lithosphere explains the recurrence interval of arc-breaking events and the separation of decoupling and lithospheric earthquakes. The viscosity found from postseismic rebound (*4*) for an arc event gives reasonable migration velocities for great earthquakes along major fault systems. Meters per year of displacement for lithospheric convergence and sinkage are appropriate after a major decoupling earthquake. Such rates with time constants of the order of 5 years reopen the question of excitation of the Chandler wobble and changes in the length of day (*2*).

DON L. ANDERSON
Seismological Laboratory,
California Institute of Technology,
Pasadena 91125

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Partial Amino Acid Sequence of Rabbit β_2-Microglobulin

Abstract. *The amino acid sequence of the first 35 residues of a low-molecular-weight protein obtained from the urine of rabbits treated with sodium dichromate was determined and shown to be identical with human β_2-microglobulin in 30 positions. Rabbit β_2-microglobulin, like the human protein, is strikingly homologous to the constant regions of rabbit immunoglobulin G, particularly the C_H3 region.*

β_2-Microglobulin is a low-molecular-weight protein found in normal biological fluids (*1*) and on the surfaces of a variety of different cell types (*2*). The complete amino acid sequence of human β_2-microglobulin (*3*) shows that it is strikingly homologous to immunoglobulin G (IgG) and in many ways resembles the individual domains of the constant region of the light and heavy chains (*4*). Recent data (*5*) indicate that β_2-microglobulin is closely associated with the histocompatibility antigens on cell surfaces and that it may represent the light chain of the human histocompatibility (HL-A) antigens. The similarity of β_2-microglobulin to IgG and its close association with the HL-A antigens has a number of important implications for the evolution of the immune and histocompatibility systems and for the structure of the HL-A antigens (*6, 7*). As a result, numerous attempts have been made to obtain similar proteins from other species for evolutionary comparisons and for functional studies.

A protein that resembles β_2-microglobulin in molecular weight, charge, and amino acid composition has been purified from the urine of rabbits treated with sodium chromate (*8*). We report here the partial amino acid sequence of this protein and show that it is very similar to both β_2-microglobulin and a homolog of β_2-microglobulin obtained from dogs (*9*). Like the human protein, rabbit β_2-microglobulin is homologous to the constant region of the heavy chain (C_H) of IgG.

The rabbit protein was prepared as described (*8*). Half-cystines were reduced in the presence of 6*M* guanidine hydrochloride and alkylated with iodoacetamide or [^{14}C]iodoacetamide (*10*). Excess reagents were removed by gel filtration on Sephadex G-25 in 5 percent formic acid. Manual sequence determinations on the intact protein (100 to 200 nmole) and on isolated peptides were carried out by the dansyl-Edman technique (*11*). Automatic sequence analysis of the reduced and alkylated protein (130 to 170 nmole) was performed in a Beckman model 890 C automatic sequencer and with the use of the Quadrol double cleavage program (Beckman Instruments).

Phenylthiohydantoins were identified quantitatively by gas chromatography on columns of Chromasorb SP400 in a Beckman GC-65 gas chromatograph. Qualitative identification of the phenylthiohydantoins was made by thin-layer chromatography on Chromagram silica gel sheets with fluorescent indicator (Eastman Kodak) with the use of solvent systems V and IV of Jeppson and Sjöquist (*12*) and by thin-layer chromatography on polyamide layers (*13*). The presence of half-cystine was determined by dissolving all samples from the sequencer in Aquasol and counting for ^{14}C in a Packard Tri-Carb 3003 scintillation counter. The presence of arginine (*14*) or histidine was determined colorimetrically.

Tryptic peptides were obtained from digests of 4 to 5 mg of the reduced and alkylated protein. Peptide TN1 ($Arg_{1.1}$ $Glu_{1.0}$ $Val_{0.9}$) (*15*) was purified initially by gel filtration of a tryptic digest on Sephadex G-25 in 0.015*M* NH_4OH which was 10 percent in 1-propanol. Extraction of the lyophilized fraction containing the material of lowest molecular weight with pyridine-acetate, *p*H 6.5 (*16*), gave peptide TN1 as a precipitate in 25 percent yield. Peptide TN2a ($Asp_{1.1}$ $Glu_{1.2}$ $Pro_{0.9}$ $Ala_{1.0}$ $Val_{0.9}$ $Tyr_{0.5}$) was obtained in 24 percent yield from a digest of the protein by high-voltage paper electrophoresis at *p*H 4.7 followed by electrophoresis at *p*H 1.9 (*17*).

The amino acid sequence of the first 35 residues of the rabbit protein is shown in Fig. 1, which also summarizes the methods used to identify each residue. The yield at each step was established from the gas chromatographic analysis. Yields were 77 percent for Val^1, 44 percent for Ala^4, 18 percent for Ser^{11} and 1 percent for Gly^{18}. Up to step 18 no contamination from previous steps or phase changes were apparent. After step 18 yields were decreased and the identification of the phenylthiohydantoins from residues 19 to 35 depended primarily upon qualitative techniques. Half-cystinyl residue 25 was determined in about 3 percent yield by counting the [^{14}C]carboxamidomethyl derivative. The sequence of residues 1 to 10 was confirmed by dansyl-Edman analysis of the intact protein and of peptides TN1 and TN2a. Peptide TN2a probably arose as the result of chymotryptic activity in the trypsin preparation. The human protein is also highly susceptible to chymotryptic cleavage at Tyr^{10} (*3*).

In Figure 1 are shown the positions where the rabbit homolog differs from human β_2-microglobulin (*3*) and from the dog homolog (*9*). Of the 35 residues compared, there are only 5 differences from the human protein, which has residues Ile^1, Thr^4, Lys^6, Ile^7, and Ser^{20} rather than those shown for the rabbit homolog. The dog homolog has Lys^6 and Ile^7 in common with the human protein and differs from the rabbit

homolog by having His[3], Pro[4], Pro[20], and Glx[34], and possibly a different, as yet unidentified residue, at position 33. The data support the conclusion that the rabbit protein is a homolog of β_2-microglobulin and indicate that all three proteins are closely related.

Rabbit β_2-microglobulin is similar in sequence to the constant region of the C_H of rabbit IgG (*18*) (Table 1) and resembles the various homology regions to nearly the same extent as the IgG homology regions resemble each other. When aligned for maximum homology the rabbit homolog of β_2-microglobulin appears to more closely resemble the C_H3 region of IgG than any of the other homology regions. The extent of homology between the amino terminal portions of rabbit β_2-microglobulin and the homology regions of rabbit IgG is similar to that found between the corresponding portions of human β_2-microglobulin and the homology regions of human IgG (Table 1). Human β_2-microglobulin also resembles C_H3 more closely than any of the other homology regions (Table 1), and the similarity extends throughout the entire sequence of β_2-microglobulin and the C_H3 region (*4, 6, 19*).

As is expected, the β_2-microglobulins are much more closely related to each other than either is related to the various portions of the immunoglobulins. Of the positions in rabbit β_2-microglobulin that differ from the human protein (Fig. 1), none improve or reduce the homology with human IgG so that both β_2-microglobulins are related to human IgG to the same extent. Rabbit β_2-microglobulin has one more identity with the C_H1 region of rabbit IgG than with human β_2-microglobulin, but the rabbit protein has one less identity with the

Table 1. Identical residues in the sequences of rabbit β_2-microglobulin and rabbit IgG. Residues 1 to 35 were compared. Sequences were aligned to maximize homology, Results obtained when comparing human β_2-microglobulin with human IgG are given in parentheses.

	β_2	C_H1	C_H2	C_H3
β_2	—			
C_H1	9(8)	—		
C_H2	6(6)	10(12)	—	
C_H3	13(16)	9(11)	10(9)	—

C_H3 region of rabbit IgG. As a result, both β_2-microglobulins are also related to rabbit IgG to about the same extent. Comparison of the amino terminal 35 residues indicates that rabbit and human β_2-microglobulin share 30 residues. Over the corresponding regions, their immunoglobulins share 23 of 36 residues in C_H1, 33 of 38 residues in C_H2, and 22 of 36 residues in C_H3. These data suggest that the β_2-microglobulins may be more highly conserved across species lines than are the immunoglobulins. The similar apparent conservation of the C_H2 region of IgG does not extend throughout the entire sequence of this homology region, and it is doubtful that the conservation of the β_2-microglobulins will be apparent when their complete sequences can be compared (*19*). The fact that the variations among the β_2-microglobulins are clustered near the amino terminus (Fig. 1) may prove significant with regard to the function of the molecule or may prove important for its interactions with other proteins, such as the histocompatibility antigens, or with the cell surface.

The extensive similarity between the β_2-microglobulins and immunoglobulins has led to two hypotheses regarding the possible evolutionary origin of β_2-microglobulin (*4*). We have suggested (*4, 6*) that the gene coding for β_2-microglobulin represents a modern descendant of a primitive precursor gene for immunoglobulins that arose prior to the duplication event (*7*). Proof of this hypothesis (*7*) may have increased significance because of the demonstration of a close association of β_2-microglobulin and the histocompatibility antigens on the cell surface (*5*). Continued structural comparisons of β_2-microglobulins within and among various species may reveal genetic polymorphisms of this protein and provide additional information on the evolution of immunoglobulins.

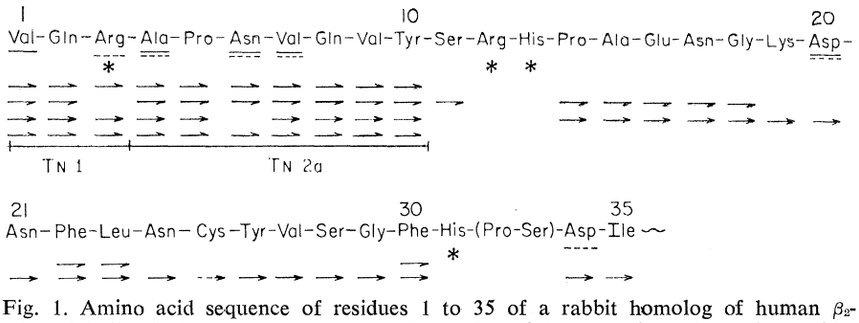

Fig. 1. Amino acid sequence of residues 1 to 35 of a rabbit homolog of human β_2-microglobulin. (→) denotes sequence analysis of the intact protein and tryptic peptides TN1 and TN2a by the dansyl-Edman technique. Other arrows indicate sequence determination in the automatic sequencer with phenylthiohydantoins identified by (⇀) thin-layer chromatography; (→) by gas chromatography; (*) by colorimetric assay, and (⇢) as [^{14}C]carboxamidomethyl derivative; (—) indicates residues differing from human β_2-microglobulin; (- - -) indicates residues differing from a dog homolog of β_2-microglobulin.

BRUCE A. CUNNINGHAM
Rockefeller University, New York 10021

INGEMAR BERGGÅRD
University of Lund, Lund, Sweden

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15. Abbreviations for amino acid residues are as follows: Ala, alanine; Arg, arginine; Asp, aspartic acid; Asn, asparagine; Cys, half-cystine; Glu, glutamic acid; Glx, glutamine or glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Phe, phenylalanine; Pro, proline; Ser, serine; Tyr, tyrosine; Val, valine.
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