Polymorphism of the group-specific component in Japanese monkeys as revealed by two-dimensional gel electrophoresis

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SUMMARY

The polymorphism of group-specific component (Gc) in Japanese monkeys (Macaca fuscata fuscata) was analyzed by two-dimensional gel electrophoresis. Two phenotypes were detected by two-dimensional gel electrophoresis. These were designated as Gc2 and Gc2−2A1 according to the nomenclature described by Constans et al.1) in comparison with the phenotypes detected by conventional isoelectric focusing electrophoresis followed by immunofixation with anti-human Gc. The phenotype frequencies in ten wild Japanese monkeys were 70% for type Gc2 and 30% for type Gc2−2A1. The Gc typing in Japanese monkeys by two-dimensional gel electrophoresis is a useful and inexpensive method than conventional isoelectric focusing electrophoresis followed by immunofixation with antiserum.

Key words: Japanese monkey, Group-specific component, Two-dimensional gel electrophoresis

INTRODUCTION

Polymorphism of the group-specific component (Gc, Vitamin D binding protein) has been demonstrated by isoelectric focusing electrophoresis followed by immunofixation with anti-human Gc in human and non-human primates1−3). Constans et al.3) examined the Gc polymorphism in 30 Japanese monkeys (Macaca fuscata fuscata) and reported that the polymorphism was observed for three alleles, Gc2, Gc2A1 and Gc2C1. In this paper, the Gc polymorphism in Japanese monkeys detected by two-dimensional gel electrophoresis is described.

MATERIALS AND METHODS

Venous serum samples were collected from 16 Japanese monkeys in Nagano Chausuyama Zoo. The serum samples were stored at −20°C until use. Two-dimensional gel electrophoresis in the absence
of denaturing agent was performed according to Manabe et al. with minor modification. Isoelectric focusing electrophoresis (IEF) in the first dimensional gel was run in 4% polyacrylamide gel containing 0.5% Ampholine pH 3.5-9.5 and 2% Ampholine pH 5-7 in a capillary column. The second dimensional gel electrophoresis was carried out in 4-17% gradient polyacrylamide gel. Gels were stained with 0.1% Coomassie brilliant blue R-250-50% methanol -7% acetic acid.

The phenotypes of Gc were determined by isoelectric focusing electrophoresis in a polyacrylamide slab gel containing 2% Ampholine pH 4-6, followed by immunofixation with anti-human Gc (DAKO PATTS a/s, Denmark) which was used for human as well as non-human primates.

RESULTS AND DISCUSSION

Figure 1 shows the two-dimensional gel elec-
phoretic patterns of the serum proteins in Japanese monkeys, together with pH profiles. Figure 2 shows the comparison of the two-dimensional gel electrophoretic patterns of two Japanese monkeys and of the patterns of the immunofixation using anti-human Gc after two-dimensional gel electrophoresis. Two phenotypes were detected by the immunofixation. The one had a single spot and the other had two spots. When isoelectric focusing electrophoresis followed by immunofixation was performed on these samples, two phenotypes were observed, which were Gc 2 and Gc 2-2A1 according to the nomenclature described by Constans et al.3) (Figure 3). By comparing the phenotypes detected by two-dimensional gel electrophoresis with those detected by isoelectric focusing electrophoresis followed by immunofixation, we propose that two spots detected by the two-dimensional gel electrophoresis were identical with Gc 2 and Gc 2A1. Gc 2 spot was the common spot and Gc 2A1 was an additional spot more anodal than Gc 2. The electrophoretic mobility of Gc 2A1 in the second dimensional gel was greater than Gc 2 spot. The phenotype frequencies in ten wild Japanese monkeys were 70% for type Gc 2 and 30% for type Gc 2-2A1. More samples must be necessary to estimate the phenotype and gene frequencies of the Gc polymorphism in Japanese monkeys.

The proportion of polymorphic loci and the average heterozygosity per individual in Japanese monkeys were estimated to be remarkably lower than those for most other animals. As regarding the polymorphism of the serum proteins in Japanese monkeys, three genetic loci, protease inhibitor (Pi), albumin (Alb) and transferrin (Tf) as detected by starch gel electrophoresis were previously reported6-8). However, Gc polymorphism is not studied extensively in terms of the genetic study. Gc phenotypes in Japanese monkeys were detected by isoelectric focusing electrophoresis followed by immunofixation with anti-human Gc5). By two-dimensional gel electrophoresis, Gc polymorphism was detectable without Gc antiserum. Simultaneously, the variation of transferrin was also found in two-dimensional gel electrophoretic patterns (Figure 2). It is thought that two-dimensional gel electrophoresis is a very useful method for genetic study of Japanese monkeys.

Fig. 3. The patterns of Gc phenotypes of Japanese monkeys detected by isoelectric focusing electrophoresis followed by immunofixation with anti-human Gc as compared with that of human Gc 1F-1S, Gc 1F-2 and Gc 2.


要 旨

2次元電気泳動法を用いてニホンザル Gc 蛋白多型の検出法を検討した。その結果、ニホンザル血清にもヒトの血清蛋白マップの Gc 蛋白領域に多型性を観察することができた。また、これらの多型を示すスポットは抗ヒト Gc 血清を用いた免疫固定法でも確認された。すなわち、検出される 2 つの表現型は従来の等電点電気泳動法と免疫固定法を組み合わせた方法により分類された表現型と比較したところ、Gc2 型および Gc2A1 型と判定され、10例の野生ニホンザルの表現型出頻度は Gc2 型は70%、Gc2-2A1 型は30%であっ た。なお、ニホンザルの Gc 型は 2 次元電気泳動法のみでも検出可能であり、従って、2次元電気泳動法は抗 Gc 血清を必要とした従来の方法より簡便でしかも経済的な検出法といえよう。
REFERENCE