Rabbit Immunoglobulin Lacking Group *a* Allotypic Specificities. II. Retention of Constant Region *d*11 and *d*12 Specificities†

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ABSTRACT: Allotypes *d*11 and *d*12 are present on the constant region of heavy chains of rabbit immunoglobulin G. The *d*11 allotype correlates with a methionine residue in position N226, adjacent and N terminal to the inter-γ chain disulfide bond. A threonine residue is found in this position in *d*12 molecules. Cyanogen bromide cleavage of *d*11 molecules results in the peptide spanning residues N227–N250. Since allotypes *a*1, *a*2, and *a*3 share the same heavy chain with *d*11 and *d*12, it was of interest to establish the presence or absence of the latter on γ chains derived from rabbits suppressed for the group *a* allotypes. The isolation of the peptide N227–N250 provided chemical evidence that allotype *d*11 was retained when *a*3,*b*5,*d*11 rabbits were suppressed for *a*3.

Allotypic specificities *d*11 and *d*12 are present on the heavy (H) chains of IgG[1] (Mandy and Todd, 1968, 1969, 1970). They are detected by inhibition of hemagglutination, as anti-*d*11 and anti-*d*12 are normally nonprecipitating. Each of these specificities is associated with each of the group *a* allotypes (*a*1, *a*2, and *a*3), and all permutations have been observed, *e.g.*, *a*1,*d*11; *a*1,*d*12; etc. Breeding studies have shown them to be linked to the group *a* allotypes (Zullo *et al.*, 1968; Mandy and Todd, 1970), but occasional recombination has been reported (Mage *et al.*, 1971; Kindt and Mandy, 1972).

Studies of the peptides derived from γ chains by cyanogen bromide cleavage and by trypsin digestion have established that *d*11 correlates with a methionine residue adjacent and N terminal to the inter-γ chain disulfide bond in the hinge region, and *d*12 correlates with the threonine residue at this position (Prahl *et al.*, 1969).

Landucci-Tosi *et al.* (1972) have reported retention of the constant region *e*15 specificity upon suppression of the *a*2 allotype in an *a*2,*b*4,*e*15 rabbit. In the present paper, chemical data will be presented which clearly establish the retention of the *d*11 allotype on non-*a* γ chains derived from *a*3,*b*5,*d*11 rabbits suppressed for the *a*3 specificity.

Materials and Methods

IgG Preparation. The preparation of IgG from the sera of normal and allotype-suppressed rabbits has been described in the previous paper (Tack *et al.*, 1973).

Reduction and Radioalkylation of γ Chains. IgG from rabbits homozygous for *d*11 and *d*12 was dialyzed against 0.5 M Tris-HCl buffer (pH 8.0), 2 mM with EDTA at a protein concentration of 20 mg/ml. Each solution was deaerated with a stream of nitrogen, and solid dithiothreitol was added to a final concentration of 10 mM. After incubation at 37° for 2 hr, each reaction mixture was cooled to 4°, and [1-^{14}C]iodoacetic acid (0.4 mCi/mmol) or [1-^{3}H]iodoacetic acid (2 mCi/mmol) in 50 mM Tris-HCl buffer (pH 8.0), 2 mM with EDTA, was added to a final concentration of 20 mM. After 1 hr, each reaction mixture was dialyzed against 10 mM Tris-HCl buffer (pH 8.0) and then against 1 N propionic acid. H and light (L) chains were separated by gel filtration on Sephadex G-100 in 1 N propionic acid.

Cyanogen Bromide Cleavage of γ Chains. Details have been presented in the previous paper (Tack *et al.*, 1973).

Isolation of Peptide C1c. The cyanogen bromide digest of $a\bar{3}$,*d*11 chains was chromatographed on a 2.5 × 100 cm column of Sephadex G-100 in 50 mM sodium formate (pH 3.2), 6 M with urea. Fractions from peak C5 and its associated radioactive peak (Figure 1) were pooled, concentrated by ultrafiltration, freed of urea by passage through Sephadex G-25, and lyophilized. Peptide C1c was resolved from peptides C4 and C5 by two successive filtrations on a 1.6 × 240 cm column of Sephadex G-50 in 50 mM ammonium hydroxide (Figure 2).

Determination of Radioactivity. Radioactivity was determined in a Beckman LS-233 liquid scintillation counter. Column effluent fractions were formulated in Aquasol (New England Nuclear).

Results

The amino acid compositions of $a\bar{2}$,*d*12 and $a\bar{3}$,*d*11 γ chains are shown in Table IV of the preceding paper (Tack *et al.*, 1973). The higher methionine content of the $a\bar{3}$,*d*11 chains was itself suggestive that the *d*11 allotype had been retained upon suppression of the *a*3 specificity.

In the reduction of rabbit IgG with dithiothreitol (10 mM), the interchain disulfide linkages are preferentially cleaved. Introduction of a radioactive alkylating agent therefore selectively radiolabeled the cysteine residues participating in the interchain disulfide bonds. Peptide C1c, which spans positions N227–N250 (see Figure 5 of Tack *et al.*, 1973) of a *d*11 chain, is readily detected upon gel filtration of the cyanogen bromide digested chain through the presence of radiolabeled cysteine at position N227.

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[1] The nomenclature employed for rabbit immunoglobulins, their chains, and fragments follows that recommended by a committee of the World Health Organization (1964).

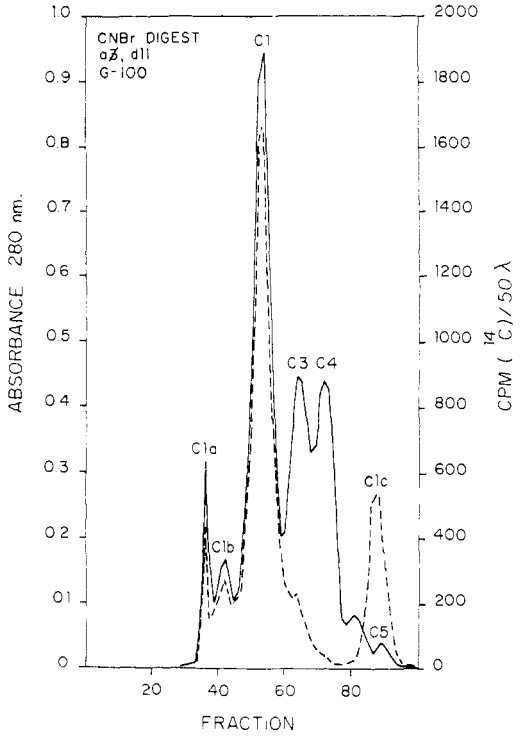

FIGURE 1: Gel filtration of cyanogen bromide reaction products through a 2.5 × 100 cm column of Sephadex G-100 in 50 mM sodium formate buffer (pH 3.2) 6 M with urea; applied 64 mg of the $a\bar{3}$,*d*11 chain in 7 M guanidine-HCl ($\bar{3}$ is represented by the number with a slant in the figure): (—) absorbance at 280 nm; (---) radioactivity.

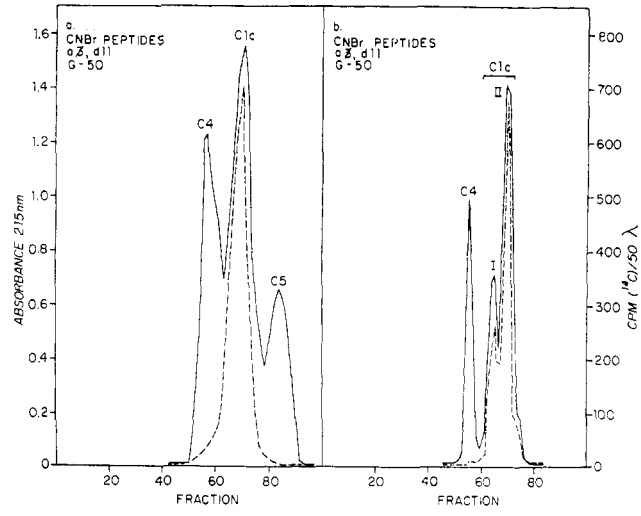

FIGURE 2: (a) Gel filtration of the C5 region and associated radioactive peak from $a\bar{3}$,*d*11 chains through a 1.6 × 240 cm column of Sephadex G-50 in 50 mM ammonium hydroxide ($\bar{3}$ is represented in the figure by the number with a slant). (b) Refiltration of the radioactive peak: (—) absorbance at 215 nm; (---) radioactivity.

The gel filtration of CNBr-fragmented $a\bar{3}$,*d*11 chains (Figure 1) revealed the presence of a radioactive component which was eluted immediately preceding the carboxy-terminal octadecapeptide, C5. Utilizing the same conditions of fractionation, Prahl *et al.* (1969) observed the same chromatographic behavior of the peptide C1c from CNBr-cleaved *a*1,*d*11 and *a*3,*d*11 γ chains. Fractions from peak C5 and the associated radioactive peak were pooled, desalted on a column of Sephadex G-25, and lyophilized.

Gel filtration of the lyophilized material on Sephadex G-50 in 50 mM ammonium hydroxide (Figure 2a) incompletely resolved the radioactive peptide from peptides C4 and C5. Refiltration of the radioactive fractions on the same column (Figure 2b) gave two radioactive peaks, I and II, which were resolved from the other peptide material as judged by the 215-nm absorbancy profile. In both this study and that of Prahl *et al.* (1969), the major radioactive peptide eluted at 57–60% of the total column volume. Fractions constituting peaks I and II were separately pooled and lyophilized.

Amino acid analyses of these two fractions revealed that they were compositionally indistinguishable. The resolution of the C1c peptide into two discrete peaks is not understood at this time, but it may be related to the tendency of the N-terminal *S*-carboxymethylcysteine residue to undergo intramolecular cyclization to yield the 3-oxo-(1,4-thiazine)-5-carboxyl derivative in acid media (Bradbury and Smyth, 1973), as was employed for the initial fractionation of the CNBr fragments. More importantly, the analyses were in agreement with the published analysis of peptide C1c from *a*3,*d*11 chains (Table I). The recovery of C1c from the $a\bar{3}$,*d*11 chains was 87.7% compared to a recovery of 87% from *a*3,*d*11 chains.

The $a\bar{2}$,*d*12 chains did not have a methionine residue at position N226 as evidenced by the absence of peptide C1c upon gel filtration of the C5 region on a 1.6 × 240 cm column of Sephadex G-50 in 50 mM ammonium hydroxide (Figure 3).

Discussion

Chemical and immunochemical data were presented in the preceding paper (Tack *et al.*, 1973), which suggested that the constant regions of *a* and non-*a* chains are identical. The isolation and characterization of a radioactive peptide from cyanogen bromide treated $a\bar{3}$,*d*11 chains as C1c further strengthen the peptide mapping and compositional studies of the Fc

TABLE I: Amino Acid Composition of a C1c Peptide.[a]

	H-Chain Allotype		
		$a\bar{3}$,*d*11	
	*a*3,*d*11	I	II
Asp	0.96	1.11	1.22
$CysCH_2COOH$	0.62	0.85	0.95
Glu	1.05	1.15	1.14
Gly	2.08	2.14	2.16
Ile	0.82	0.97	0.96
Leu	2.92	2.90	2.86
Lys	1.99	1.92	1.92
Phe	1.71	1.74	1.69
Pro	7.09	6.40	6.51
Ser	1.09	1.15	1.23
Thr	0.92	0.99	1.04
Val	0.95	1.00	1.05
Homoserine	0.94	0.99	0.98
Recovery (%)	87.0	87.7	

[a] Compositions are reported as amino acid residues per mole of peptide. Recoveries are based on the yield of C1c peptide relative to C5. The composition and recovery of C1c derived from a *a*3,*d*11 H chain are those previously reported by Prahl *et al.* (1969).

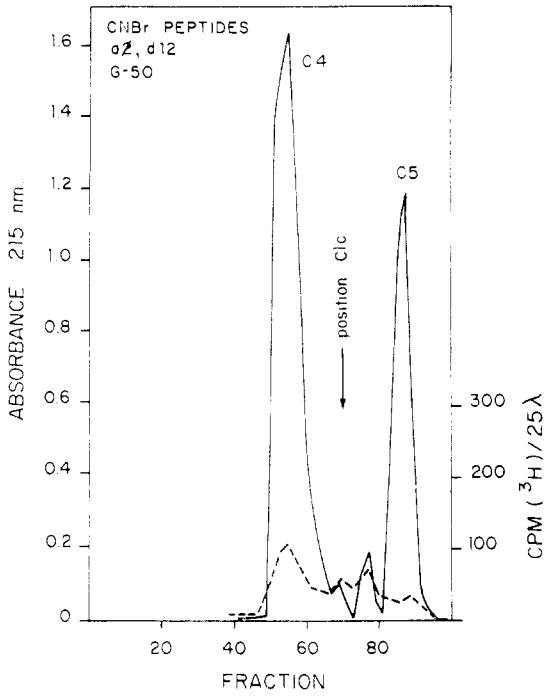

FIGURE 3: Gel filtration of the C5 region from $a\bar{2}$,d12 chains through a 1.6 × 240 cm column of Sephadex G-50 in 50 mM ammonium hydroxide ($\bar{2}$ is represented in the figure by the number with a slant): (—) absorbance at 215 nm; (---) radioactivity.

regions from both types of chains. The constant region allotype e15 was present on $a\bar{3}$,d11 IgG (S. Dubiski, personal communication), as has been reported for $a\bar{2}$ IgG by Landucci-Tosi *et al.* (1972). Thus, the present chemical evidence is in agreement with the serologic data. Accordingly, it seems established that the allotypic markers of groups d and e, which are characteristic of γ chains, are present whether the group a markers, which are shared by the several classes of H chains, are present or absent. Further genetic implications of a and non-a chains sharing apparently identical constant regions will be considered in the Discussion of the following paper (Prahl *et al.*, 1973).

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