Restriction fragment length polymorphism of the rat albumin gene in Sprague-Dawley rats and its application in genetic study of analbuminemia

Hiroyasu Esumi1, Yuri Takahashi2, Shigeaki Sato2 and Takashi Sugimura2

1 Virology Division, and 2 Biochemistry Division, National Cancer Center Research Institute, 5-1-1, Tsukiji, Chuo-ku, Tokyo, Japan

Received 20 April 1982; Revised and Accepted 14 June 1982

ABSTRACT

Restriction fragment length polymorphism of the rat albumin gene was discovered in a stock of Sprague-Dawley rats by Southern blots of rat liver DNAs using cloned albumin cDNA, prAlb-1 (1), as a probe. The polymorphic DNA fragments were observed when rat DNAs were digested with either Hind III or Pst I and the difference in length of the DNA fragments in Hind III or Pst I digests was estimated as 1.4 kbp. When DNAs were digested with EcoRI, restriction fragment length polymorphism was not observed. Therefore, this polymorphic DNA was concluded to be located in the flanking sequence. Structural analysis of the cloned albumin gene showed that the polymorphism was located in the 3'-flanking sequence. With this polymorphism as a marker of the albumin structural gene, the phenotype of analbuminemia, which is an autosomally recessive trait, was found to be linked to the structural gene of albumin.

INTRODUCTION

Analbuminemic rats (2) are useful for studies on mRNA processing, because recently it was found that the nuclear RNA fraction of analbuminemic rat liver contains about the normal amount of albumin mRNA precursors (3) in spite of the absence of cytoplasmic albumin mRNA (4). Similar findings were made in β+ thalassemia recently (5-7). In β+ thalassemia it was suggested that a single base substitution in an intervening sequence of the β-globin gene is responsible for disorder of mRNA processing (8,9). In analbuminemic rats, we recently found a 7 bp deletion in an intron of the albumin gene of analbuminemic rats (10). Thus it was necessary to determine whether the disorder of albumin mRNA processing is caused by the albumin structural gene itself.

Genetic polymorphism of various gene products has been
useful in studies on genetic control of various genes. In mice, several hundred loci have already been mapped on the basis of genetic polymorphisms, such as coat color and polymorphism of enzymes. However, in rats only a few alleles have been noticed and thus genetic studies are very difficult. Until recently, only genetic polymorphisms of gene products could be used as markers in genetic studies. But in the present study we found genetic polymorphism at the level of DNA structure and used it as a marker in segregation studies. Polymorphism detected by Southern blot, particularly using cloned cDNA, as demonstrated in this paper, is a promising tool for use in molecular genetic studies. Indeed, Kan and Dozy found a restriction fragment length polymorphism in the 3'-flanking sequence of α-globin gene and clearly showed the usefulness of this polymorphism in studying sickle cell anemia (11). More recently Bell et al. (12) found restriction fragment length polymorphism at the insulin locus in humans. They used polymorphism as a marker of the insulin gene and examined the correlation between the polymorphism and diabetes mellitus. Several other workers have also reported polymorphism of DNA sequences and indicated the importance of these polymorphic DNAs as a basis for genetic studies of humans (13) especially of mitochondrial DNA (14).

Here we report a new polymorphism of rat DNA at the albumin structural gene and show the usefulness of this polymorphism in segregation studies. We also report that an mRNA processing mutation, analbuminemia in rats, is closely linked to the albumin structural gene.

MATERIALS AND METHODS

Animals

Normal Sprague-Dawley rats were obtained from the stock of rats in which the first analbuminemic rat was found. Analbuminemic rats were maintained in the Sasaki Institute, Tokyo, Japan and have been described (2).

Structural analysis of the albumin gene

The albumin gene was analyzed by the Southern blot technique with cloned albumin cDNA, prAlb-1 (1) as a probe by the method
of Southern (15) with the slight modification described previously (3). Rat DNA was prepared from liver tissue obtained by partial hepatectomy. DNA from the partial hepatectomized tissue was extracted by the method of Marmur (16). Before partial hepatectomy, all rats used were checked for the presence of serum albumin by the single radial immunodiffusion method (17) with anti-rat serum albumin antiserum. Partial hepatectomy of F1(Alb−/Alb− × Alb+/Alb+) rats and offspring of F1 × Alb−/Alb− backcrosses was performed 5 weeks after birth when the serum albumin concentration had already reached the adult level in normal rats.

Albumin cDNA clone

Cloned albumin cDNA, prAlb-1, was a generous gift from Dr. S.M. Tilghman, Fels Institute for Cancer Research, Philadelphia. This cloned cDNA was recently found to be a chimeric albumin cDNA, not colinear with albumin mRNA, consisting of approximately 600 bp cDNA from the 614th base from the ATG initiation codon and approximately 600 bp cDNA from the 1261st base from the ATG codon in the opposite direction (3). Therefore, prAlb-1 covers approximately two thirds of the albumin coding region.

Albumin gene clone

The albumin structural gene of normal Sprague-Dawley rats was cloned by the method of Sargent et al. (18). Charon 4A was used as a vector, prAlb-1 as a probe. Subcloning of the EcoR I fragment of 1.6 kbp of the 3'-end portion (19) of this albumin gene was carried out with pKH47 (20) as a vector. Details of this cloning were described elsewhere (10).

The gross restriction map is shown in Fig. 1. This is consistent with that reported by Sargent et al. (18) except for the 3'-flanking sequence. Nucleotide sequencing of the 3'-end portion of the albumin gene was carried out by the chemical method of Maxam and Gilbert (21). The nucleotide sequence of the 3'-end of albumin mRNA has been determined by Sargent et al. (19).

Chemicals


4249
Fig. 1: Restriction map of cloned rat albumin gene from ff type normal Sprague-Dawley rats. The albumin gene was cloned in Charon 4A as partially digested DNA fragment with EcoR I. The restriction map was constructed from 3 overlapping albumin clones. Lengths of fragments are expressed in kbp. R; EcoR I, H; Hind III sites. The locations of exons were determined by partial DNA sequencing with reference to the DNA sequence of cloned albumin cDNA reported by Sargent et al. (18). The 5'-end of albumin gene has not been determined yet. Therefore, the names of exons are tentatively expressed according to the report of Sargent et al. (17).

RESULTS
Digestion pattern of albumin gene of normal Sprague-Dawley rats by restriction enzymes

Among the stock of normal Sprague-Dawley rats, we found three patterns of Southern blot of the albumin gene. Representative examples of two of the three patterns are shown in Fig. 2. As clearly demonstrated, the longest fragments obtained by digestion with either Hind III or Pst 1 differed in mobility in these two types. The third pattern was a mixed pattern of these two (data not shown). The difference in mobilities of the longest fragments of the two types in Hind III and Pst 1 digests corresponded to 1.4 kbp. Apart from this difference in the longest fragment generated by Hind III or Pst 1 digestion, the fragments of the two types were indistinguishable. Moreover, when the DNAs were cleaved with EcoR I, all the fragments of the two types were indistinguishable. Therefore, the difference in pattern must be due to a difference in structure of a single site, probably of a flanking sequence. From the structure of the cloned albumin gene, the longest fragment in the Southern
Fig. 2: Southern blots of ff and ss types of rat albumin genes. DNAs were digested with EcoR I (a,b), Hind III (c,d) and Pst I (f,g and h) and subjected to electrophoresis on 1% agarose gel in parallel with end-labeled EcoR I and Hind III digests (e) or Hind III digests (i) of λ phage. The albumin gene was located by hybridization to cloned albumin cDNA, prAlb-1. a, c and f; ss type, b, d and h; ff type and g; mixture of ss type and ff type DNAs.

blot with Hind III seemed to be the 3'-fragment (Fig. 1). If the polymorphic DNA sequence is located in the 3'-flanking sequence, it would not be detected by EcoR I digestion because the EcoR I fragment containing the polymorphic DNA sequence would not hybridize to cDNA probe. We tentatively named these two polymorphic fragments s(slow moving) and f(fast moving).

Localization of the polymorphic DNA sequence

To confirm that the polymorphic DNA was the 3'-flanking sequence, we examined the pattern of the Southern blot using the 3'-end EcoR I fragment of cloned albumin gene as a probe. Results are shown in Fig. 3. When genomic DNAs of the s and f types were cleaved with EcoR I, their 1.6 kbp fragments were identical. However, on Hind III digestion, the s type DNA generated an s type fragment, and the f type an f type.
Therefore, as predicted from the structure of the cloned albumin gene (Fig. 1), the polymorphic DNA sequence must be located at the 3'-end. The nucleotide sequence of the 1.6 kbp fragment which we used as a probe in Southern blot analysis was partially determined to confirm that it contained the 3'-end of the albumin gene. The nucleotide sequence of part of this fragment is shown in Fig. 4. By referring to the nucleotide sequence of cloned albumin cDNA (19), we could identify the 3'-end of the albumin gene in this fragment. As reported in the case of many eukaryotic genes, it contained an AATAAA sequence, a poly(A) addition signal, and the 3'-end of albumin cDNA, AAAGAATATA, proposed by Sargent et al. (19). These results show that this 1.6 kbp fragment did in fact contain the 3'-end of the albumin gene. From these findings and the fact that Southern blot of EcoR I with cDNA as a probe did not detect the polymorphic DNA sequence, the polymorphic DNA sequence was concluded to be located in the 3'-flanking sequence of the albumin gene.

Digestion pattern of the albumin gene of (s x f)F1 offspring

Next we examined whether this polymorphism in length of the restriction fragment of the albumin gene with Hind III or Pst I was heritable, by making heterozygotes of the s type and f type. As described in the Materials and Methods, ss type and ff type homozygotes were selected from a stock of Sprague-Dawley rats by Southern blots of the albumin gene with Hind III after
Fig. 4: Nucleotide sequence of the 3'-end of the albumin gene
The nucleotide sequence was determined by a chemical method of Maxam and Gilbert (20). The 3'-end of the albumin gene was determined with reference to the published albumin cDNA sequence (18). Line indicates exon N. Stars indicate poly(A) addition signal.

extraction of liver DNA from partially hepatectomized liver tissue. A female ss type homozygote and a male ff type homozygote were mated and the digestion patterns of the albumin gene of four offspring were screened. All were found to be of the fs type. A representative Southern blots of the albumin gene of an F1 hybrid is shown in Fig. 5. Thus, we concluded that restriction fragment length polymorphism of the rat albumin gene was a heritable genetic polymorphism.

Southern blot of the albumin gene of analbuminemic rats and their heterologous offsprings

The structure of the albumin gene of an analbuminemic rat was indistinguishable from that of ff type Sprague-Dawley rats (Fig. 6, a). The structure of the 3'-flanking sequence of the albumin gene was of the ff type. Therefore, we made F1 hybrids by crossing ss type Sprague-Dawley rats with analbuminemic rats (ff type). As expected, all the F1 hybrids were found to have the fs type structure. A representative result on a F1 rat is shown in Fig. 6, b. Since analbuminemia has been found to be an autosomally recessive trait, all F1 hybrids were wild type
Segregation of analbuminemia and restriction fragment length polymorphism of the albumin gene

Next we examined whether analbuminemia is determined by the albumin structural gene by a segregation study of analbuminemia and restriction fragment length polymorphism in offsprings of (alb^-/alb^- x alb^+/-alb^+)F_1 (fs type) x alb^-/alb^- (ff type) backcrosses. As described above, (alb^-/alb^- x alb^+/-alb^+)F_1 rats were obtained by crossing (alb^-/alb^-)(ff type) and (alb^+/-alb^+)(ss type) rat and found to have the fs type of restriction fragment length polymorphism. Examination of two litters of backcrosses of alb^-/-alb^- and F_1 hybrids showed that 5 of 12 rats were wild type as to the presence of serum albumin and 7 were analbuminemic (Fig. 6). All the wild type offsprings of backcrosses were found to have the fs type of restriction

Fig. 5: Southern blots of rat albumin genes of the ss type, ff type and F_1 hybrids of the two. Blot hybridization was carried out as in Fig. 2. a, b and c; digests with Hind III, d, e and f; digests with Pst I. a, d; ss type, b, e; ff type, c, f; F_1 hybrid of ss and ff types.

with regard to the presence of serum albumin.
fragment length polymorphism and all those with analbuminemia were ff type. Therefore, analbuminemia seems to be completely linked to the albumin gene.

DISCUSSION

The present data clearly show the presence of restriction fragment length polymorphism of the rat serum albumin gene in a stock of normal Sprague-Dawley rats. This polymorphism was found to be inherited genetically. The structure of the rat serum albumin gene has been determined by Sargent et al. (18) who observed heterogeneity of the rat serum albumin gene in Southern blots using total genomic DNA. They suggested that the heterogeneity of the albumin gene might indicate the presence of more than one copy of the albumin gene. However, from the present data, the heterogeneity which they observed seems to be due to heterogeneity of the rats.

The rat serum albumin gene seems to be located in one locus on the genome, because in the offspring of fs type ×

Fig. 6: Segregation of analbuminemia and the polymorphic DNA sequence in alb−/alb− × alb+/alb− offspring. Southern blot of the albumin gene was carried out as described in Fig. 2 and serum albumin was determined by single radial immunodiffusion. Symbols of + and − indicate presence and absence of serum albumin, respectively. a; analbuminemic rat, b; F1 rat, c-n; offsprings of backcross.
ff type back crosses we obtained 5 fs type and 7 ff type; there were no intermediates. If albumin genes had two loci with an identical structure in homozygotes, restriction fragment length polymorphism should segregate as 3 heterozygotes and 1 homozygote. Moreover, heterozygotes should be divided into two groups, one with the same intensities of s and f fragments, and the other with three times stronger intensity of f fragments than s fragments. But this was not the case. All the bands that we obtained by Southern blots with total genomic DNA were found to be present in one contiguous albumin structural gene, judging from the cloned rat albumin gene. Therefore, in homozygotes the rat serum albumin gene must be one species. These findings indicate that the rat serum albumin gene is a unique gene: there is no indication of more than one copy.

Our data also indicate that analbuminemia is closely linked to the albumin structural gene, probably the structural gene itself. Indeed, we recently found a 7 base pair deletion in the HI intron of the albumin gene of analbuminemic rats located 5 base pairs from the 5'-end of the intron. During the "maturation" of mRNA in the nuclei of analbuminemic rat liver cells, the intron HI sequence was found not to be excised out (10). Of course we cannot conclude that analbuminemia is caused by a mutation in the albumin structural gene only from the present segregation study, which however, strongly support the possibility that analbuminemia is caused by this mutation.

ACKNOWLEDGMENTS

This work was supported by Grants-in-Aid from the Ministry of Education, Science and Culture of Japan and from the Japanese Association for the Study of Metabolism and Disease.

REFERENCES

4. Esumi, H., Okui, M., Sato, S., Sugimura, T. and Nagase, S.