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Amino acid sequence of the first constant region domain and the hinge region of the δ heavy chain of human IgD

(antibody structure/IgM/protein evolution/galactosamine oligosaccharides/lymphocyte receptor)

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ABSTRACT We have determined the amino acid sequence of the first constant (C) region domain ($C_\delta1$) and the hinge region of the δ heavy chain of human IgD WAH and also the sequence of the adjacent COOH-terminal portion of the variable (V) region, including the J_H region. Together with the sequence of the Fc fragment already reported, this establishes the complete amino acid sequence of the C region of the human δ chain and confirms the presence of three C region domains in human IgD. Although the C_H1 domains of the five classes of human heavy chains have the expected degree of homology (≈30%), the homology of the $C_\delta1$ domains of the human and mouse chains is less than that exhibited by the C_H1 domains of other pairs of human and mouse heavy chains. The hinge region of the human δ chain has an unusual structure; the NH_2-terminal half has four (or five) GalN oligosaccharides attached, whereas the COOH-terminal half lacks carbohydrate, is dissimilar in sequence, and has a high charge. A computer search verified that the GalN-rich segment has a high degree of identity in sequence with the middle portion of the human $C_\mu2$ domain and that the high-charge segment is related to the same sequence. We propose that the two segments of the human δ hinge have a common evolutionary origin and arose by duplication and independent mutation of a hinge exon derived from the ancestral gene for the $C_\mu2$ domain.

Although the primary structure of the Fc fragment region of human IgD has been determined (1, 2), IgD is the only one of the five classes of human immunoglobulins for which the complete amino acid sequence has not been reported. Yet, interest in its structure has intensified because of evidence that IgD exists in two forms (3, 4): the IgD secreted into the serum and the IgD bound to the surface membrane of B lymphocytes, where it functions as a receptor (5). Whereas the δ heavy chain of human IgD has a four-domain structure consisting of a variable (V_H) region and three constant (C) region domains ($C_\delta1$, $C_\delta2$, and $C_\delta3$) (1, 2, 6), DNA sequence analysis of the gene coding for a mouse δ chain (7) indicates the presence of only two C region domains, designated $C_\delta1$ and $C_\delta3$ with an apparent deletion of $C_\delta2$. Although a deletion of $C_\delta2$ has also been postulated in rat IgD on the basis of partial amino acid sequence data (8), the latter were not placed correctly by homology (9).

We report here the tentative amino acid sequence of the J_H region, the $C_\delta1$ domain, and the hinge region of the human δ chain WAH. These results, together with the Fc sequence of 226 residues (1), give the complete amino acid sequence of the C region of a human δ chain and establish the presence of three C region domains and an unusual hinge region structure. The hinge region is remarkable for its length (≈64 residues), the presence of four or five GalN oligosaccharides, the paucity of cysteine, the presence of a highly charged region, and the fact that it appears to be divided into two structurally distinct segments each of ≈30 residues. We call the NH_2-terminal half the GalN-rich segment because it contains all the GalN and the COOH-terminal half the high-charge segment because of its high content of glutamic acid and lysine. The GalN-rich segment has a high degree of identity with a segment from the middle of the second constant region domain (C_H2) of human and animal μ heavy chains ($C_\mu2$). A computer search verified the statistical significance of this identity and indicated that the high-charge segment of the hinge is related to the same $C_\mu2$ sequence. We propose that the human δ hinge is coded for by two separate exons (or a tandem union of two exons), one for the GalN-rich segment and one for the high-charge segment, and that the two exons arose by duplication and subsequent mutation of a common ancestral exon that originated from the gene for the primordial $C_\mu2$ domain.

EXPERIMENTAL PROCEDURES

Methods described by Lin and Putnam (6) were used to purify WAH IgD and to prepare the Fab(t), Fc(t), Fab(p), and Fc(p) fragments and the λ light and δ heavy chains. The Fd(p) fragment was prepared from aminoethylated Fab(p). When CNBr cleavage was done as described (1, 6), there was little cleavage of the Met-Gly (C44 to C45) bond. To cleave this bond the sample was reduced with 6 M thioglycolic acid before treatment with CNBr. The CNBr fragments were separated on Sephadex G-75 and purified with a high-performance liquid chromatography system consisting of a Beckman model 421 controller and model 110A pump. The columns (Synchropak RP-P and ULTRASPHERE ODS) were equilibrated with 0.1% trifluoroacetic acid or 0.1% hexafluorobutyric acid and eluted at a flow rate of 0.7 ml/min with an isocratic or programmed gradient of *n*-propanol or acetonitrile.

Peptides were prepared by digestion with trypsin, chymotrypsin, or *Staphylococcus aureus* V8 protease (1). The GalN-rich hinge peptide was resistant to these enzymes and so was also digested with proline-specific endopeptidase (EC 3.4.21.26) (Seikagaku Kogyo, Tokyo, Japan) (10). Peptide digests were separated by gel filtration and ion-exchange chromatography (1) and purified by high-performance liquid chromatography as described above. The methods for amino acid analysis and sequence determination of the peptides with a Beckman model 890C sequencer have been described (1, 6). To determine the

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Abbreviations: Fab(t) and Fc(t), tryptic Fab and Fc fragments of IgD; Fab(p) and Fc(p), papain Fab and Fc fragments of IgD. Abbreviations for classes, fragments, regions, and domains of immunoglobulins accord with official World Health Organization recommendations for human immunoglobulins published in ref. 35.

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amino sugar content, the sample was hydrolyzed in 4 M HCl at 110°C and analyzed with a Beckman model 121M amino acid analyzer. Several methods were tried in attempts to remove sugar from glycopeptides to facilitate enzymatic digestion and sequence analysis. The hinge glycopeptides were treated with HF at 0°C for 6 hr or at room temperature for 3 hr or with trifluoromethanesulfonic acid for 10 hr at room temperature. Also, Jacques Baenziger of Washington University of St. Louis School of Medicine digested the hinge glycopeptides for us with neuraminidase and *endo*-α-galactosaminidase.

RESULTS AND DISCUSSION

Primary Structure of the $C_\delta 1$ Domain and the Hinge Region. The amino acid sequence of the $C_\delta 1$ domain and the hinge region of the WAH δ chain is given in Fig. 1. The C region is assumed to begin at Ala-C1 after the Val-Ser-Ser sequence that ends the J_H region (12–14). When these results are added to the 226 residues of the $Fc_\delta(t)$ sequence that begins at Thr-C158 (1), the C region of the WAH δ chain has a length of 383 residues. Thus, it is longer than the C regions of the γ and α chains, which have three C_H domains (15–18), but shorter than the C regions of the μ and ε chains, which have four C_H domains (19–21) (Table 1). The δ chain is similar in length to $\gamma 3$, which has a four-fold repeating 15-residue sequence (22), but the only repeat in the δ hinge is the Ala-Thr-Thr sequence.

The NH_2-terminal sequence of $C_\delta 1$ of WAH is identical with the partial sequence given through Val-C38 for human IgD AM; the latter was determined by both protein sequence analysis and prediction from the nucleotide sequence (14). However, both of these δ chains lack two amino acids reported in the protein sequence for the ErI δ chain (23). There are other discrepancies in our sequence for WAH and the sequence of ErI, which was reported only through Met-C44. This is a difficult area for sequence determination because of poor cleavage of the Met-Gly bond by CNBr, and this methionine was missed in tentative structural models of human IgD (1, 6, 9).

By homology to the nucleotide sequences of the DNA exons for the C_H1 domain of the mouse $\gamma 1$ (24), $\gamma 2a$ (25), and $\gamma 2b$ (26) chains, the $C_\delta 1$ domain is taken to be $\approx$98 residues long. This estimate is supported by the homology of the C_H1 domains of the human γ and δ chains (Fig. 2) and agrees with the $C_\delta 1$ domain of 101 residues predicted from the mouse DNA sequence (7). By similar reasoning, the exon of the human $C_\delta 2$ domain was assumed to begin with Ser-C163 of Fig. 1, which corresponds to the sixth position in the $Fc_\delta(t)$ sequence (1, 2). By difference, this requires the human δ-hinge region to have 64

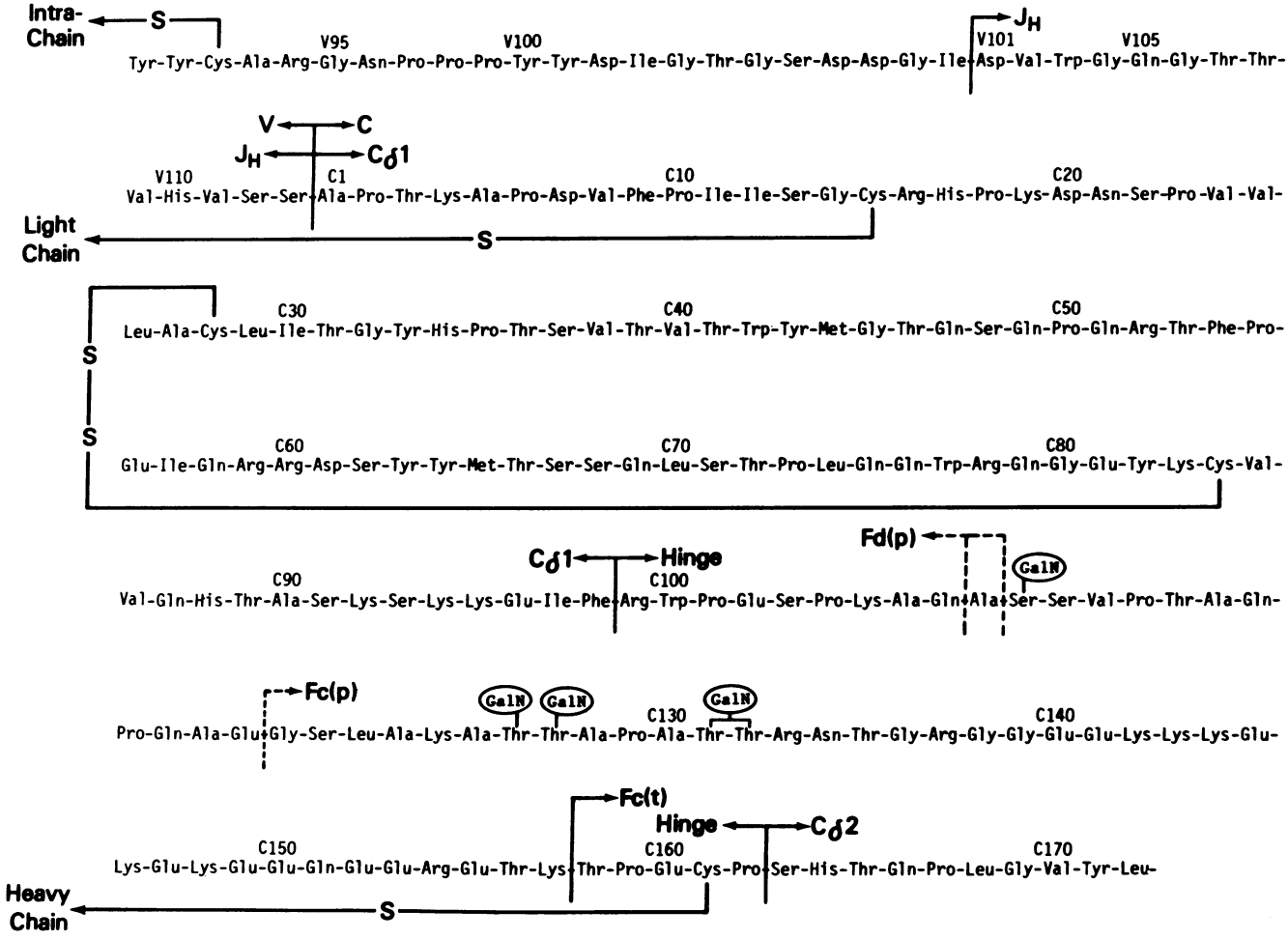

FIG. 1. Amino acid sequence of the $C_\delta 1$ domain and the hinge region of the δ heavy chain of IgD WAH and the COOH-terminal portion of the V region and the NH_2 terminus of the Fc(t) fragment (1). Because V regions vary in length, several characteristic residues are designated V and to facilitate comparison are numbered according to the adjusted numbering system of Kabat *et al.* (11). Thus, Cys-V92 is the invariant second half-cystine in the intrachain disulfide bridge of all V_H regions and Asp-V101 marks the beginning of the J_H region (12–14). The C region residues are designated C and are numbered consecutively beginning with Ala-C1. The hinge region has four (or five) GalN oligosaccharides attached. Dashed vertical lines indicate multiple sites of cleavage of IgD by papain to yield δ chain fragments called Fd(p). The tryptic Fc(t) fragment begins at Thr-C158 and has an interchain disulfide bond at Cys-C161 just before the start of the $C_\delta 2$ domain at Ser-C163.

Table 1. Number of amino acid residues and carbohydrate content of the C regions of human heavy chains

Chain	C_H domains, no.	Residues, no.		Oligosaccharides, no.	
		Hinge*	C region	GalN (hinge)	GlcN (domains)
δ	3	64	383	4 or 5	3
$\gamma 1$	3	15	329	0	1
$\gamma 2$	3	12	325	0	1
$\gamma 3$	3	62	375	0	1
$\alpha 1$	3	26	353	5	2
$\alpha 2$	3	13	340	0	4,5†
μ‡	4		450		5
ε‡	4		420		6

* Estimates of the length of the human hinge regions are approximate. Lengths given for the $\gamma 3$ and ε chains are estimates because the sequences are incomplete (21, 22).

† The A2m(1) allotype of the $\alpha 2$ chain has four GlcN oligosaccharides, and the A2m(2) allotype has five (17, 18).

‡ The μ and ε chains lack a hinge region but have an extra (fourth) C_H domain (19–21).

amino acid residues—i.e., to extend from Arg-C99 through Pro-C162. This needs verification by DNA sequence analysis of the δ-chain gene.

Comparison of the C_H1 Domains of Human Heavy Chains. The amino acid sequence of the WAH $C_\delta 1$ domain is compared with the sequences of the C_H1 domains of human μ (19, 20), $\gamma 1$ (15), $\alpha 1$ (16), and ε (21) heavy chains in Fig. 2. The C_H1 domains of the five human heavy chains have about the same degree of homology when compared pairwise with each other (≈30%). In this alignment, 55 residues of the δ chain are identical to one or more residues at the same position in the other four chains. Only 10 positions are identical in all five chains; 9 of these are located in the β strands of the β-pleated sheet structure determined for IgG1, and the 10th is at the end of β strand 4-2. Hence, these conserved positions probably constitute the framework of the three-dimensional structure of the C_H1 domain, which has the function of interaction with the C_L domain of the light chain. The degree of identity of the $C_\delta 1$ domain with the C_H1 domain of other chains is μ, 29%; $\gamma 1$, 32%; $\alpha 1$, 33%; and ε, 26%. This exceeds the degree of identity of the three C_H domains of the δ chain for each other; in an alignment set to maximize their similarity (not shown), $C_\delta 1$, $C_\delta 2$, and $C_\delta 3$ exhibit only 17–21% identity. Neither the results for $C_\delta 1$ nor those for Fc_δ support the suggestion (3) that IgD is more closely related to IgE than to the other three classes.

As in IgM, IgA, and IgE (but unlike IgG), the light chain of IgD is linked by a disulfide bond to the first half-cystine in the C_H1 domain (Cys-C15). Although μ, $\alpha 2$, and ε have a GlcN carbohydrate attached at a homologous position in the C_H1 domain, this is absent in δ, $\alpha 1$, and γ. This carbohydrate and others present in the C_H1 domains of the α and ε chains are located in bends of the polypeptide chain between the β strands; hence, the C_H1 domains of all five chains can probably assume similar conformations (the immunoglobulin fold) that enable all of them to pair in a combinatorial manner with both κ and λ light chains. However, some preference for λ chains has been noted for IgD (3, 4).

Homology of the $C_\delta 1$ Domains of Human and Mouse δ Chains. It is surprising that the $C_\delta 1$ domains of the human and mouse δ chains have only ≈25% homology. In an alignment in which five gaps were inserted into the mouse δ chain (7) to achieve maximum homology, only 24 positions of 95 compared were identical. This contrasts with the much greater homology of other pairs of human and mouse C_H1 domains—i.e., $\gamma 1$, 60%; μ, 48%; and $\alpha 1$, 40%. Also, the mouse $C_\delta 1$ domain has three possible sites for attachment of GlcN to the signal sequence Asn-X-Thr/Ser, whereas none is present in human $C_\delta 1$. Because the mouse δ chain also appears to lack the $C_\delta 2$ domain, the primary structure predicted from the nucleic acid sequence should be verified by sequence analysis of the protein.

Characteristics of the Hinge Region. The hinge region is an unusual structure in the segment of the heavy chain that joins the Fd and Fc regions of immunoglobulin classes having only three C_H domains (IgG, IgA, and IgD) but is absent in IgM and IgE, which have four C_H domains (Table 1). The amino acid

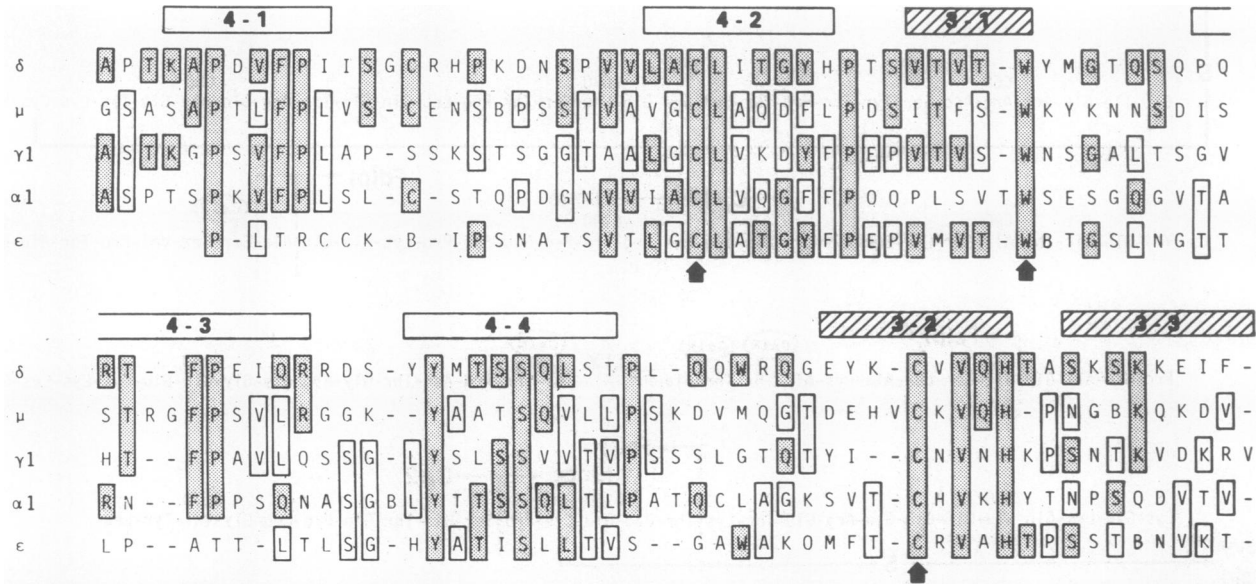

FIG. 2. Comparison of the amino acid sequences of the C_H1 domains of the five classes of human immunoglobulins. The one-letter notation for amino acids is given in ref. 27. The boundaries of the C_H1 domains are based on structural characteristics and comparison with the known junctional sequences of the exon for the C_H1 domain of mouse γ chains (24–26). Gaps have been inserted to maximize the homology. The three invariant cysteine (C) and tryptophan (W) residues in each domain were used to place the alignment in register, and they are indicated by arrows. Residues in the μ, γ, α, or ε sequences that share identity with the corresponding residues in the δ chain are outlined by shaded boxes; residues that are identical in sequences other than the δ chain are outlined in open boxes. The β strands (28) are numbered according to Edmundson *et al.* (29) with the four-stranded β-sheet elements in open bars and the three-stranded β-sheet elements in hatched bars.

sequence of the hinge region is unique for each class, differs markedly even for subclasses, and appears to be unrelated to the rest of the chain; that is, the hinge region lacks the characteristic structural features of the immunoglobulin fold. The function of segmented flexibility has been ascribed to the hinge, and this idea is supported by the fact that the hinge region is disordered in the crystal structure (28). The hinge of the δ chain differs from the hinges in γ and α in four notable characteristics: (*i*) its length (≈64 residues), which is about 4 times the length of the γ1, γ2, and α2 hinges and twice that of the α1 hinge, the only other one to contain carbohydrate (Table 1); (*ii*) its division into two distinct segments (the GalN-rich NH_2-terminal half and the high-charge COOH-terminal half); (*iii*) its dominant composition and repetitive pattern (alanine and threonine in the first half and glutamic acid and lysine in the second half versus half-cystine and proline in the γ and α chains); and (*iv*) presence of only one half-cystine.

Predicted Conformation of the Human IgD Hinge Region. The surprising division of the human δ hinge into two dissimilar halves led us to attempt to predict the conformation by the procedure of Chou and Fasman (30). No clear result was obtained for the GalN-rich segment, which appears to have a random structure. The potential for both the β sheet and the α helix are low because of the frequency of proline and serine. The frequency of GalN oligosaccharides probably contributes to the apparent disorder in the first part of the human IgD hinge. In contrast, the high-charge region from Glu-C140 through Glu-C155 appears to form an ideal α-helical segment. Glutamic acid has the highest conformational parameter for the α helix and the lowest for β sheet of any amino acid (30). Lysine is also favorable for the α helix but not for the β sheet. The rigid highly charged α helix may be separated from the GalN-rich segment by a β turn induced by glycine in the sequence Gly-Arg-Gly-Gly and would be followed by the first β-sheet strand of the $C_\delta 2$ domain. Because α-helical segments are rare in immunoglobulins, the presence of one in human IgD is probably associated with specific biological function. Thus, its apparent absence in the mouse IgD hinge is surprising.

Number and Location of Oligosaccharides in the Hinge Region. The presence of four or five GalN-oligosaccharides in the human δ hinge is of interest because of the rarity of GalN in immunoglobulins, the high local concentration of GalN, the effect this has on secondary structure and conformation, and its possible biological significance. The human α1 hinge, which has an octapeptide duplication, has five GalN-Gal disaccharides; one is attached in *O*-glycosidic linkage to each of the five serines within a sequence of only 17 amino acid residues (16), but the human α2 hinge lacks GalN because of a deletion that includes all the threonine and serine residues (17, 18). In contrast to the highly charged α-helical section of the δ hinge, which is easily cleaved by proteolytic enzymes, the GalN-rich segment of δ (like the α1 hinge) was very resistant to all enzymes we tried. This impeded determination of the protein sequence and of the number, location, and structure of the GalN oligosaccharides. Our attempts to remove the carbohydrate from hinge glycopeptides by chemical or enzymatic treatment also were only partly successful. However, the partially deglycosylated glycopeptides were more amenable to purification, digestion, and sequence determination.

The tentative location of four or five GalN oligosaccharides was assigned by difference in the GalN content of various subpeptides and by the failure to detect serine or threonine at a given step in the operation of the protein sequencer. This approach allowed assignment of one GalN to Ser-C109 and one to each of the threonines in the first Ala-Thr-Thr sequence. It is uncertain whether the second Ala-Thr-Thr sequence has GalN on both threonines or only one.

Origin of the δ-Chain Hinge. Although the boundaries of the hinge region were originally based on structural characteristics, it is now known that, in the mouse γ-chain subclasses, the hinge region is precisely defined by a DNA coding segment (exon) that is separated by intervening noncoding DNA sequences (introns) from the exons that code for the C_H1 and C_H2 domains (24–26). The evolutionary origin of the hinge is unknown, and the hinge is the most mutable region in immunoglobulin chains, as shown by the frequency of deletions and duplications. These facts and the distinguishing characteristics of the δ hinge caused us to search for possible regions of homology in other classes of heavy chains. Because the μ chain is thought to be the prototype heavy chain and lacks a hinge but has an extra domain ($C_\mu 2$), we searched that domain for possible homology to the δ hinge, with the result shown in Fig. 3. In the GalN-rich segment of the human δ hinge, 12 of 36 successive residues are identical to the sequence shown from the middle of the C_H2 region of the human μ chain except for uncertainty at Gln-C117 and Glu-C119. The same positions are identical in the dog μ chain; seven of them also are identical in the mouse μ chain. It is unlikely that a 33% identity in sequence occurs by chance in two unrelated proteins. To ascertain its significance, we asked Winona C. Barker of the National Biomedical Research Foundation to make a computer search for similarity to the entire collection of protein sequences of the Atlas of Protein Sequence and Structure (33). Using the unitary matrix that scores only identities, the search program (34) examined a segment of 30 residues in the δ hinge (see Fig. 3) and made 179,748 comparisons. The highest score (10 identities) was registered for the 30-residue segment of the human and dog μ chains shown in Fig. 3. (A segment of the β chain of rabbit tropomyosin where the sequence is proposed but is incomplete also gave a score of 10.) A score of 9 was given for the mouse δ-chain hinge and also for segments from eight other proteins. However, by inserting some gaps in the human and mouse δ hinges to maximize the

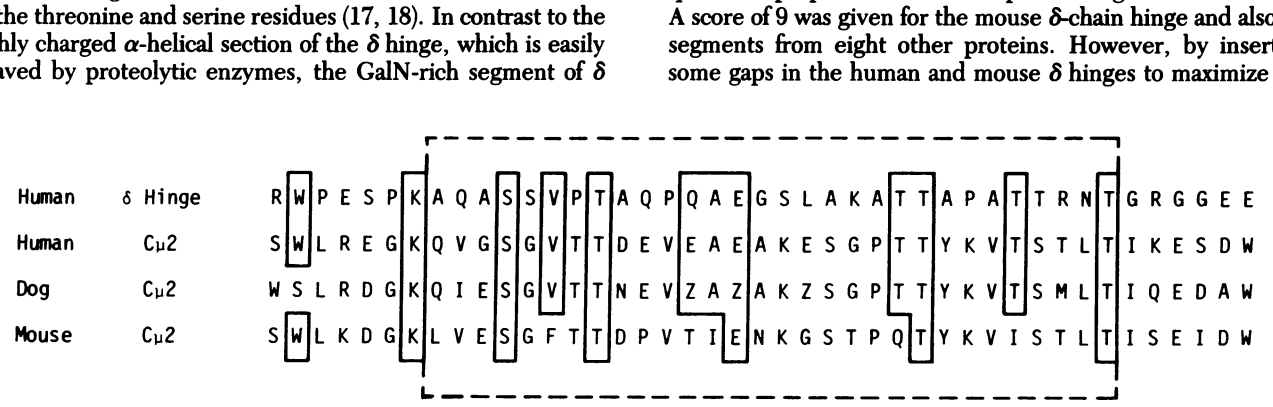

FIG. 3. Identities in amino acid sequence of the GalN-rich segment of the hinge region of the human δ chain and the $C_\mu 2$ domain of human (19, 20), dog (31), and mouse (32) μ chains. Positions having residues identical to those in the δ chain are enclosed in solid boxes. Sequences aligned as having highest similarity by a computer search using a unitary matrix that scores only identities but does not differentiate Q, E, and Z (33, 34) are enclosed by dashed lines. The human μ chain sequence begins at position 270 and has been updated.

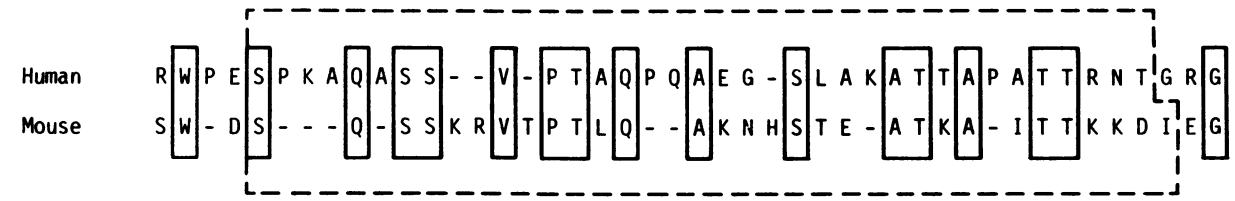

FIG. 4. Similarity between the amino acid sequence of the GalN-rich segment of the hinge region of the human δ chain and that of the entire hinge region of the mouse δ chain deduced from the DNA sequence (7). Identical residues are enclosed in solid boxes. Gaps have been inserted to maximize the homology. Segments aligned by a computer search using a scoring matrix based on the genetic code with no gaps inserted are enclosed by dashed lines.

homology, we had previously obtained 17 identities (Fig. 4). Many protein segments gave a score of 8, including fragments of fish antifreeze glycoproteins that have a repeating structure of Ala-Ala-Thr with GalN attached to every threonine. Thus, high scores may be given not only by proteins that are related genetically but also by proteins that have achieved similar structural features through convergent evolution. One score of 8 was for a 30-residue segment in the C_H3 domain of the human ε chain that is homologous to the segment of the $C_\mu2$ domain shown in Fig. 3.

Possible Duplication of the Human δ Hinge. Although there is no apparent similarity in sequence of the first and second segments of the human δ hinge, the two may have a common evolutionary origin. When the computer search was made for the high-charge region using the genetic code mutation matrix instead of the unitary matrix, the only immunoglobulin region that scored high was exactly the one in the $C_\mu2$ domain that scored highest for the first segment of the human δ hinge (Fig. 3). Although there are only five identities, the score was 58, compared with a largest possible score of 90, a maximum observed of 61, and an average of 44, with a SD of ±3.8. The high-charge segment was listed 18th in nearly 180,000 comparisons. This suggests that, whereas the mouse δ hinge consisting of 35 amino acids is encoded by only one DNA exon, the human δ hinge may be coded for by two separate exons (or by two joined in tandem), one for the GalN-rich region, the other for the high-charge region. The two human δ-hinge exons may have arisen by duplication and subsequent mutation of a common ancestral exon derived from the primordial gene for the $C_\mu2$ (or $C_\varepsilon3$) domain, whereas evolutionary deletion of one hinge exon may have led to the half-size murine δ hinge. This hypothesis is supported by the existence of duplications and deletions in the hinge region of human γ and α chains (12) and the quadruplication in the γ3 hinge (22). If this hypothesis is correct, the hinge regions of human and mouse IgD may have acquired somewhat different biological functions. Alternatively, part of the mouse δ hinge may have been deleted or missed in the DNA sequences or the two forms of IgD may have different functions.

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