Studies on the Electrophoretic Variation of Soluble Glutamic-Oxaloacetic Transaminase (s-GOT) in Non-Human Primates.

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ABSTRACT The electrophoretic variation of soluble glutamic-oxaloacetic transaminase (s-GOT) in red cells of 26 species of non-human primates including one hybrid species and those in liver homogenates of 9 *Macaca* species were investigated. It was demonstrated that red cell GOT is present widely in the non-human primates so far examined and the strength of the zymograms were nearly equal. The intraspecific variation of s-GOT was confirmed in *Macaca cyclopis* and further discovered in *Aotes trivirgatus*, *Macaca fascicularis* and *Pan troglodytes* by starch gel electrophoresis. Besides the intraspecific variation, the *Macaca* species examined were divided into two groups according to the electrophoretic mobility of the common s-GOT isozyme: in the one group (*M. arctoides*, *M. assamensis* and *M. radiata*), it is faster than that in the other group (*M. fuscata*, *M. fascicularis*, *M. cyclopis*, *M. mulatta*, *M. nemestrina* and *M. niger*).

INTRODUCTION

Glutamic-oxaloacetic transaminase (GOT), known as aspartate aminotransferase (EC. 2. 6. 1. 1), catalyzes reversible conversion of L-aspartate and α -ketoglutarate to oxaloacetate and L-glutamate. This enzyme exists in both cytoplasmic (soluble) and mitochondrial fractions. The electrophoretic variation of soluble GOT was reported in the human red cells, especially in Mongoloid populations (CHEN and GIBLETT, 1971). Two infrequent variants, designated GOT 2-1 and GOT 3-1, have been found by starch gel electrophoresis,

which are controlled by an autosomal, codominant inheritance. The structural gene for human s-GOT was assigned to the chromosome 10 by somatic cell hybridization (CREAGAN *et al.*, 1973).

In primates, the electrophoretic variations of red cell GOT have so far been reported in *M. cyclopis*, *M. fuscata* and *M. nemestrina* (ISHIMOTO and KUWATA, 1974; ANDERSON and GIBLETT, 1975). Furthermore, it was demonstrated that in enzyme activities of red cell glutamic-pyruvic transaminase (GPT) which is one of the transaminases like GOT there were significant differences among primate species, not reflecting the phyloge-

netic relationships (UEDA *et al.*, 1980). In this paper, we have investigated the distribution of red cell GOT and its electrophoretic variations among 26 species of non-human primates including one hybrid species and those in liver homogenates of 9 *Macaca* species.

MATERIALS AND METHODS

The species of non-human primates and the number of samples examined in red cell GOT are shown in Table 1. Fresh blood was anticoagulated with heparin and the red cells were washed three times

with physiological saline and kept frozen at -20°C before use. Besides red cell samples, liver specimens of 9 *Macaca* species were obtained and stored frozen at -85°C until use. The liver samples were homogenized with 3 to 5 volumes of cold distilled water and the supernatants were used after centrifugation.

Horizontal starch gel electrophoresis was carried out for 18 hr at 4°C at 6–8 V/cm, using 0.1 M Tris–0.1 M maleic acid–0.01 M EDTA–0.01 M MgCl_2 buffer (pH 7.4) as the bridge buffer. The gels were prepared containing 12% starch in a 1 in

Table 1 Materials examined in red cell GOT.

Genus and Species		Number
<i>Tupaia glis</i>	(common treeshrew)	1
<i>Galago crassicaudatus</i>	(grand galago)	7
<i>Nycticebus coucang</i>	(slow loris)	3
<i>Saimiri sciurea</i>	(common squirrel monkey)	5
<i>Ateles geoffroyi</i>	(Central American spider monkey)	1
<i>Aotes trivirgatus</i>	(night monkey)	14
<i>Cebus capucinus</i>	(white-throated capuchin)	1
<i>C. apella</i>	(tufted capuchin)	3
<i>Saguinus oedipus</i>	(cotton-top tamarin)	17
<i>Callithrix argentata</i>	(silvery marmoset)	6
<i>Cercopithecus aethiops sabaesus</i>	(green monkey)	2
<i>Erythrocebus patas</i>	(patas monkey)	3
<i>Papio hamadryas</i>	(Hamadryas baboon)	2
<i>Theropithecus gelada</i>	(gelada)	2
<i>Macaca fuscata fuscata</i>	(Japanese monkey)	30
<i>M. f. yakui</i>	(Yaku Japanese monkey)	11
<i>M. mulatta</i>	(Rhesus monkey)	9
<i>M. fascicularis</i>	(crab-eating monkey)	8
<i>M. arctoides</i>	(red-faced macaque)	7
<i>M. nemestrina</i>	(pig-tailed macaque)	5
<i>M. cyclopis</i>	(Formosan monkey)	5
<i>M. radiata</i>	(bonnet monkey)	3
<i>E. patas</i> × <i>C. aethiops sabaesus</i>		2
<i>Hylobates lar</i>	(white-handed gibbon)	2
<i>H. agilis</i>	(agile gibbon)	1
<i>Pan troglodytes</i>	(chimpanzee)	15

15 dilution of the bridge buffer. In addition to the buffer system mentioned above, the another system was used in some instances following CHEN and GIBLETT (1971). The latter system consisted of 0.1 M Tris-citrate buffer (pH 7.5) as the bridge buffer and a 1 in 10 dilution of the bridge buffer as the gel buffer. The specific staining of GOT was performed as described by CHEN and GIBLETT (1971).

RESULTS AND DISCUSSION

In contrast to the significant differences in the enzyme activities of red cell GPT in primates, it was demonstrated that red cell GOT occurred widely in the primates so far examined and the strength of the zymograms were nearly equal. The electrophoretic variations of soluble GOT have so far been reported in *M. cyclopis*, *M. fuscata* and *M. nemestrina* (ISHIMOTO and KUWATA 1974; ANDERSON and GIBLETT, 1975). In this study, the polymorphism was confirmed in *M. cyclopis* and further discovered in *Aotes trivirgatus*, *Macaca fascicularis* and *Pan troglodytes*. In the other species examined in this study, no electrophoretic variation was observed.

The slow variant (type 1-S) described by ISHIMOTO and KUWATA (1974) was found in 2 out of 5 specimens of *M. cyclopis* (Fig. 1) and the fast variant (type 1-F) in 4 out of 8 of *M. fascicularis* (Fig. 2). Three phenotypes of red cell GOT were observed in *Aotes trivirgatus*. As seen in Fig. 3, the zymograms indicate the presence of three phenotypes: one with relatively fast moving bands (type F),

one with relatively slow moving bands (type S) and one with both bands (type FS). Among 14 specimens, 3 were of type F, 2 were of type S and 9 were of type FS. There was one fast variant (type 1-F) in 15 specimens of *Pan troglodytes* (Fig. 4). These findings suggest that genetic polymorphism of s-GOT is widely present among non-human primate species.

Moreover, s-GOT zymograms were obtained using the liver homogenates of 9 species of the genus *Macaca*. The *Macaca* specimens comprise *M. f. fuscata* (25), *M. f. yakui* (5), *M. radiata* (7), *M. mulatta* (9), *M. cyclopis* (3), *M. assamensis* (3), *M. fascicularis* (5), *M. arctoides* (3) and *M. niger* (1). The zymograms of liver homogenates were clearly detected in all the *Macaca* species examined and the intraspecific variation was found in one of the *M. fascicularis*. The fast variant was observed in a triple banding pattern,

Fig.1. Zymograms of s-GOT (RBC) in *Macaca cyclopis*.

Fig.2. Zymograms of s-GOT (RBC) in *Macaca fascicularis*.

Fig.3. Zymograms of s-GOT (RBC) in *Aotes trivirgatus*.

Fig.4. Zymograms of s-GOT (RBC) in *Pan troglodytes*.

Fig.5. Zymograms of s-GOT (liver) in *Macaca fascicularis*.

suggesting a heterozygote (Fig. 5).

Although the Tris-citrate buffer system was originally used for electrophoresis by CHEN and GIBLETT (1971), the Tris-maleic acid-EDTA-MgCl₂ (TMEM) buffer system was found to give better separation, but tended to show strongly stained minor components anodal to the main GOT component. As seen in Figs. 2 (red cells) and 5 (liver), the enzyme patterns of s-GOT were different between red cells and liver homogenates under the TMEM buffer system used; there were faster migrating components of s-GOT in red cells while only one major component could be detected in liver homogenates. Using the Tris-citrate buffer system, however, no additional bands in red cells were observed in most of samples examined except the heterozygotes as previously reported by ISHIMOTO and KUWATA (1974).

The interspecific variation of GOT

mobilities has been reported in 8 species of non-human primates (KÖMPF *et al.*, 1971). In 28 non-human primate species examined in this study, only the intra-genic variation of the GOT mobility in the *Macaca* species seemed to be of systematic importance. The *Macaca* species examined in this study was divided into two groups according to the electrophoretic position of the common GOT isozyme using the TMEM buffer system. The zymograms obtained using red cells are shown in Fig. 6 and those using liver homogenates in Fig. 7. In the one group (*M. arctoides*, *M. assamensis* and *M. radiata*), the common s-GOT isozymes migrate faster than those in the other group (*M. f. fuscata*, *M. f. yakui*, *M. fascicularis*, *M. cyclopis*, *M. mulatta*, *M. nemestrina* and *M. niger*). The same results were obtained in all the *Macaca* species in which both liver homogenates and red cells were examined.

Fig. 6. Zymograms of s-GOT (RBC) in the *Macaca* species. Left to right: *M. cyclopis*, *M. arctoides*, *M. nemestrina*, *M. fascicularis* and *M. mulatta*.

Fig. 7. Zymograms of s-GOT (liver) in the *Macaca* species. Left to right: *M. f. fuscata*, *M. arctoides*, *M. fascicularis*, *M. assamensis*, *M. cyclopis*, *M. mulatta*, *M. radiata*, *M. f. yakui* and *M. f. fuscata*.

In the past, *M. assamensis* was considered to be closely related to *M. mulatta* (POCOK, 1926; MILLER, 1933). HILL and BERNSTEIN (1969) and FOODEN (1971), however, postulated from the behavioural and morphological studies that *M. assamensis* was more similar to *M. radiata* than to *M. mulatta*. The same conclusion was drawn recently from the data of the distribution of 30 genetic markers (SHOTAKE, 1979). The present finding on s-GOT agrees well with these observations. Moreover, FOODEN (1976) divided roughly the *Macaca* into two groups, based primarily on the structure of adult male external genitalia: *M. fuscata*, *M. fascicularis*, *M. cyclopis*, *M. mulatta*, *M. nemestrina* and *M. niger* were involved in one group (*fascicularis* and *silenus-sylvanus* group) and *M. arctoides*, *M. assamensis* and *M. radiata* in the other (*sinica* and *arctoides* group). It is interesting that this classification is consistent with the one based on the mobility of s-GOT isozymes detected in the present study.

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REFERENCES

- ANDERSON, J.E. and E.R. GIBLETT, 1975: Intraspecific red cell enzyme variation in the pig-tailed macaque (*Macaca nemestrina*). *Biochem. Genet.*, 13: 189-212.
- CHEN, S.H. and E.R. GIBLETT, 1971: Genetic variation of soluble glutamic-oxaloacetic transaminase in man. *Am. J. Human Genet.*, 23: 419-424.
- CREAGAN, R.J. TISCHFIELD, F.A. McMORRIS, S. CHEN, M. HIRSCHI, T. R. CHEN, E. RICCIUTI and F.H. RUDDLE, 1973: Assignment of the genes for human peptidase A to chromosome 18 and cytoplasmic glutamic oxaloacetate transaminase to chromosome

- 10 using somatic-cell hybrids.
Cytogenet. Cell Genet., 12: 187-198.
- FOODEN, J., 1971: Female genitalia and taxonomic relationships of *Macaca assamensis*. Primates, 12: 63-73.
- FOODEN, J., 1976: Provisional classification and key to living species of macaque (Primates: *Macaca*). Folia Primat. 25: 225-236.
- HILL, W.C.O. and I.S. BERNSTEIN, 1969: On the morphology behaviour and systematic status of the Assam macaque (*Macaca assamensis* MCCCELLAND, 1839). Primates, 10: 1-17.
- ISHIMOTO, G. and M. KUWATA, 1974: Studies on human glutamic-oxaloacetic transaminase variation, including enzyme patterns in some non-human primates. Jpn. J. Human Genet., 18: 364-372.
- KÖMPF, J., RITTER, and J. SCHMITT, 1971: Transspecific variability of glutamic oxaloacetic transaminase (E.C. 2.6.1.1.) in primates. Humangenetik, 13: 72-74.
- MILLER, G.S., 1933: The groups and names of macaques. In: The Anatomy of the Rhesus Monkey, C. G. Hartmann and W. L. Struss (eds.), Baillière, Tindall and Cox, London.
- POCOK, R.I., 1926: The external characters of the catarrhine monkeys and apes. Proc. 2001. Soc. Lond. for 1925, pp. 1479-1579.
- SHOTAKE, T., 1979: Serum albumin and erythrocyte adenosine deaminase polymorphism in Asian macaques with special reference to taxonomic relationships among *Macaca assamensis*, *M. radiata*, and *M. mulatta*. Primates, 20: 443-451.
- UEDA, S., O. TAKENAKA and K. OMOTO, 1980: Glutamic-pyruvic transaminase (GPT) in non-human primates, I. its activities and electrophoretic variation in red blood cells. J. Human Evol., 9: 333-336.

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電気泳動法による霊長類 s-GOT の変異の検索

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可溶性分画に存在するグルタミン酸オキザロ酢酸アミノ基転移酵素 (s-GOT) の、ヒト以外の霊長類における変異に関し、デンブングル電気泳動法にて検索を行なった。

26種の霊長類の赤血球ならびに9種のマカク属の肝を調べた結果、すべての種で s-GOT ザイモグラムがみられ、先に報告のあるタイワンザルで種内変異が観察された。また、あらたにヨザル、カニクイザル、チンパンジーで種内変異が観察され、s-GOT の多型がヒト以外の霊長類においても広く存在することが示唆された。

更に、種内変異とは別に、s-GOT の一般にみられる表現型の電気易動度の差異により、今回調べられたマカク属は2つのグループにわけられた。すなわち、ベニガオザル、アッサムモンキー、ボンネットモンキーにおける s-GOT は、ニホンザル、カニクイザル、タイワンザル、アカゲザル、ブタオザル、クロザルのそれよりも速い易動度を示している。この結果は、HILL と BERNSTEIN (1969), FOODEN (1971) (1976) の、形態学的特徴から為されたマカク属の分類ならびに遺伝標識による SHOTAKE (1979) の結果と良く一致している。