BRIEF COMMUNICATION

A Salivary Protein Polymorphism Found in the Extra-Parotid Secretion of Macaques

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Abstract Macaque salivary proteins corresponding to human proline-rich proteins showed monomorphic in all 38 salivas, but another far-anodally migrating protein, absent in parotid salivas, exhibited a polymorphism in the samples of *Macaca fuscata*.

Keywords Salivary protein, Polymorphism, Macaque

Introduction

As genetic markers for population surveys, most of attention has been concentrated on the blood group antigens, serum proteins, and red cell enzymes. For the last decade, however, genetic studies of human salivary proteins have been developed, and several polymorphisms have been reported (AZEN, 1978). In man some salivary proteins are proving to be promising genetic markers for population genetic surveys, for paternity testing as well as anthropological investigations (IKEMOTO *et al.*, 1979). In order to find out such polymorphisms in non-human primates, we have examined salivary proteins in the secretion of macaque monkeys. It is the purpose of this paper to describe the existence of intra-species variation in a salivary acidic protein observed in Japanese macaques (*Macaca fuscata*).

Material and Methods

Thirty-seven individuals of Japanese macaque were used in this investigation. They were maintained in the University Animal Center on a normal monkey diet, after having captured in and around Suzuka district, Mie Prefecture. Another animal of *Macaca fascicularis*, imported from Java, was also included for comparison. Prior to saliva collection, the animals were anesthetized by ketamine HCl injection each at dosages of 0.1 ml per kg body weight. Salivary flow was stimulated by approximately 1 mg/kg body weight of pilocarpine HCl, and whole salivas were obtained into glass beakers by drooling. At each collection, approximately 20 ml or more liquids were obtained per animal within 20 minutes. The solution consisted of parotid, submandibular, sublingual and minor salivary gland

secretions. In some selected individuals (bigger animals) parotid samples were also collected by inserting small plastic tubes into the salivary ducts. Usually less than 2 ml of the parotid secretion was obtained. The samples were immediately lyophilized after centrifugation and kept frozen. The dried samples were dissolved in distilled water with five times concentration just before use.

Electrophoresis in 10% polyacrylamide gels (PAG) was performed as described by TAGGART *et al.* (1978). The tris buffer, pH 8.8, of their System A was used throughout. Two staining procedures were employed after electrophoresis: One was a dimethoxybenzidine (DMB) method for detecting acidic proline-rich proteins. The solution contained 0.02 M DMB in 0.02 M acetate buffer, pH 3.3, with 0.1 ml of hydrogen peroxide per 100 ml (AZEN & DENNISTON, 1974). The protein patterns appeared as white band against a dark brown background. The other was a coomassie brilliant blue R-250 method for ordinary protein staining. The method described by STECK *et al.* (1980) was utilized, thus the solution contained 0.12% dye in 5% formaldehyde. As far as our experience was concerned, salivary proteins were best visualized by this method.

Results and Discussion

Figure 1 shows the electrophoretic pattern of acidic proline-rich proteins in macaque and human saliva samples, after staining with DMB. Using alkaline PAG electrophoresis and a DMB stain, salivary polymorphic proteins, such as PR, PA and DB (not shown), are easily recognized in human parotid salivas. Under the condition, at most 4 bands for PR, 2 for DB and

1 for PA are visible in an individual human sample. On the other hand, the present macaque samples disclosed only one major, somewhat diffuse, zone of the proline-rich protein. The same pattern was observed in all 38 different macaques in both whole salivas and parotid samples.

OPPENHEIM *et al.* (1982, 1985) have purified and characterized a macaque proline-rich phosphoglycoprotein (MPRP) from the parotid saliva of *Macaca fascicularis*. We believe the salivary protein showing a single diffuse zone on PAG corresponds to the MPRP of OPPENHEIM *et al.* According to them, the broad band can be explained by the fact that MPRP is a glycoprotein whose electrophoretic pattern may be the result of microheterogeneity in the carbohydrate units.

LEVINE *et al.* (1978) have observed several immunological similarities between human and macaque (*Macaca arctoides*) salivary proteins, and OPPENHEIM *et al.* (1982) have demonstrated that the chromatographic patterns of parotid saliva proteins of both human and *M. fascicularis* are of sufficient closeness. However, the electrophoretic patterns from the two species revealed considerable differences in most salivary proteins. For instance, the MPRP was dense in intensity and appeared to be one of the main constituents of macaque salivas, while the human counterpart was divided into several discrete bands on the middle parts of PAG. There were two main amylase bands, each accompanying with a few minor isoenzymes, in Japanese macaques. They show an extensive polymorphism, as already reported (UDA *et al.*, 1985, 1986; ISHIMOTO, 1986). In contrast, the human enzymes were present in multiple forms constantly in most human samples.

Another difference between the macaque

Fig. 1. Photograph showing acidic proline-rich protein phenotypes in human and macaque salivas. The PAG was stained with a DMB solution. Anode is at the top. Samples are from the left; human PR 1 PA 0, human PR 1-2 PA 1, human PR 2 PA 1, and four macaques.

and human secretions was seen on the anodal part of PAG. In macaque samples one or two main bands each with faster minor ones were visualized in the more anodally migrating region than that of MPRP, whereas the human analogues could not yet be identified clearly. This far-anodally migrating protein seemed to show a polymorphism, as can be seen in Fig. 2. Three phenotypes could be classified among 37 whole saliva samples of *M. fuscata*: Presumed homozygotes exhibited a single fast, more anadally migrating or slow, less anodally migrating band and presumed heterozygotes exhibited roughly equal intensities of both bands. One more sample of another macaque had the same phenotype as the most common of *M. fuscata*. The result is given in Table 1. Although the sample number is not large enough, there is a possibility to utilize the "Fast" protein

Fig. 2. Photograph showing salivary "Fast" protein polymorphism in the macaques. The PAG was stained with Coomassie Blue. Only the distal one-third from the origin is shown. The dark zones at the bottom of the figure are MPRPs, the samples of the left edge is from *M. fascicularis*, and the others are all from *M. fuscata*.

Table 1. Distribution of salivary "Fast" protein types in the macaques.

	Types		
	F	FS	S
<i>Macaca fuscata</i>	19	13	5
<i>Macaca fascicularis</i>	1		

system as a genetic marker.

Interestingly, we found that this polymorphic protein is absent or extremely diminished in parotid salivas. This suggests that it may be synthesized and secreted by the submandibular and/or sublingual gland. Our preliminary investigation indicates that the "Fast" protein has less molecular weights than those of MPRP, and contains half-cystines in the amino acid composition. Therefore, we consider that the present macaque "Fast" protein might be an analogue of one of the cysteine-containing phosphoproteins obtained from human submandibular-sublingual saliva (SHOMERS *et al.*, 1982).

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抄 録

マカック全唾液に見出された唾液タンパク多型

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唾液タンパク変異を37例のニホンザルで調べたところ, ヒトの多型性 proline-rich proteins に相当する MPRP は全く変異が見られなかったが, より陽極側に位置する酸性タンパクに個体差を見出した. ニホンザルでアミラーゼ以外にも唾液タンパクの遺伝標識が存在することになる. このタンパクは耳下腺液に殆んど認められず, ヒトのシステイン含有りんタンパクの1種に相当すると推測している.

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