[Research Paper]

Designation of the *B* Haplotypes by Restriction Fragment Length Polymorphism and its Application to Native Chicken Populations

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Abstract The *B-G* region of the major histocompatibility complex (MHC) of chickens was analyzed for restriction fragment length polymorphism (RFLP), to establish standard RFLP patterns for designating the *B* haplotypes. Chickens from 8 lines having known *B* haplotypes were used to establish standard RFLP patterns.

All *B* haplotypes were clearly differentiated by RFLP pattern, except for *B*²¹. The pattern of *B*²¹/*B*²¹ in the Cornell-N line was differed from that in the GSP line. To ascertain the usefulness of RFLP analysis for designating *B* haplotypes, DNA samples from another two lines with unknown *B* haplotypes were analyzed. A total of 10 different *B* haplotypes were differentiated when DNAs were digested with the *Hae*III restriction endonuclease.

The method was applied to analyze *B* haplotypes in native chicken populations. Each RFLP pattern was considerably complicated, consisting of 4 to 21 bands. Many haplotypes were detected, although the haplotype designation based on the RFLP analysis alone was difficult. It was shown that genetic distances estimated from RFLP patterns provide a reliable measurement for phylogenetic studies.

Key words : major histocompatibility complex, B-G antigen, RFLP, genetic distance, chicken

Introduction

The major histocompatibility complex (MHC) in chickens was initially described as the B blood group system (Briles *et al.*, 1950). The MHC consists of three regions designated *F*, *L* and *G* which encode *F*, *L* and *G* antigens, respectively (Pink *et al.*, 1977). Among these, the *G* antigen is expressed mainly at the cell surface of erythrocytes and corresponds to the *B* haplotype determined by serological methods (Kuragaki *et al.*, 1991; Okada, 1992).

The reagents for typing *B* haplotypes are usually prepared by alloimmunization. However, it is difficult to prepare antisera with identical specificity by alloimmunization. Furthermore, the antisera are usually crossreactive to several B antigens. Such crossreactivity causes problems in

identifying *B* haplotypes by hemagglutination only, particularly in populations of chickens with unknown *B* haplotypes.

Recently, restriction fragment length polymorphism (RFLP) has attracted attention as a method for characterizing differences among *B* haplotypes. Kuragaki *et al.* (1991) examined the *B* haplotypes of 16 different chicken lines by RFLP analysis with a probe for the MHC *B-G* region. In most instances, the RFLP patterns were the same within a *B* haplotype, and different *B* haplotypes showed different RFLP patterns. The authors concluded that RFLP analysis would be useful as a supplementary means for designating the *B* haplotype of chickens. Usefulness of RFLP typing *B* haplotypes was also confirmed by Uni *et al.* (1992) and Landesman *et al.* (1993). The purpose

of this study was to establish standard RFLP patterns for designating *B* haplotypes and to apply the RFLP typing in a study of phylogenetic relationships among native breeds of chickens.

Materials and Methods

Chickens

Chickens from 8 lines were used to establish standard RFLP patterns for designating the *B* haplotypes. The list of these lines is given in Table 1.

Detailed descriptions of the GVHR-HA, GVHR-HG, CB and GSP lines were previously presented by Kuragaki *et al.* (1991), whereas descriptions of the RPRL-15 I_s, Cornell-P and Cornell-N lines were given by Somes (1984). The DWR line was established by selection for delayed-type wattle reaction of chickens to BCG antigen (Afraz *et al.*, 1994).

To test the application of the RFLP to typing of *B* haplotypes, two non-inbred strains, N (White Leghorn) and C (crossbreds), and 6 populations of Japanese native chickens were used. The breeds of Japanese native chickens were Onaga-dori (Kochi and Kagoshima populations), Sado-higejitori (Sado and Echigo populations), Shibatori and Echigo-nankin.

Blood samples were collected from each chicken and DNA was isolated from blood cells as described by Kuragaki *et al.* (1991). The DNA samples were digested with a restriction endonuclease prior to electrophoresis on 1% agarose gel. The restriction endonucleases used were *Hind* III, *Pvu* II, *Bgl* II and *Hae* III. After electrophoresis, the DNA was transferred to nylon membranes by blotting buffer. Probe labeling and hybridizations were carried out using a rapid hybridization system, Multiprime (Amersham). The *B-G* probe was a 600bp *Eco*RI fragment cloned in pUC19 corresponding to gene 8.5 (Kaufman *et al.*, 1991).

Blood typing and electrophoresis

Four blood group systems, A,B,D and E, were examined by hemagglutination. Blood plasma proteins were analyzed by starch or polyacrylamide gel electrophoresis (Okada *et al.*, 1993). The

loci examined are albumin, *Alb*; alkaline phosphatase, *Akp* and *Akp-2*; amylase, *Amy-1*; esterase, *Es-1*; postalbumin, *Pas*; prealbumin, *Pa-1*; and transferrin, *Tf* loci.

Estimation of gene frequencies and genetic distances

Gene frequencies were estimated by the direct counting method for the loci consisting of only codominant alleles and by the method of Ito and Kanemaki (1987) for the loci including recessive alleles.

Genetic distances were measured by the jackknife method (Mueller and Ayala, 1982) using the Nei's genetic distance (Nei, 1975) calculated by the method modified for small populations (Nei, 1978). The genetic distances from the RFLP pattern were calculated on the basis of the frequency of each band in the populations. The dendrogram was constructed from the genetic distance matrix by the unweighted pair-group method (Sneath and Sokal, 1973).

Results

Analysis of the RFLP pattern for individual *B* haplotypes

The RFLP patterns in the 8 lines with known *B* haplotypes are shown in Fig. 1. Comparison of

Fig. 1. RFLP patterns of different *B* haplotypes digested with *Hae* III. Line abbreviation; A:GVHR-HG, B:GVHR-HA, C:CB, D:DWR, E:RPRL-15 I_s, F:Corneil-P, G:Corneil-N, H:GSP

DNAs digested with 4 different restriction endonucleases showed that clearest RFLP pattern was obtained with *Hae* III (data obtained with other restriction enzymes are not shown). Each *B* haplotype was clearly differentiated. For example, the $B^{15}B^{15}$ samples from the GVHR-HA, DWR and RPRL-15 I_s lines, which have different origins, showed the same RFLP pattern. In the DWR line in which three *B* haplotypes were segregated, the pattern of heterozygotes consisted of common bands to both or either one of the two *B* haplotypes. On the other hand, a line difference was found in the pattern of $B^{21}B^{21}$ from the Cornell-N and GSP Lines. The line difference was also confirmed when the DNA was digested with *Pvu* II or *Bgl* II (data are not shown).

For examining usefulness of the *B* haplotype designation by RFLP and the inheritance of the RFLP patterns, DNAs from the N and C lines having unknown *B* haplotypes were analyzed. RFLP patterns of chickens within a sire family in the N line are shown in Fig. 2. The *B* haplotype was tentatively designated by its RFLP pattern and checked by serological typing. All bands in the progeny were clearly explained by inheritance from the parents.

In the N and C lines, a total of 10 different *B* haplotypes were found. The complete RFLP pattern for these 10 haplotypes, as well as those for the known *B* haplotypes shown in Fig. 1, are

Fig. 2. RFLP patterns of a sire family in the N line. DNA was digested with *Hae* III. The top numbers show the *B* genotypes. S: sire, D₁-D₄: dams, others: offspring, ►: bands from sire, ●: bands from dam

Fig. 3. Schematic representation of RFLP patterns of DNA from 16 different *B* haplotypes. DNAs were digested with *Hae* III. The $B^{GSP^{21}}$ indicates the B^{21} haplotype of GSP line. The number with symbol* has the same serological specificity with the same number without*.

given in Fig. 3. With digestion by *Hae* III, 33 polymorphic fragments with sizes ranging from 1.1 to 10.3 kb were detected. The 2.5 kb fragment was found in all *B* haplotypes except B^1 . On the other hand, several fragments were found only in a specific *B* haplotype. For example, the 2.0 and 2.7 kb fragments were specific to B^{15} , and the 4.1 kb fragment was specific to B^{21} .

Application of RFLP typing to native chicken populations

A total of 166 birds belonging to 6 populations from 4 Japanese native breeds were examined. The RFLP patterns of these chickens are summarized in Fig. 4. Two to 9 patterns were found in each population. The total number of the patterns was 39. Individual RFLP patterns consisted of 4 to 21 bands. Therefore, it was difficult to identify the *B* haplotypes by the RFLP pattern only. Considering the results of serological typing together with the RFLP patterns, tentative *B-G* genotypes were assigned. The nomenclature of Okada and McDermid (1970) was used, except for the symbol X, Xs were tentative symbols in the present paper. Some of them are shown in Table 2. Some *B* phenotypes were assigned to the same *B*

[illegible]

Fig. 4. Schematic representation of RFLP patterns of DNA from Japanese native chickens. The DNA was digested by *Hae*III. ^{a-d)} RFLP patterns with the same superscript are identical.

Using the frequencies of RFLP bands in each population, genetic distances among these populations were estimated. The dendrogram drawn from the genetic distance based on the RFLP analysis of *B* haplotypes, is given in Fig. 5A, and that obtained from gene frequencies at blood group and blood protein loci in Fig. 5B. Both dendrograms were similar to each other, even though the former was based only on the *B-G* locus and the latter on data from 12 loci.

LP analysis for designation of

haplotype into 5 subtypes by *B-L* and *B-F* RFLPs. Kuragaki *et al.* (1991) also found that the *B⁹* haplotype in a line was divided into two types by *B-G* RFLP, although both types showed the same serological specificity.

In the present study, the RFLP typing was also applied to non-inbred lines N and C, and to 6 populations of Japanese native chickens. In accordance with the results of Uni *et al.* (1992) and Landesman *et al.* (1993), RFLP patterns in these populations consisted of many bands. Therefore, it was difficult to designate the *B* haplotypes without pedigree information. In the N and C lines in which both pedigree and serological information was available, the RFLP typing was successful as shown in Fig. 2. However in the Japanese native breeds, in which pedigree information was not available, considerable differences between the RFLP patterns and serological types were found (Table 2). It is likely that these patterns reflect variation in the non-expressed or non-serologically defined regions of the *B-G* DNA. Although some of the differences could have been caused by errors or crossreactions in serological typing.

Fig. 5. Dendrograms showing genetic relationships among 6 populations of Japanese native chickens. A: dendrogram based on RFLP analysis of *B-G* locus. B: dendrogram based on 12 blood groups and blood protein loci. Abbreviations; Ona (A): Onaga-dori (Kochi), Ona (B): Onaga-dori (Kagoshima), SAD (A): Sado-higejitori (Sado), SAD (B): Sado-higejitori (Echigo), SHIBAT: shibatori, ECHIGO: Echigo-nankin.

The dendrogram based on genetic distances derived from the frequencies of the *B-G* RFLP bands showed good agreement with the dendrogram based on gene frequencies at 12 blood group and blood protein loci. The dendrogram also reflected generally accepted relationships among these populations. Kuhnlein *et al.* (1989) also estimated genetic distance from the frequencies of bands in DNA fingerprints, and found that the distances correctly reflected the history of the strains examined. Similar results were also obtained by Gilbert *et al.* (1990). Thus, measurements of genetic distances using RFLP patterns may provide useful means for studying phylogenetic interrelationships of animal populations.

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Table 1. Chicken lines were used to establish standard RFLP patterns for designating the *B* haplotype

Line	<i>B</i> haplotype	Breed
GVHR-HG	B^9B^9	White leghorn
CB	$B^{12}B^{12}$	White leghorn
GVHR-HA	$B^{15}B^{15}$	White leghorn
RPRL-15 I _s	$B^{15}B^{15}$	White leghorn
Cornell-P	$B^{19}B^{19}$	White leghorn
Cornell-N	$B^{21}B^{21}$	White leghorn
GSP	$B^{21}B^{21}$	Fayoumi
DWR	Segregated ¹⁾	White leghorn

1) Three *B* haplotypes, B^9 , B^{12} and B^{15} were present.

Table 2. Comparison of *B* haplotypes tested by hemagglutination with *B* haplotypes estimated by RFLP analysis in the Kochi population of Onagadori

RFLP pattern ¹⁾	<i>B</i> haplotype ²⁾	<i>B</i> genotype ³⁾
1	C(2) ⁴⁾	B^cB^{x1}
2	C(2)	B^cB^{x2}
3	COT(11),CO(6),CT(3),others(6)	$B^{\sigma\tau}B^{x3}$
4	OT(31),T(3),CT(1)	$B^{\sigma\tau}B^{\sigma\tau}$
5	CL(4),others(3)	$B^{x3}B^{x4}$
6	OT(4)	$B^{\sigma\tau}B^{x5}$
7	--(3)	B^bB^b
8	O(1)	?
9	MOT(1)	$B^{\sigma\tau x}/B^{x6}$

¹⁾ See Fig.4. ²⁾ Examined by hemagglutination.

³⁾ Estimated by RFLP analysis. ⁴⁾ Number of chickens detected.

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