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β_2-Microglobulin—A Free Immunoglobulin Domain

(light and heavy chain/amino-acid sequence)

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ABSTRACT **Analysis of the primary structure of β_2-microglobulin indicates that this human protein is homologous in sequence to the constant portion of immunoglobulin light chains (C_L), and to the homology regions (C_H1, C_H2, and C_H3) of the constant portion of γ1 (heavy) chains of immunoglobulin G. Homology with the C_H3 region is particularly striking. No convincing homology could be demonstrated by similar comparisons with the variable regions of immunoglobulin light and heavy chains. β_2-Microglobulin contains an intrachain disulfide loop of 57 amino-acid residues that is similar in size to disulfide loops found in the constant regions of immunoglobulin G. These findings suggest that β_2-microglobulin is a free immunoglobulin domain, possibly serving an effector function similar to that of the C_H3 domain of γ1 chains of immunoglobulin G.**

The distribution of homologous sequences and the arrangement of the intrachain disulfide bonds in the light and heavy chains of the myeloma protein Eu led to the prediction (1, 2) that antibody molecules are composed of a series of compact domains (Fig. 1), each contributing to an active site. V-region domains (V_L and V_H) mediate antigen-binding functions, while C-region domains (C_L, C_H1, C_H2, and C_H3) mediate effector functions. Each domain contains about 100 amino-acid residues and has a single disulfide loop of about 60 residues. The overall structural features of γG1 immunoglobulins have been confirmed by analysis of the primary structure of the heavy chain of another myeloma protein, He (3). Strong confirmation of the domain hypothesis has come from x-ray crystallographic studies on whole immunoglobulin G (IgG) (4) and on crystals of Fab fragments (5), from electron microscopic studies of IpA (6, 7), and from the results of limited proteolysis of immunoglobulins (8–11). Additional support has come from the reconstitution of heavy chain dimers (12) and analysis of the properties of isolated V_L and C_L regions (9, 10, 13).

As shown by Berggård and Bearn (14), the human protein β_2-microglobulin contains about 100 amino-acid residues and has a single disulfide bond. This protein has no known function but appears in low amounts in the serum, urine, and cerebrospinal fluid of normal individuals and in increased amounts in the urine of individuals with malfunction of the renal tubules. Because of the size and amino-acid composition of this protein, it was reasonable to ask whether β_2-microglobulin is related in any way to the polypeptide chains of immunoglobulins. Smithies and Poulik (15) have recently reported the amino-acid sequence of 44 of the first 46 residues of β_2-microglobulin and compared their sequence with that of protein Eu. On the basis of their data, they have postulated that the gene for this protein arose by an evolutionary event that resulted in a deletion of a large segment of an Eu-like ancestral gene.

We report here the tentative amino-acid sequence of the entire β_2-microglobulin molecule. The sequence obtained is homologous to all of the constant domains (C_L, C_H1, C_H2, and C_H3) of the immunoglobulin Eu, particularly to C_H3. The protein also contains a disulfide loop similar in size to those found in each of the IgG domains. We suggest that β_2-microglobulin is a free immunoglobulin domain, and propose that it represents a product of lymphoid cells that probably performs an effector or regulatory function in the immune system.

MATERIALS AND METHODS

β_2-Microglobulin was isolated from the urine of patients with chronic cadmium poisoning, as described by Berggård and Bearn (14). The amino-acid sequence was determined by isolation and characterization of tryptic, chymotryptic, thermolytic, and peptic peptides. Actual sequence determination was performed on peptides by the dansyl-Edman technique (16–18), and positions of asparaginyl and glutaminyl residues were determined by the electrophoretic mobility of the appropriate peptides at pH 6.5 (19). Details

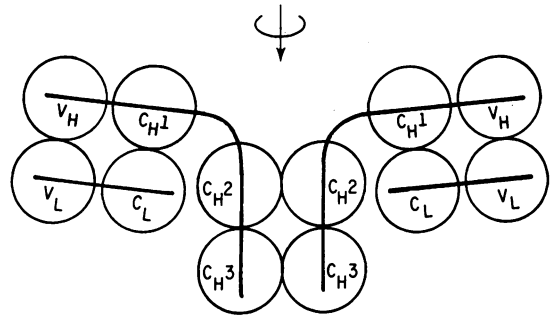

FIG. 1. Diagram of the overall structure of immunoglobulin G illustrating the domain hypothesis (1, 2). Light chain (V_L, C_L) and heavy chain (V_H, C_H1, C_H2, and C_H3) domains are denoted by *circles;* the dyad axis is indicated by Ψ. Each domain is connected to the succeeding domain by a less tightly folded region of the polypeptide chain. V-region domains mediate antigen-binding functions, while C-region domains mediate effector functions.

Abbreviations: Ig, immunoglobulin; PBS-BSA, phosphate-buffered saline (800 g NaCl, 1.15 g Na_2HPO_4, 0.20 g KH_2PO_4, 0.20 g KCl per liter of H_2O, pH 7.4) containing 0.2% bovine serum albumin.

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1                                   10                                  20
ILE-GLN-ARG-THR-PRO-LYS-ILE-GLN-VAL-TYR-SER-ARG-HIS-PRO-ALA-GLX-ASX-GLY-LYS-SER-

21                                  30                                  40
ASX-PHE-LEU-ASN-CYS-TYR-VAL-SER-GLY-PHE-HIS-PRO-SER-ASP-ILE-GLU-VAL-ASP-LEU-LEU-

41                                  50                                  60
LYS-ASP-GLY-GLU-ARG-ILE-GLX-LYS-VAL-(ASX, HIS, SER, GLX)-LEU-SER-PHE-SER-LYS-ASN-SER-

61                                  70                                  80
TRP-PHE-TYR-LEU-(LEU, TYR, SER)-TYR-THR-GLU-PHE-THR-PRO-THR-GLU-LYS-ASP-GLU-TYR-ALA-

81                                  90                                 100
CYS-ARG-VAL-ASX-HIS-VAL-THR-LEU-SER-GLX-PRO-LYS-ILE-VAL-LYS-TRP-ASP-ARG-ASP-MET
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FIG. 2. Amino-acid sequence of β_2-microglobulin.

of the sequence determination will be reported elsewhere (manuscript in preparation).

Estimates of the number of β_2-microglobulin molecules on leukocytes were made with ^{125}I-labeled rabbit antibodies to the human protein. Antisera were obtained by injection of β_2-microglobulin in complete Freund's adjuvant intramuscularly into rabbits. IgG was purified from the antisera by precipitation with ammonium sulfate (37% saturation) at 21° followed by chromatography on diethylaminoethyl (DEAE)-cellulose (20). The rabbit anti-β_2-microglobulin was labeled with ^{125}I (21) (3.1×10^5 cpm/μg). Leukocytes were prepared from whole human blood by differential sedimentation in 3% gelatin prepared with mammalian Ringer's solution (22). The binding of ^{125}I-labeled anti-β_2-microglobulin to human leukocytes was determined by incubation of 2×10^6 leukocytes with various concentrations of the labeled antibody in 750 μl of phosphate-buffered saline (pH 7.4)–0.2% bovine serum albumin (PBS–BSA) for 30 min at room temperature (21°). The cells were then washed four times with PBS–BSA, and counted for radioactivity in a Nuclear Chicago model 4320 gamma spectrometer.

RESULTS AND DISCUSSION

The tentative amino-acid sequence of β_2-microglobulin is shown in Fig. 2. The protein contains 100 amino-acid residues and the sequence is in excellent agreement with the reported composition (14). The sequence within the first 46 residues is identical with that determined by Smithies and Poulik (15), and the two residues [31 and 45], not identified in their study have now been determined. The single outstanding feature of the structure is a cluster of residues with aromatic side chains near the center of the molecule [residues 61–71]. Because the protein exists predominantly as a monomer of molecular weight 12,000 and contains no free-SH groups (14), we conclude that the two half-cystinyl residues at positions 25 and 81 participate in an intrachain bond, forming a loop of 57 amino-acid residues.

When aligned as shown in Fig. 3, the sequence of β_2-microglobulin is homologous with the constant region of the Eu light chain (C_κ) and with the homology regions of the Eu heavy chain (C_H1, C_H2, and C_H3). Of the 93–97 positions compared (Table 1), 26 are identical with C_L (C_κ), 22 with C_H1, 22 with C_H2, and 28 with C_H3. A similar comparison with C_λ (23) indicated identical residues in 21 positions. The same comparisons among the various homology regions of protein Eu (Table 1) yielded identical residues in 28–34 positions (2); when similarly aligned, C_κ and C_λ are identical in 38 positions. No convincing homology was obtained with the variable regions.

β_2-Microglobulin is therefore homologous to all of the constant homology regions in κ, λ, and γ chains, but appears to be most closely related to the C_H3 region of $\gamma1$ chains. On the basis of the number of identical residues, β_2-microglobulin is as closely related to the C_H3 region as the various homology regions of Eu are related to each other. When the sequences C_κ, C_H1, C_H2, and C_H3 are aligned as shown in Fig. 3, all four sequences give identical residues in eleven positions. β_2-Microglobulin has identical residues in 10 of these positions, the outstanding exception being the absence of a tryptophanyl residue at position 39. The tryptophanyl residues in protein Eu show a characteristic linear distribution (2) that is not apparent in the sequence of β_2-microglobulin (Fig. 2). While this difference requires nonconservative mutations and might have functional significance, none is apparent at this time.

Comparison of the same sequences (Fig. 3, Table 1) by the method of Fitch (24) emphasized the similarity between β_2-microglobulin and the C_H3 region. When no deletions were made (Fig. 4), the homology with C_H3 was particularly outstanding. When the sequences are aligned as shown in Fig. 3, all the regions show homology with β_2-microglobulin, but that for C_H3 is still particularly pronounced.

The similarity of β_2-microglobulin to the homologous regions of light and heavy chains suggests that this protein might be the product of proteolytic cleavage of a larger immunoglobulin. Limited proteolysis of immunoglobulins has been shown to lead to cleavage between homologous segments of both light and heavy chains (8–11), and the products are often seen in urine. Although we cannot rigorously rule out this possibility, it seems unlikely for several reasons. There is no indication of internal cleavage or of fraying at the carboxyl- or amino-terminal ends as is often seen in such proteolytic cleavages. Moreover, the amino-acid sequence does not correspond exactly to any known immunoglobulin sequence nor does β_2-microglobulin react with antisera to any of the known classes of immunoglobulins, to the J chain, or to the transport piece of secretory IgA (14, 15). Similarly, antisera against β_2-microglobulin do not react with

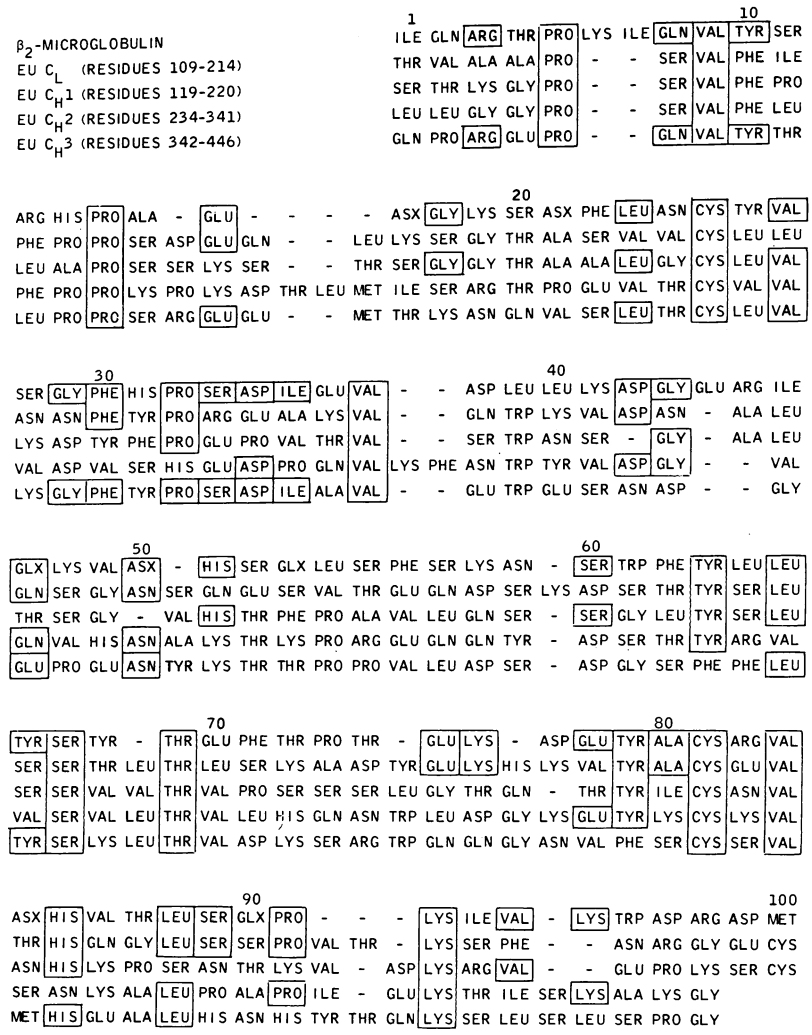

FIG. 3. Comparison of the amino-acid sequence of β_2-microglobulin with the homology regions C_L, C_H1, C_H2, and C_H3 of the γG1 immunoglobulin Eu. Deletions, indicated by *dashes*, have been inserted to maximize homologies. Identical residues are enclosed in *boxes*. Numbering is for β_2-microglobulin.

purified IgG, or with any other serum protein. This would suggest that if β_2-microglobulin does come from a larger molecule, it would have to be derived from an as yet unknown immunoglobulin *via* a relatively specific cleavage. Finally, β_2-microglobulin does not appear to bind γG1 immunoglobulins, even if the light and heavy chains are separated and then reconstituted in its presence.

If we assume that β_2-microglobulin is the product of a distinct structural gene, two questions arise: (*a*) how is the protein related to immunoglobulins?, and (*b*) more important, what is its function? Analysis of the structural features of the myeloma protein Eu has provided convincing support for the hypothesis (25, 26) that immunoglobulins evolved by the duplication of a precursor gene of a size sufficient to specify a polypeptide chain of 100-110 residues—the size of β_2-microglobulin. Many schemes can be envisioned to explain the evolution of the gene for β_2-microglobulin. These range in complexity from direct evolution from the immunoglobulin precursor gene to evolution via some mechanism that occurs after the duplication event. The data provide no direct support for more complex mechanisms, such as that suggested by Smithies and Poulik (15). The choice between the simple mechanism that we favor and more complicated schemes will depend upon genetic studies of this protein in various species, as well as upon knowledge of its function.

Studies of the function of β_2-microglobulin are hampered by the fact that this protein has been found only in humans. Our preliminary experiments to establish the exact location and possible function of this protein suggest that the molecule may be localized specifically in lymphoid tissue. Attempts to

TABLE 1. *Number of identical residues among the sequences of β_2-microglobulin and the constant homology regions of protein Eu**

	β_2	Eu C_L	Eu C_H1	Eu C_H2	Eu C_H3
β_2	—				
Eu C_L	26	—			
Eu C_H1	22	32	—		
Eu C_H2	22	34	28	—	
Eu C_H3	28	32	30	29	—

* Sequences were aligned as shown in Fig. 3, and the alignment was kept constant for all comparisons.

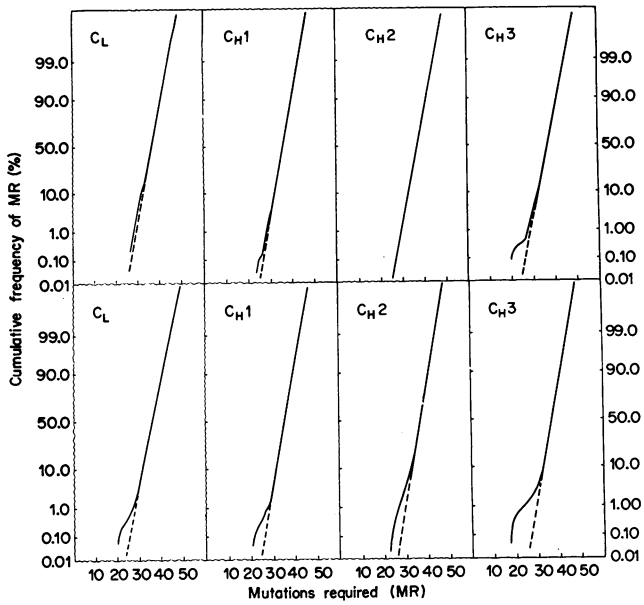

FIG. 4. Cumulative probability plots obtained in the comparison of β_2-microglobulin with the C_L, C_H1, C_H2, and C_H3 regions of the myeloma protein Eu according to the method of Fitch (24). In all cases, length of sequence examined (LSE) = 25. The *dashed line* denotes the normal probability distribution. *Top row*, comparison made with no deletions; *bottom row*, sequences aligned as shown in Fig. 3.

quantitate the number of β_2-microglobulin molecules on peripheral leucocytes (Fig. 5) were made with ^{125}I-labeled rabbit anti-β_2-microglobulin. If we assume uniform labeling of all cells, the results indicate an upper limit of 6×10^7 molecules per cell. Binding of the antibodies was inhibited by unlabeled β_2-microglobulin, and nonspecific rabbit immunoglobulins similarly labeled with ^{125}I gave essentially no binding. Experiments to determine whether this labeling is restricted to any special class of lymphocytes are in progress.

Although it seems clear that V-region domains of immunoglobulins serve antigen-binding functions, the effector functions served by the individual C-region domains have not been completely defined. It has been suggested (27) that the C_H2 domains are involved in complement binding, but no function has been assigned to the C_H3 domain. Because of their similarity, it is tempting to suggest that this domain and β_2-microglobulin serve a similar function, such as binding to the cell surface, although β_2-microglobulin may bind to a different population of cells than does C_H3.

One structural feature clearly distinguishes β_2-microglobulin from domains in the immunoglobulin molecule: in the immunoglobulin molecule domains occur in pairs (V_LV_H, C_LC_H1, C_H2C_H2, and C_H3C_H3), whereas the predominant form of β_2-microglobulin is the monomer (14). This suggests the possibility that the excreted β_2-microglobulin differs from the functional species that may exist as a dimer.

Regardless of such differences, β_2-microglobulin is strikingly similar to the domains of IgG and the evidence is consistent with the hypothesis that it represents a free domain. From its homology with the C_H3 domain, we suggest that it serves a similar function. A more detailed interpretation must await efforts to analyze its function and its three-dimensional structure. Evidence for the genetic polymorphisms and for the existence of this protein in other species will be key elements in extending these studies.

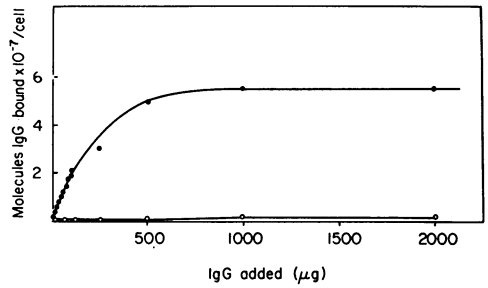

FIG. 5. Binding of anti-β_2-microglobulin IgG (*dark circles*) and nonspecific IgG (*open circles*) to human peripheral leukocytes. Various concentrations of rabbit [^{125}I]IgG obtained from antisera directed against β_2-microglobulin and nonspecific rabbit [^{125}I]IgG, respectively, were added to 2×10^6 leukocytes in a total volume of 750 μl of PBS–BSA.

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