PRIMATE PLACENTAL ALKALINE PHOSPHATASE

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1. Introduction

In [1] we examined placental alkaline phosphatases of the 3 ape species chimpanzee, gorilla and orangutan. The chimpanzee and orangutan placental phosphatases resembled the human isoenzyme, in that they were heat-stable, phenylalanine-sensitive and cross-reactive with antibody to human placental phosphatase. The gorilla placental extracts had almost 3 orders of magnitude lower enzyme activity than the human, chimpanzee and orangutan placental extracts and the gorilla enzyme did not resemble the human.

These results complemented other reports [2–5] of the absence of the 'human' isoenzyme in lower primates and a number of other mammalian species. This suggested to us that the human placental isoenzyme may have appeared relatively late in primate evolution, but the absence of a similar isoenzyme in gorillas was puzzling.

Here, we extend our previous results on ape species and examine the placental phosphatases of several other lower primates.

2. Methods

Enzyme assay, isolation of the IgG fraction, and assay of immunochemical reactivity of the alkaline phosphatases were as in [6]. Briefly, the assay of immunochemical reactivity is performed by binding of enzyme–antibody complexes to formalin-fixed *Staphlyococcus* A cells.

3. Results and discussion

A summary of our characterization of the primate placental phosphatases is given in table 1. Both chimpanzee species and 2 out of 3 orangutans had a heat-stable placental alkaline phosphatase at levels within that found in the human population. Three gorilla placentae had 3 orders of magnitude less heat-stable enzyme than the average human placenta. The 3 orangutan placentae had a range of heat-stable activity of 2 orders of magnitude, from within 1 SD of the mean human assay value to 2 orders of magnitude lower.

Consistent with [2–5], the old world monkey species Sumatran Macaque, African green, Langur and Colobus had very low levels of heat-stable enzyme but had readily measurable levels of heat-labile enzyme

In the spider monkeys, the levels of heat-stable enzyme varied over 4 orders of magnitude, from 1/10th the mean level in humans to levels comparable to those in old world monkeys. Heat-stable enzyme was also found in the single squirrel monkey placenta tested.

Two characteristics used to distinguish the human placental isoenzyme, in addition to heat stability, are its sensitivity to amino acid and peptide inhibitors, and its reactivity with specific antisera. We used the 2 inhibitors L-phenylalanine [7] and L-phenylalanylglycylglycine [8] and rabbit and rhesus monkey antisera to the human placental enzyme to compare the heat-stable enzymes from these sources.

Fig.1 contains the inhibition data. All enzymes were sensitive to inhibition by L-phenylalanine, 50% inhibition being attained at 1 mM (human)–5 mM (orangutan). L-Phenylalanylglycylglycine was a much more selective inhibitor of the human and ape enzymes, in that 50% inhibition was attained at 0.1 mM for the human, chimpanzee (not shown) and pygmy chimpanzee enzymes, 0.6 mM for the orangutan enzyme, and 10 mM for the squirrel and spider monkey enzymes. In this respect, the enzyme from

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Table 1
Levels of alkaline phosphatase and heat-stable alkaline phosphatase in placental extracts from primate species

Primate	Total activity	Heat-stable activity[b]	
Human	4.0 ± 1.8 (0.2 – 14)[a]		(>95)[c]
Chimpanzee	8.0	7.5	(94)
Pygmy chimpanzee	0.91	0.84	(92)
Gorilla	0.01	0.0023	(23)
	0.003	0.0009	(31)
	0.002	0.0005	(25)
Orangutan	2.7	2.3	(85)
	0.42	0.21	(50)
	0.37	0.04	(9)
Sumatran macaque	1.2	–	(<0.5)
	0.7	–	(<0.5)
African green monkey	0.39	–	(<0.5)
Colobus monkey	1.2	–	(<0.5)
Douc langur	0.44	–	(<0.5)
	0.08	–	(<0.5)
Hanuman langur	1.34	–	(<0.5)
	0.19	–	(<0.5)
Spider monkey	0.54	0.46	(85)
	0.46	0.41	(89)
	0.40	0.30	(75)
	0.06	0.034	(56)
	0.04	0.015	(38)
	0.02	0.002	(10)
	0.01	0.0009	(9)
	0.03	0.0015	(5)
	0.03	–	(<0.5)
	0.02	0.001	(5)
Squirrel monkey	0.11	0.057	(52)

[a] Value is mean ± SD for 200 placental extracts assayed at random – range of values is in parentheses; [b] heated at 65° C for 10 min; [c] percent heat-stable activity in parentheses

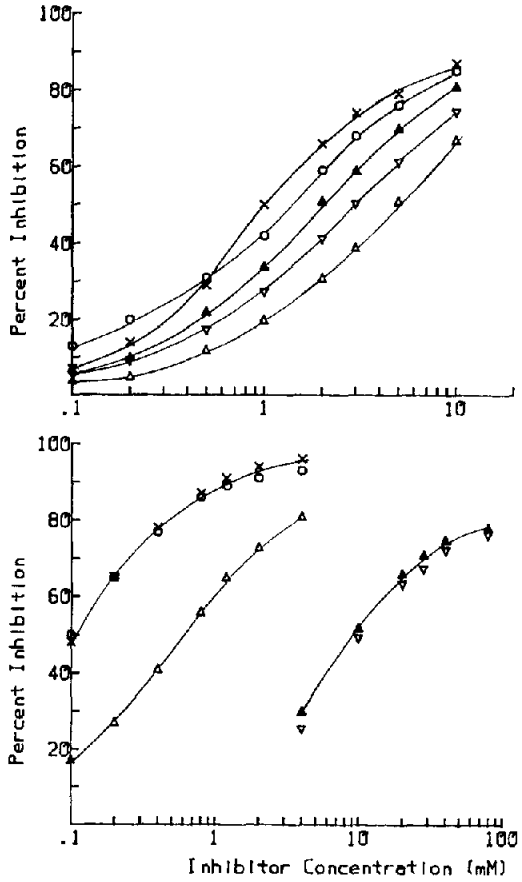

Fig.1. Inhibition of heat-stable placental alkaline phosphatases from primate species by L-phenylalanine (top) and L-Phe–Gly–Gly (bottom). Enzymes tested were: (×) human; (○) pygmy chimpanzee; (△) orangutan; (▽) spider monkey; (▲) squirrel monkey.

the 2 new world monkey species more closely resembled the 'Nagao' isoenzyme found in some cancer patients (50% inhibition at 3 mM) than the human placental enzyme.

Three antisera were used to investigate the homology of primate placental phosphatases. Early (3 week) and hyperimmune (6 month) antisera each pooled from 3 rabbits, and early (6 week) antiserum from a single rhesus monkey were tested. Early and late antisera were compared, since we had found with the placental and intestinal phosphatases that partial homology could readily be identified with hyperimmune antisera, and differences with early antisera [9].

Fig.2 shows the result of this experiment. Using the monkey and the early rabbit antisera, significant

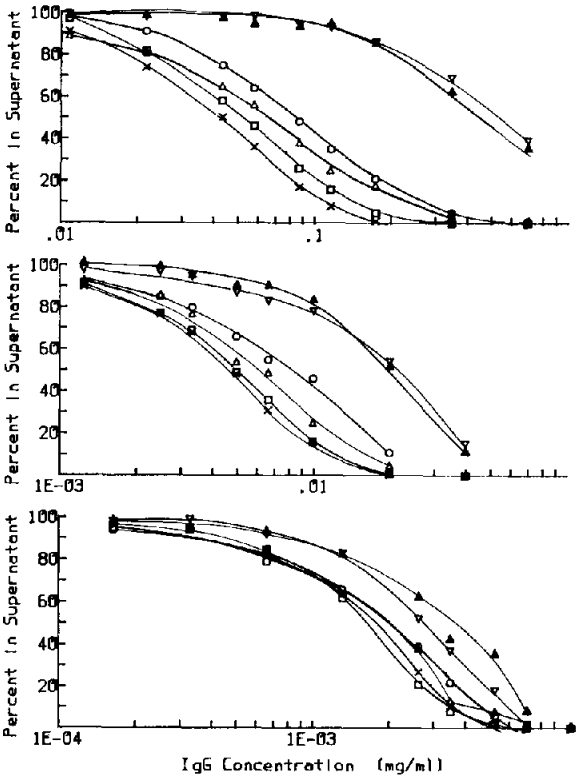

Fig.2. Binding of heat-stable placental alkaline phosphatases from primate species to antibody prepared against human placental alkaline phosphatase: (top) rhesus monkey antiserum collected 6 weeks after primary immunization; (middle) rabbit antiserum, 3 weeks after primary immunization; (bottom) rabbit antiserum, 6 months after primary immunization, with repeated booster injections. Enzymes used are: (×) human; (□) chimpanzee; (○) pygmy chimpanzee; (△) orangutan; (▽) spider monkey; (▲) squirrel monkey.

differences among the placental phosphatases of the distantly related species are demonstrated. These differences are much less remarkable when hyperimmune rabbit antiserum is used to test for homology. Table 2 shows that an order of magnitude more monkey IgG was needed to achieve the equivalent binding of the squirrel and spider monkey enzymes than the homologous human enzyme antigen using hyperimmune rabbit antiserum.

Comparing the 'early' antisera, the rhesus monkey antiserum was obtained 6 weeks after primary immunization with 200 μg enzyme in incomplete Freund's adjuvant and the rabbit antiserum was obtained 3 weeks after immunization with 50 μg enzyme in complete Freund's adjuvant. The differences in the reactivity of antisera produced from these 2 protocols could be due to the species immunized, the adjuvant used, or the amount injected. We do not have a preferred explanation of the differences in relative reactivity of these two antisera. We note the similarity between them; i.e., the ratio of antiserum concentrations required for 50% binding follows the pattern human < chimpanzee < orangutan < pygmy chimpanzee ≪ spider monkey = squirrel monkey. This strongly suggests a pattern of relative structural homology of these placental phosphatases, confirmed using 2 very distantly related species for immunization.

These results argue against the hypothesis that appearance of 'human' placental phosphatase is a late evolutionary event restricted to several apes and man [1,4]. The orangutan and spider monkey samples were particularly interesting since the levels of 'human' placental-type phosphatase varied over 2 and 3 orders of magnitude, respectively, in these 2 species.

In only 2 cases in humans was there an apparent

Table 2
Antibody concentration at which 50% of the placental phosphatases from various species were bound

Enzyme	Rhesus monkey antiserum	3-week rabbit antiserum	6-month rabbit antiserum
Human	0.04	0.005	0.002
Chimpanzee	0.05 (1.25)	0.005 (1.0)	0.002 (1)
Pygmy chimpanzee	0.08 (2.0)	0.007 (1.4)	0.002 (1)
Orangutan	0.06 (1.5)	0.006 (1.2)	0.002 (1)
Spider monkey	0.5 (12.5)	0.02 (4)	0.003 (1.5)
Squirrel monkey	0.5 (12.5)	0.02 (4)	0.003 (1.5)

Ratio in parentheses is the ratio of the antibody concentration to that for human enzyme; Data of fig.2

complete absence of placental alkaline phosphatase, in twins both suffering from Crouzon's disease [10]. Based upon a relative overabundance of rare 'homozygous' phenotypes, a null allele was suggested [11]. Among common human allozymes, the 'I' variant is known to have a lower enzyme activity in placentae [12], which appears due to a lower level of enzyme synthesis, since specific activity of pure enzyme is the same as for more common variants [13]. Several rare allozymes also appear to have lower enzyme activity [14]. These examples in the human population could account for some of the variation on other primates, if we assume that analogues of rare variants in humans are more common in some of these species. Two alternatives to allozyme differences are regulation of enzyme level by physiological influences or a regulatory locus which has polymorphic variants.

The 'human' placental phosphatase has an unusually variable tissue-specific isoenzyme expression in related species. It is reasonable to argue that it is not essential for 'primate' placental function. Teleologically, it is difficult to accept the idea that an enzyme which contributes 1% of the total membrane protein is not functional. The apparent selection against some allozymes [15–17] also suggests a role for this enzyme in human pregnancy. One hypothesis is that this role is in the regulation of some aspect of placental metabolism. An alternative to this hypothesis, is that an essential metabolic function is catalyzed either by the placental phosphatase or some alternative pathway, which in the absence of the phosphatase is the principal route. In some old world monkeys, high levels of an alkaline phosphatase which does not resemble the human enzyme are present, but this is not the case in gorillas and some spider monkeys.

The most important, hitherto unanswered, question in all investigations on alkaline phosphatase remains its function. This study points to 2 interesting primate 'models' in elucidating this function for the human isoenzyme, the spider monkey and the squirrel monkey.

Acknowledgements

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