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Genes encoding α-heavy chains of rabbit IgA: characterization of cDNA encoding IgA-g subclass α-chains

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ABSTRACT

cDNA molecules encoding rabbit IgA α-heavy chains have been synthesized and six of these have been characterized. The complete nucleotide sequence of one cDNA, p 19 (942bp), showed that it encoded all but the N-terminal 57 amino acid residues of the constant region of α-chains. The cDNA molecules were subcloned into the expression vector pUC8 and E. coli were transformed. Radioimmunoassay of the molecules synthesized by these clones showed that all six cDNA molecules encoded α-chains of the IgA-g subclass. Comparison of the amino acids encoded by the α-cDNA with the amino acid sequence of mouse and human α-chains showed that although all of the intradomain disulfide bonds appear to be conserved, some positions, probably involved in interchain disulfide bonds, are not conserved. We propose that secretory component is covalently bound to cysteine 299 and/or cysteine 301 of the CH2 domain of mouse and human α-chains.

The results from Southern blot analysis of genomic DNA with ^{32}P-cDNA suggests that the rabbit genome has multiple Cα genes.

INTRODUCTION

Two subclasses of rabbit IgA, IgA-f and IgA-g, have been identified and several allotypes of each subclass are known. The f69, f70, f71, f72 and f73 allotypes are controlled by allelic genes at the Cαf locus and the g74, g75, g76 and g77 allotypes are controlled by allelic genes at the Cαg locus (1). These loci are closely linked to the genes controlling other heavy chain allotypes such as those of VH, Cγ and Cμ(2). Immunochemical analyses of proteolytic fragments of the IgA-f and IgA-g molecules have shown that the allotypic specificities are found on both the Fc and Fd portions of the α-heavy chains (3,4). Partial amino acid sequence analyses of the Fc fragment of IgA-g allotypes indicates that the allotypic specificities reflect amino acid sequence differences (5). Further analyses of the structural basis of the IgA allotypic specificities as well as of the subclasses have been hampered by the difficulty in separating IgA-f and IgA-g subclass molecules.

Recent serological studies have indicated that the IgA-f subclass

molecules can be separated into at least two subpopulations based on differences in their reactivity with α-chain specific alloantisera (6). Because it appears that rabbit IgA may be comprised of more than two subclasses, there are expected to be more than two genes encoding Cα in the rabbit genome. To determine the number of rabbit Cα genes and to analyze the structural basis for the various allotypes, we have begun to clone rabbit Cα genes.

A cDNA library was constructed from poly(A)$^+$ RNA of mammary tissue of a lactating rabbit and from this library we have isolated six plasmid clones, each of which encodes Cα molecules. All six cDNA molecules were cloned into the expression vector pUC8, and the recombinant bacterial clones were shown to synthesize molecules of the IgA-g subclass. Southern blot analysis of rabbit genomic DNA with ^{32}P-labeled cDNA encoding α-heavy chains indicates that the rabbit genome contains several Cα genes.

MATERIALS AND METHODS

Construction of cDNA Library

RNA was isolated from mammary tissue of a lactating rabbit, U193-1, homozygous for the heavy chain haplotype $\underline{a}^2\underline{n}^{80}\underline{de}^{12,15}\underline{f}^{71}\underline{g}^{75}$. The tissue, obtained 5 days post-parturition, was homogenized in 4M guanidinium thiocyanate/1M 2-mercaptoethanol (7,8) and the RNA was obtained after equilibrium centrifugation in CsCl. Poly(A)$^+$ RNA was selected by oligo-(dT)-cellulose chromatography and double-stranded cDNA was synthesized using oligo(dT) as primer (9). The larger double stranded cDNA molecules were selected by passage over a Bio-Gel A-150m column (Bio-Rad). Terminal deoxynucleotidyl-transferase (P-L Biochemicals) was used to add 15-30 dCMP molecules to each 3' end (10). The dCMP-tailed cDNA was annealed to Pst I digested, dGMP-tailed pBR322 (11) and E. coli MC1061 were transformed with the plasmids (11,12).

Selection of Cα cDNA and Nucleotide Sequence Analysis.

Tetracycline-resistant bacterial colonies were grown on nitrocellulose filters (13) and the colonies were screened by hybridization with a ^{32}P-labeled cDNA probe, kindly provided by J. Ellison and L. Hood, which encodes the CH2 and CH3 domains of mouse α-heavy chain. Six colonies which hybridized with the probe were picked and grown for further study. The nucleotide sequence of one cDNA clone, pα19, was determined by the method of Maxam and Gilbert (14) from the original clone (in pBR322) or from subclones made in pUC8 as described below.

Hybridization Analysis of Rabbit Genomic DNA

High molecular weight DNA was isolated from sperm cells (15) of rabbit X314-6 (homozygous for the heavy chain haplotype $\underline{a}^{3}\underline{n}^{80}\underline{de}^{12,15}\underline{f}^{71}\underline{g}^{75}$). Ten micrograms of DNA were digested with the restriction endonucleases Bam HI and Hind III and analysed by the Southern Blot procedure using ^{32}P-labeled pα19 (8,16). The hybridized blot was autoradiographed on Kodak XAR-5 film.

Expression of cDNAs

The cDNAs of 6 different recombinant clones encoding α-heavy chains (pα6, pα8, pα17, pα19, pα21 and pα26) were digested with Pst I and the 725 bp fragment encoding the CH2 and CH3 domains of the α-chain, as well as the 3' untranslated region of the mRNA, was ligated into the Pst I site of the expression vector pUC8. This vector contains a single Pst I site 3' to the lac promoter region (17); insertion of a cDNA molecule within the lac Z gene results in the synthesis of a hybrid molecule composed of 5 amino acids of β-galactosidase and the CH2 and CH3 domains of the α-heavy chain. E. coli JM83 cells were transformed with the recombinant plasmids (18), and subclones were selected by plating with 5-bromo-4-chloro-3-indolyl-β-d-galactoside (Bachem) on L agar plates containing 15 μg/ml ampicillin (Sigma).

Individual colonies were picked and grown in L broth containing 20 μg/ml ampicillin and 10 mM isopropyl-β-D-thiogalactopyranoside (P-L biochemicals) to induce the lac operon. Cells from stationary phase cultures (10 ml) were resuspended in 1 ml of PBS (pH 7.4) containing 2 mg/ml lysozyme (Sigma), and were lysed by four cycles of freezing and thawing. The lysates were centrifuged for 30 minutes at 10,000 rpm and the supernatant fluids were tested by radioimmunoassay for the presence of molecules with α-heavy chain antigenic determinants.

Solid Phase Radioimmunoassay.

Anti-Fcα was purified from goat anti-rabbit IgA antiserum (19). The serum was passed through a column of IgG-coupled Sepharose 4B, and the effluent was then passed through a column of IgA-coupled Sepharose 4B. Purified anti-α chain antibody was eluted with 0.2M glycine sulfate, pH 2.3 (3).

Anti-g75 allotype antibody was prepared by passage of anti-g75Fc antibody (3) through a column of Sepharose to which f71,g75 sIgA had been coupled. The specific anti-g75 antibody was eluted with 0.2M glycine sulfate, pH 2.3.

Cross-reactive anti-f71 allotype antibody was prepared from anti-f70,g76 antiserum. Our previous observation that there exists extensive

cross reactivity between the f70 and f71 allotypic markers (6), and no apparent cross reactivity between the g76 and g75 allotypic markers (20), allowed us to use the anti-f70g76 antiserum for the isolation of an antibody specific for f71 IgA molecules. The use of the anti-f70g76 antiserum was necessary as it has been difficult to prepare anti-f71 antibody free of anti-g75 antibodies. The serum was passed through a column of Sepharose to which f71,g75 sIgA was coupled. The purified antibody, designated anti-f71, was eluted with 0.2M glycine sulfate pH 2.3. All purified antibodies were concentrated in an Amicon ultrafiltration cell containing a PM10 membrane, and dialysed extensively against borate saline buffer (0.16M borate, 0.13M NaCl, pH8.0). The specifically purified anti-Fcα, anti-f71 and anti-g75 antibodies were radiolabeled with ^{125}I by the method of McFarlane (21).

For the radioimmunoassay, 100 µl of anti-Fcα (50 µg/ml) was added to each well of a 96 well mictrotiter plate (Falcon) and allowed to incubate overnight at room temperature. Plates not used immediately were stored at $4^{o}C$ up to 60 days. The plates were treated for 1 hour with phosphate buffered saline containing 1% bovine serum albumin (Sigma) (PBS-BSA) and washed 3 times with the same buffer. The lysates (100 µl) prepared from the recombinant clones and control samples were added and allowed to incubate overnight at room temperature. The plates were then treated with PBS-BSA and washed as above. ^{125}I-labeled antibody (20 µg/ml), approximately 100,000 CPM, was added to each well and incubated 4 hours at $37^{o}C$. The plates were then washed four times with PBS-BSA, and dried for 30 minutes at $37^{o}C$. The dried plates were cut and counted in a γ-type scintillation counter. Samples were first screened for the expression of Fcα antigenic determinants. Those samples which exhibited a 4-fold or greater increase over control samples in binding of the labeled anti-α were considered positive for α-chain expression and further tested for expression of specific allotype. Samples that bound greater than or equal to 2 times as much radiolabel as control samples were considered positive for the expression of the specific allotype tested. The data are reported as the mean counts per minute (CPM) of triplicate samples plus or minus the standard deviation of the samples.

RESULTS

Identification and Characterization of Cα cDNA clones.

Six bacterial clones, pα6, pα8, pα17, pα19, pα21 and pα26, containing

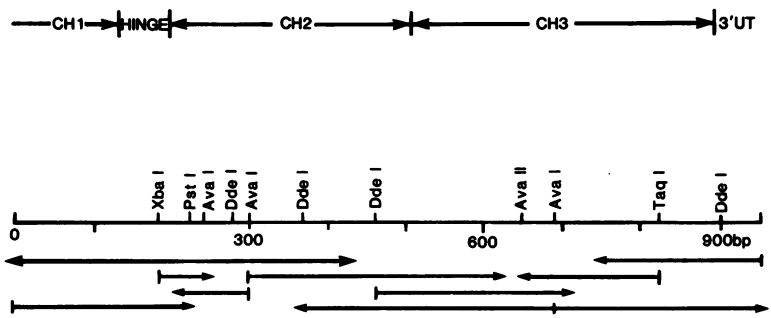


Figure 1. Restriction map and strategy for nucleotide sequence analysis of pα19 cDNA. Arrows below map indicate end labeled restriction sites and direction of sequence analysis. The 5' 225bp were sequenced from pUC8 after subcloning the 225bp Pst I fragment of pα19 into the Pst I site of pUC8.

plasmid DNA which hybridized with a probe encoding mouse CH2 and CH3 domains, were selected from the cDNA library prepared from mammary tissue of a lactating rabbit. Plasmid DNA from each of these clones was digested with Pst I and analyzed by polyacrylamide and agarose gel electrophoresis and Southern blot hybridization (16). Each clone contained a 725 bp fragment and one other fragment ranging from 100 bp (pα17) to 230 bp (pα 19). A restriction map of the largest cDNA clone, pα19, was determined (Figure 1) and the nucleotide sequence of the DNA was obtained (Figure 2). Translation of the DNA sequence into amino acid sequence and comparison with the sequences of mouse (24) and human (25) α-heavy chains showed that pα19 encodes from amino acid 176 (Bur numbering system) of the CH1 domain to the COOH-terminal end of the CH3 domain (897 bp), the 3' untranslated region of α-heavy chain mRNA (45 bp) and 25 residues of the poly (A) tail.

Allotypes Encoded by Cα cDNA Clones.

Limited amino acid sequence data for g74, g75 and g76 Fcα allotypes have been published (5). Comparison of the amino acid sequence encoded by pα19 with sequences of the g74, g75 and g76 α-heavy chains shows that 60 of 65 compared positions in the CH2 domain are identical between pα19 and one or more of the IgA-g allotypes (Figure 3). Due to lack of available data for amino acid sequences of the IgA-f subclass heavy chains, it is not possible to determine which subclass pα19 encodes based on amino acid comparisons. Thus, the subclass encoded by pα19, as well as the other five

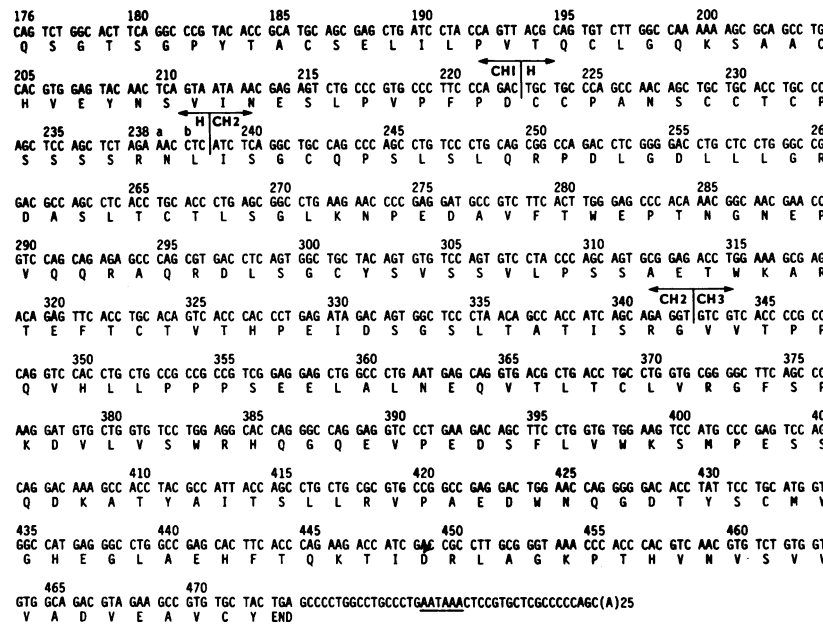


Figure 2. Nucleotide sequence of pα19 cDNA. The amino acid translation of the nucleotide sequence is shown on the second line. The amino acid numbering system is based on protein BUR and the division into CH1, hinge, CH2 and CH3 is based on Kabat et. al. (22) and on Tucker et. al. (23).

cDNA clones, was investigated by radioimmunoassay of molecules synthesized by bacteria containing the Cα recombinant plasmids. The 725 bp Pst I fragment of each of the six Cα encoding cDNA molecules was cloned into the Pst I site of the expression vector pUC8 and E. coli strain JM83 was

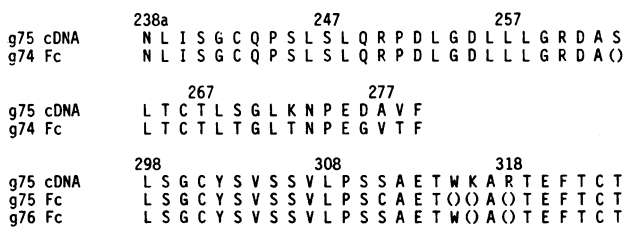


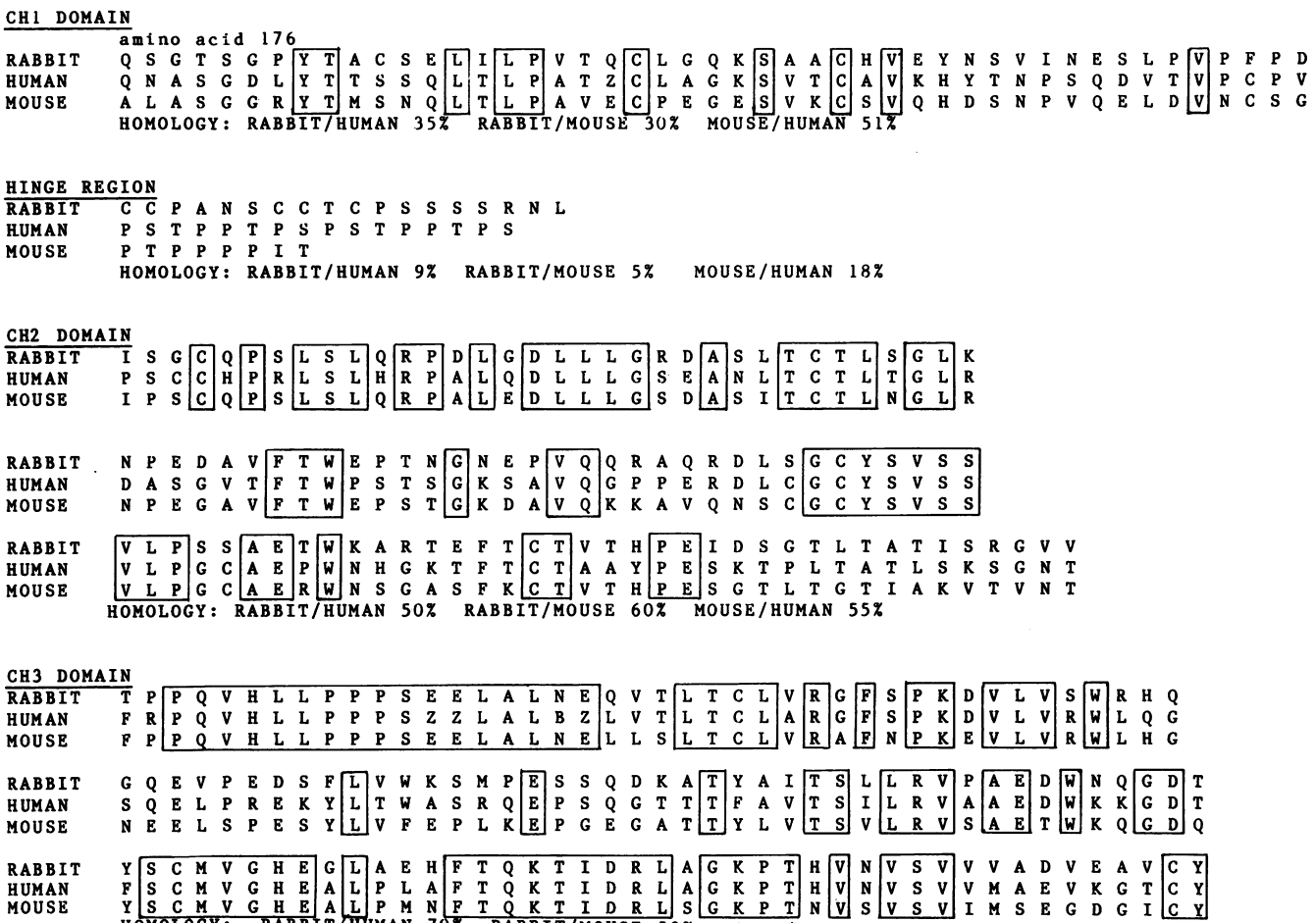
Figure 3. Comparison of amino acid sequences of g74, g75 and g76 α-chains. The amino acid numbers of the α-chains are given above the one letter amino acid code. The g74Fc, g75Fc and g76Fc sequences are from Malek et. al. (5).

Table I Radiobinding Analysis of Cell Lysates from E. coli transformed with recombinant plasmids encoding the CH2 and CH3 domains of rabbit α-chains.

	ISOTYPE EXPRESSION	ALLOTYPE EXPRESSION	
LYSATES	^{125}I-anti-Fc (cpm ± S.D.)	^{125}I-anti-f71 (cpm ± S.D.)	^{125}I-anti-g75 (cpm ± S.D.)
pα6-1	11,327 ± 297	1,310 ± 31	7,513 ± 114
pα8-1	16,797 ± 95	1,233 ± 51	10,062 ± 81
pα8-2	17,276 ± 385	1,345 ± 59	10,598 ± 81
pα17-1	11,983 ± 89	1,321 ± 16	7,663 ± 54
pα17-2	11,555 ± 76	1,357 ± 21	7,681 ± 161
pα19-1	17,790 ± 142	1,380 ± 8	11,442 ± 99
pα21-1	15,055 ± 174	1,263 ± 84	9,362 ± 122
pα21-2	12,444 ± 277	1,093 ± 40	7,242 ± 149
pα26-1	15,831 ± 256	1,320 ± 58	10,035 ± 35
pα26-2	12,972 ± 106	1,357 ± 56	8,917 ± 46
pα8-4	782 ± 59	1,540 ± 53	3,003 ± 28
CONTROLS			
PBS[1]	1,303 ± 174	1,923 ± 146	3,243 ± 61
IgG_2 10 μg	1,048 ± 31	1,895 ± 85	2,954 ± 48
IgA[2] 10 μg	19,614 ± 817	7,786 ± 195	13,260 ± 77
IgA 1 μg	19,199 ± 375	6,834 ± 91	11,749 ± 152
IgA 0.1 μg	13,671 ± 282	6,243 ± 75	8,483 ± 98
IgA 0.05 μg	9,324 ± 148	5,652 ± 54	5,908 ± 75
IgA 0.01 μg	3,244 ± 45	3,110 ± 67	3,806 ± 89
IgA 0.005 μg	2,147 ± 76	2,532 ± 25	3,560 ± 46

1 = phosphate buffered saline
2 = f71g75 sIgA

transformed with each recombinant plasmid. Nucleotide sequence analysis showed that translation of these clones would occur in the correct reading frame. The cell lysates of approximately half of the various recombinant clones reacted with anti-Fcα antibody, indicating that the cloned genes directed synthesis of molecules which have Fcα-like antigenic determinants. The results of the radiobinding assays for one or two such positive transformants of each of the six cDNA subclones are shown in Table 1. Comparison of these results with those of the IgA standards indicates that the clones synthesize on the order of 0.1 to 1.0 μg Fcα-like molecules per ml culture. The other one-half of the transformants did not react with anti-Fcα (data not shown) and presumably reflect clones in which the cDNA had been inserted in the opposite orientation. As a control, one clone, pα8-4, which had been shown by nucleotide sequence analysis to be in the


CH1 DOMAIN
amino acid 176
RABBIT Q S G T S G P Y T A C S E L I L P V T Q C L G Q K S A A C H V E Y N S V I N E S L P V P F P D
HUMAN Q N A S G D L Y T T S S Q L T L P A T Z C L A G K S V T C A V K H Y T N P S Q D V T V P C P V
MOUSE A L A S G G R Y T M S N Q L T L P A V E C P E G E S V K C S V Q H D S N P V Q E L D V N C S G
HOMOLOGY: RABBIT/HUMAN 35% RABBIT/MOUSE 30% MOUSE/HUMAN 51%
HINGE REGION
RABBIT C C P A N S C C T C P S S S S R N L
HUMAN P S T P P T P S P S T P P T P S
MOUSE P T P P P P I T
HOMOLOGY: RABBIT/HUMAN 9% RABBIT/MOUSE 5% MOUSE/HUMAN 18%
CH2 DOMAIN
RABBIT I S G C Q P S L S L Q R P D L G D L L L G R D A S L T C T L S G L K
HUMAN P S C C H P R L S L H R P A L Q D L L L G S E A N L T C T L T G L R
MOUSE I P S C Q P S L S L Q R P A L E D L L L G S D A S I T C T L N G L R
RABBIT N P E D A V F T W E P T N G N E P V Q Q R A Q R D L S G C Y S V S S
HUMAN D A S G V T F T W P S T S G K S A V Q G P P E R D L C G C Y S V S S
MOUSE N P E G A V F T W E P S T G K D A V Q K K A V Q N S C G C Y S V S S
RABBIT V L P S S A E T W K A R T E F T C T V T H P E I D S G T L T A T I S R G V V
HUMAN V L P G C A E P W N H G K T F T C T A A Y P E S K T P L T A T L S K S G N T
MOUSE V L P G C A E R W N S G A S F K C T V T H P E S G T L T G T I A K V T V N T
HOMOLOGY: RABBIT/HUMAN 50% RABBIT/MOUSE 60% MOUSE/HUMAN 55%
CH3 DOMAIN
RABBIT T P P Q V H L L P P P S E E L A L N E Q V T L T C L V R G F S P K D V L V S W R H Q
HUMAN F R P Q V H L L P P P S Z Z L A L B Z L V T L T C L A R G F S P K D V L V R W L Q G
MOUSE F P P Q V H L L P P P S E E L A L N E L L S L T C L V R A F N P K E V L V R W L H G
RABBIT G Q E V P E D S F L V W K S M P E S S Q D K A T Y A I T S L L R V P A E D W N Q G D T
HUMAN S Q E L P R E K Y L T W A S R Q E P S Q G T T T F A V T S I L R V A A E D W K K G D T
MOUSE N E E L S P E S Y L V F E P L K E P G E G A T T Y L V T S V L R V S A E T W K Q G D Q
RABBIT Y S C M V G H E G L A E H F T Q K T I D R L A G K P T H V N V S V V V A D V E A V C Y
HUMAN F S C M V G H E A L P L A F T Q K T I D R L A G K P T H V N V S V V M A E V K G T C Y
MOUSE Y S C M V G H E A L P M N F T Q K T I D R L S G K P T N V S V S V I M S E G D G I C Y
HOMOLOGY: RABBIT/HUMAN 70% RABBIT/MOUSE 59% MOUSE/HUMAN 69%

opposite orientation, did not synthesize a molecule with Fcα determinants (Table 1).

The clones which synthesized molecules reacting with anti-Fcα were tested for their reaction with the anti-f71 and anti-g75 anti-allotype antibodies. All recombinant clones shown to synthesize molecules with Fcα-like determinants also synthesize molecules which reacted with anti-g75 allotype antibodies and not with anti-f71 allotype antibodies (Table 1). These data indicate that all six of these cDNA molecules encode molecules of the IgA-g subclass (g75 allotype) and not the IgA-f subclass (f71 allotype).

Phylogenetic Comparison of Cα Heavy Chains.

Mouse α, human α1 and rabbit αg heavy chains are from 50 to 70% homologous in the CH2 and CH3 domains (Figure 4). The hinge regions of the three α-chains differ considerably in length and in amino acid content. Whereas mouse α and human α1-hinge regions are proline-rich, and are 8 and 16 residues, in length, respectively, the rabbit αg hinge appears to be 18 residues in length and contains 5 half-cystine residues. Four of these five half cystines presumably are involved in interheavy chain disulfide bonds. The C-terminal 43 residues of the CH1 domain of rabbit αg are less homologous to mouse or human α-chains (30-35%) than mouse and human are to each other (51%). Outside of the hinge region, all of the half-cystine residues encoded by pα19 are conserved in mouse and human; however, cysteine 299 of human and mouse CH2 domains has been substituted by serine in the rabbit.

Estimation of the Number of α-genes in Rabbit Genome.

Sperm DNA from an $\underline{a}^{3}\underline{f}^{71}\underline{g}^{75}/\underline{a}^{3}\underline{f}^{71}\underline{g}^{75}$ homozygous rabbit was digested with Bam HI, Hind III, and Eco R1 and analyzed by Southern blot hybridization using ^{32}P-labeled pα19 as probe. From six to nine fragments ranging from 2.1 to greater than 23kb were detected (Figure 5). These data indicate that the rabbit genome contains multiple Cα genes.

DISCUSSION

Six cDNA molecules that encode most of the constant region of α-chains

Figure 4. Comparison of amino acid sequences of mouse α (24), human 1 (25) and rabbit g75 α-chains (5). The amino acid homology for each domain and the hinge region is shown for each region. The "extra" 18 amino acid residues at the C-terminal end of CH3 are included with the CH3 domain. Residues which are identical in all three species are enclosed in boxes.

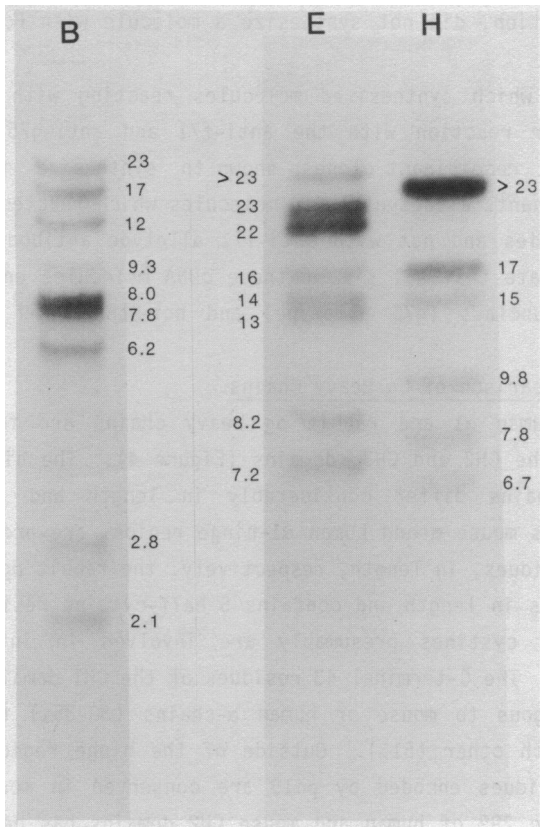


Figure 5. Hybridization of rabbit sperm DNA to ^{32}P-labeled pα19 cDNA. DNA was digested with Bam HI (B), Hind III (H) and Eco R1 (R). Specific activity of the ^{32}P-labeled pα19 was 1X10^{8} cpm/μg. Sizes (kb) were estimated from a Hind III digestion of bacteriophage λ DNA.

of rabbit IgA-g subclass molecules have been synthesized from poly(A)$^{+}$ RNA of mammary tissue of a lactating rabbit. The nucleotide sequence of one of these, pα19, shows that it encodes 47 amino acids at the C-terminal end of the CH1 domain, the entire hinge, CH2 and CH3 domains, and the "extra" 18 amino acids at the C-terminal end of the CH3 domain, to which J chain is disulfide bonded. Bacterial cells transformed with each of the Cα cDNAs in the expression vector pUC8, all synthesized fusion proteins containing α-heavy chains bearing g75 allotypic determinants as detected in radioimmunoassay by their reactivity with a heterologous anti-Fcα, and with anti-g75 allotype antibody. These molecules did not react with anti-f71

allotype antibody. The anti-f71 antibody was, in fact, anti-f70 antibody which has previously been shown to cross-react extensively with f71 molecules (6) and was specifically purified by elution from an f71 immunosorbent. Although we cannot rule out the possibility that the molecules synthesized were in fact, f71 and that the anti-f70 cross-reacts only with the Fd portion of f71 molecules (not with the Fc portion) thereby not reacting with the fusion molecules synthesized by the transformed E. coli, we think this is unlikely since these fusion molecules reacted strongly with the anti-g75 antibodies; the anti-g75 antibody has been shown in numerous experiments not to react with f71 molecules (3). In addition, nucleotide sequence data were obtained for all six cDNA clones from the hinge-encoding region which is usually the most diverse region among subclasses. All six cDNA molecules appeared to be identical in this region, thus supporting the radioimmunoassay data that all six cDNA molecules encoded heavy chains of the same subclass.

Since the ratio of f71 to g75 sIgA molecules in colostrum of an $\underline{f}^{71}\underline{g}^{75}$ homozygous rabbit is generally >1 (3), it is surprising that all of the cDNA clones encode the α-g heavy chains and none encode the α-f heavy chains. No amino acid sequence data for f-subclass α-chains are available, and it is possible that the mouse probe encoding the CH2 and CH3 domains, used to screen the cDNA library, is more homologous to α-g heavy chain genes than to α-f heavy chain genes and thus preferentially hybridized to clones encoding α-g chains. We are currently rescreening the cDNA library with rabbit α-g cDNA in an attempt to identify additional cDNA clones, some of which may encode α-f molecules.

The amino acid sequence of the Fc region of rabbit α-g heavy chains shows approximately 50% homology to the sequences of α-chains in mouse and human; this value is comparable to the homology between mouse and human Fc regions. The C-terminal 18 residue "tail" found in mouse and human α-heavy chains is present in rabbit α-chains and is 68% homologous with human and 44% homologous with mouse α-chains in that region. The intra-domain disulfide bonds appear to be conserved among the three species, including the "extra" intradomain disulfide bond in the CH2 domain. The cysteine at position 299 has been tentatively assigned to the C-terminal end of this "extra" intradomain disulfide bond in human IgA and the cysteine at position 301 has been assigned to an interheavy chain disulfide bond (26). Since the rabbit α-cDNA encodes serine instead of cysteine at position 299, we suggest that it is cysteine 301 rather than cysteine 299,

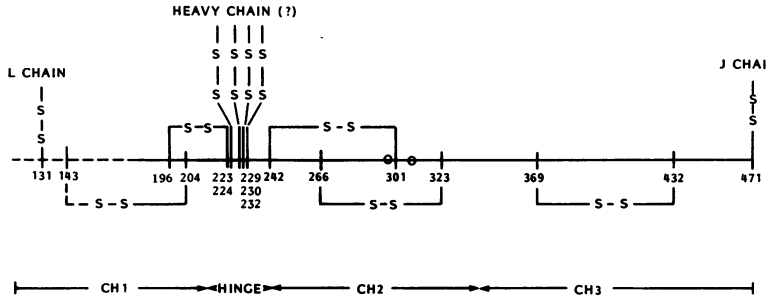


Figure 6. Proposed disulfide bond structure of rabbit g75 α-chains. The dashed lines represent unknown amino acid sequences of rabbit α-chains. The circles represent cysteine 299 and 311 postulated to be involved in interchain disulfide bonds in mouse and human IgA; these positions are serine in rabbit αg chains.

which is involved in the intradomain disulfide bond; furthermore, we suggest that cysteine 299 of human and mouse α-chains is part of an interchain disulfide bond, which is absent from rabbit IgA-g molecules (Figure 6). Within the Fc portion of human and mouse α-chain there are two cysteine residues as yet unassigned to a specific disulfide bond, cysteine 299 (or cysteine 301) and cysteine 311, both in the CH2 domain (25,26). In mouse and human sIgA, the secretory component (SC) is disulfide bonded to the Fc part of the α-chain, apparently to the CH2 domain (27). It is likely that one or both of these cysteine residues is involved in the disulfide bond between SC and α-chains. Since in rabbit α-g molecules the cysteine residues at positions 299 and 311 are substituted by serine, SC would be unable to disulfide bond to these residues. In fact, we have previously shown that SC is non-covalently bound in sIgA-g subclass molecules whereas sIgA-f molecules have covalently bound SC (28). Therefore, we predict that the amino acids at positions 299 and/or 311 will be cysteine in α-f chains. The presence of cysteine at position 311 in one of three αg subclass molecules (Figure 3) may reflect genetic polymorphism and may indicate that cysteine 299 is more important in SC binding.

One other amino acid residue, cysteine 241, presumably involved in interchain disulfide bonds in human IgA (25,26), is also substituted in other species. Position 241 is glycine in rabbit and serine in mouse α-chains (23). The significance of this substitution is not clear.

The hinge regions of mouse, human and rabbit α-chains show major differences in their cysteine content; whereas the hinge region of mouse and human α-chains have no cysteine residues, the rabbit hinge has five

cysteine residues, four of which are likely involved in interchain disulfide bonds. [The fifth cysteine may be part of a disulfide bond between the CH1 domain and the hinge region (25)] Previous studies have identified from six to nine free sulfhydryl (-SH) residues in rabbit sIgA and these were localized to the Fab portions of the molecule (29). Since the isolated Fcα fragments of rabbit IgA do not include the hinge region (5), it is likely that the hinge region is found in the Fab fragments. Thus, we suggest that the large number of free -SH groups in rabbit sIgA may be due to the cysteine residues in the hinge region and that there may be considerable disulfide bond exchange occurring, leaving some cysteine residues with free -SH groups at any given time.

Recent serological evidence has indicated that rabbit IgA may be comprised of at least three subclasses, IgA-f1, IgA-f2 and IgA-g (6). Hybridization of the α-cDNA probe to restriction digests of genomic DNA isolated from sperm supports the view that there are multiple Cα genes in the rabbit germline DNA. The precise number of Cα genes cannot be determined from these experiments since we cannot be certain that a particular restriction enzyme does not cleave within an intron or exon of the Cα gene, resulting in more than one band for each gene on the Southern blot. However, in all experiments, regardless of which restriction enzymes are used, multiple (6 to 9) hybridizing fragments are found. These results indicate that rabbit IgA is more complex than mouse and human IgA where one and two α-chain genes, respectively, have been found (30,31). Further understanding of the complexity of rabbit IgA will come from the isolation and characterization of the germline genes encoding α-chains.

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