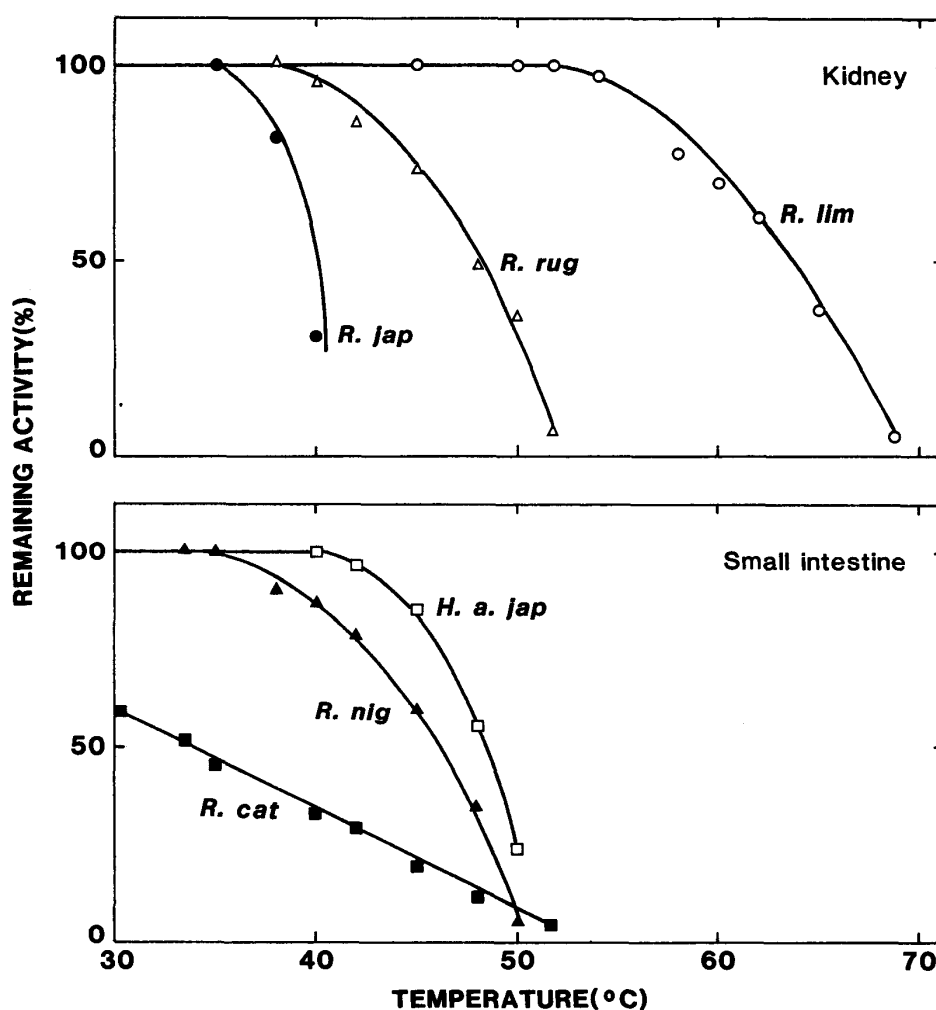

Fig. 2. Inactivation of alkaline phosphatases of the kidney and small intestine by heating for 30 minutes in amphibian species.
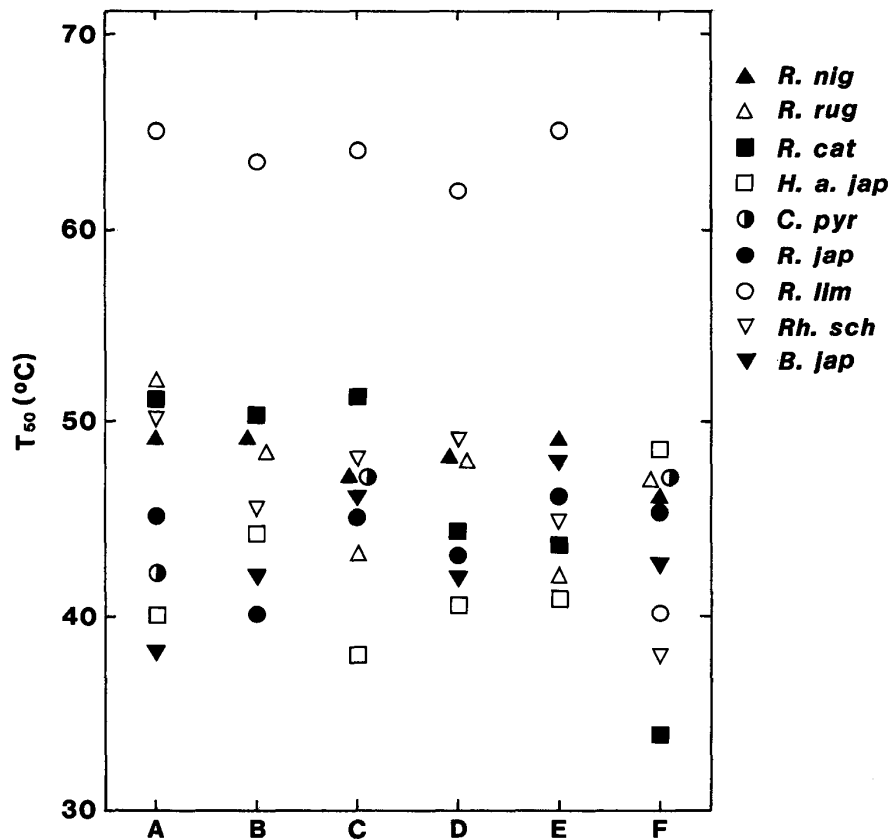
Fig. 3. T_{50} values of six organs from nine amphibian species.
A. Liver. B. Kidney. C. Skin. D. Lung. E. Ovary. F. Small intestine.
R. nig, Rana nigromaculata. R. rug, Rana rugosa. R. cat, Rana catesbeiana. H. a. jap, Hyla arborea japonica. C. pyr, Cynops pyrrhogaster. R. jap, Rana japonica. R. lim, Rana limnocharis. Rh. sch, Rhacophorus schlegelii. B. jap, Bufo japonicus.

far greater than the T_{50} values of those of the other species, while the alkaline phosphatase from the small intestine of Rana limnocharis was 40.1.   The T_{50} values of the alkaline phosphatases from the liver, kidney, skin, lung, ovary and small intestine of Rana rugosa were between 42.3 and 51.8.

The T_{50} values of the alkaline phosphatases from the liver, kidney, skin, lung and ovary of Rana catesbeiana were from 43.5 to 51.3, while that from the small intestine was 33.9.   The T_{50} values of the alkaline phosphatases from all the six organs of Rana nigromaculata were from 46.0 to 49.0.

The T_{50} values of the alkaline phosphatases from all these organs of Rana japonica were between 40.1 and 46.3.   The T_{50} values of the alkaline phosphatases from the organs other than the small intestine of Rhacophorus schlegelii were from 45.4 to 50.0, while that from the small intestine was 37.0.

The T_{50} values of the alkaline phosphatases from the organs other than the small intestine of Hyla arborea japonica were between 37.8 and 44.0, while that of the small intestine was 48.4.   The T_{50} values of the alkaline phosphatases from all the six organs of Bufo japonicus were between 38.0 and 48.3.   The T_{50} values of the alkaline phosphatases from the liver, skin and small intestine of Cynops pyrrhogaster

were 42.3, 47.1 and 47.0, respectively.

The foregoing results show that *Rana limnocharis* remarkably differs from the other eight species in temperature stability of the alkaline phosphatases from various organs, although the alkaline phosphatase from only the small intestine differs from those of the other organs. A similar difference in temperature stability of alkaline phosphatases between the small intestine and the other organs is also found in *Rana catesbeiana* and *Rhacophorus schlegelii*.

Hyla arborea japonica differs from all the other species in that the T_{50} value of the alkaline phosphatase of the small intestine is higher than those of the other organs in contrast to the above three species.

DISCUSSION

FISHMAN (1974) has documented that the alkaline phosphatases extracted from human liver, bone, placenta and small intestine exist in multiple forms. By quantitative studies of five inhibitors on the alkaline phosphatases in various human organs, MULIVOR, PLOTKIN and HARRIS (1978) have confirmed there are three clearly distinct categories of alkaline phosphatases, one being in the liver, bone and kidney, another being in the intestine and the remainder being in the placenta. McKENNA, HAMILTON and SUSSMAN (1979) and SEARGEANT and STIN-SON (1979) have also recognized three forms of the alkaline phosphatases in human organs, 1) bone, kidney and liver, 2) placenta and 3) intestine. GOLDSTEIN, ROGERS and HARRIS (1980) have confirmed by quantitative inhibition and thermostability studies that there are only two forms of alkaline phosphatases in mammalian organs, 1) liver, bone, kidney and placenta and 2) intestine. While in the first form, alkaline phosphatases show no significant variation among different species, the I_{50} values for the intestinal alkaline phosphatases in the second form vary with species to a much greater extent.

YORA and SAKAGISHI (1986) have clarified the inhibitory effects of L-homoarginine and L-phenylalanine on the alkaline phosphatases in the livers, kidneys and intestines of three amphibians, *Rana catesbeiana*, *Xenopus laevis* and *Cynops pyrrhogaster*. The same authors showed that the alkaline phosphatases in the livers and kidneys of these three amphibian species were sensitive to L-homoarginine than to L-phenylalanine and the alkaline phosphatases in the intestine of *Rana catesbeiana* were more sensitive to these two inhibitors than those in the intestine of *Cynops pyrrhogaster*.

In the present study, the alkaline phosphatases of the skins, lungs, livers, kidneys and ovaries from nine species exhibited nearly the same sensitivity to four kinds of inhibitors, L-homoarginine (*Har*), L-phenylalanine (*Phe*), L-phenyl-alanylglycylglycine (*PheGlyGly*) and L-leucine (*Leu*), although all the organs of *Rana japonica* and *Rana rugosa* showed slightly higher I_{50} values for *Phe* than those for the other inhibitors. The inhibiting effects of *Har* and *PheGlyGly* were generally strong, *Phe* was moderate and *Leu* was slight. The I_{50} values of the small intestines for all the four inhibitors were equivalent as a whole to those of the other

organs from six species, *Rana nigromaculata, Rana japonica, Rana rugosa, Rana limnocharis, Rana catesbeiana* and *Rhacophorus schlegelii*, although the I_{50} values for all the inhibitors of *Rana japonica* were somewhat higher. In three species, *Hyla arborea japonica, Bufo japonicus* and *Cynops pyrrhogaster* on the other hand, the alkaline phosphatases of the small intestines differed markedly from those of the other organs in insensitivity to all the four inhibitors.

While YORA and SAKAGISHI (1986) have reported that the alkaline phosphatases in the intestine of *Cynops pyrrhogaster* were more sensitive to *Har* than to *Phe*, the present study has clarified that they are more sensitive to *Phe* than to *Har*. Such a difference may suggest a different molecular structure of the intestinal alkaline phosphatases between these two populations.

The present study on the inhibitors on the alkaline phosphatases of various organs from nine amphibian species seems to indicate that there are two forms in inhibitor-binding sites. The first form in which the concentrations of inhibitors required for I_{50} were nearly the same was found in the alkaline phosphatases of the skins, livers, lungs, kidneys and ovaries of all the species and the small intestines of *Rana nigromaculata, Rana rugosa, Rana limnocharis, Rana catesbeiana* and *Rhacophorus schlegelii*. The second form in which the concentrations of inhibitors required for I_{50} remarkably differed from one another was found in the alkaline phosphatases of the small intestines of *Hyla arborea japonica, Bufo japonicus* and *Cynops pyrrhogaster*. This seems somewhat agreeable with the alkaline phosphatases observed in mammalian organs by GOLDSTEIN, ROGERS and HARRIS (1980). While the alkaline phosphatases of the skins, livers, lungs, kidneys and ovaries of all the species of amphibians have remained fairly constant during evolution, those of the small intestines seem to have diverged into two forms, one of which has remained almost constant, while the other has made a distinct divergence. According to CEI (1963) and SALTHE and KAPLAN (1966), the genera *Rana* and *Rhacophorus* are widely distant in evolution from the genera *Hyla* and *Bufo* which are primitive among the anura. The alkaline phosphatases of the intestines in *Hyla arborea japonica* and *Bufo japonicus* seem to have changed into a different way from those of *Rana* and *Rhacophorus*.

The alkaline phosphatases in the intestine of *Rana japonica* showed higher I_{50} values for all the inhibitors than those in the intestines of *Rana nigromaculata, Rana rugosa, Rana limnocharis, Rana catesbeiana* and *Rhacophorus schlegelii*. On the other hand, the alkaline phosphatases in the skins, lungs, livers and kidneys of *Rana japonica* and *Rana rugosa*, as well as in the ovary of *Rana rugosa* were less sensitive to *Phe* than those of the same organs of the other species. It is likely that such insensitive enzymes have arisen more recently than the sensitive enzymes which are characteristic to the other species.

KURAMOTO (1978) examined embryonic tolerance to heat in the anuran species distributed in Japan. He has shown that *Rana limnocharis* is the most heat tolerant with an upper limit of about 43°C. The present investigation on the temperature stability of the alkaline phosphatases gives a similar result. In eight of the nine species examined in the present study, alkaline phosphatases of all the six organs

lost about 50% of their activity at the temperatures between 35°C and 50°C for 30 minutes, in contrast to that those of all the organs other than the small intestine of *Rana limnocharis* did not lose their activity at these temperatures. While in *Rana limnocharis*, the alkaline phosphatase in the small intestine lost about 50% of its activity at about 40°C, those of the other organs showed 50% inhibition at the temperature of roughly 65°C. According to NAKAMURA and UENO (1963), *Rana limnocharis* is a comparatively southern species as compared with the other Japanese species used in the present study. It is probable that the alkaline phosphatases of all the organs other than the intestine adapted to higher temperatures, while the alkaline phosphatase of the intestine remained at nearly the same degree of heat resistance as those of all the organs of the other amphibian species.

It is remarkable that the difference in temperature tolerance between the alkaline phosphatase of the small intestine and those of the other organs was also confirmed in *Rana catesbeiana*, *Rhacophorus schlegelii* and *Hyla arborea japonica*.

ACKNOWLEDGMENTS

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