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The Complete Amino Acid Sequence of β_2-Microglobulin†

Bruce A. Cunningham,* John L. Wang, Ingemar Berggård, and Per A. Peterson

ABSTRACT: The complete chemical structure of the human protein β_2-microglobulin has been determined. The protein contains 100 amino acid residues and one intrachain disulfide bond forming a loop of 57 residues in the polypeptide chain. Although the function of β_2-microglobulin is unknown, it is homologous in amino acid sequence to the homology regions C_L, C_H1, C_H2, and C_H3 of the human γG1 immunoglobulin Eu. The sequence homology between β_2-microglobulin and the C_H3 region of IgG1 is particularly striking, suggesting that β_2-microglobulin may serve a function related to that of the C_H3 domain of IgG, even though β_2-microglobulin is not restricted to the immune system. The available data suggest that the gene coding for β_2-microglobulin evolved directly from a primitive precursor gene for immunoglobulins. The complete sequence reported here provides a reference structure for evolutionary comparisons and for defining similar proteins in other species.

The human protein β_2-microglobulin is found in the serum of normal individuals and in elevated amounts in the urine of patients with Wilson's disease, chronic cadmium poisoning, and other conditions leading to renal tubular malfunction (Berggård and Bearn, 1968; Peterson *et al.*, 1969). The protein is a single polypeptide chain of molecular weight 11,600; it contains two half-cystinyl residues and no free sulfhydryl groups (Berggård and Bearn, 1968). Preliminary studies of the chemical structure of this protein (Smithies and Poulik, 1972a; Peterson *et al.*, 1972) suggested that it is homologous in sequence to the constant homology regions of γG1 immunoglobulins (Cunningham *et al.*, 1971).

The fact that β_2-microglobulin is comparable in size and homologous in sequence with the homology regions of the γG1 immunoglobulin Eu prompted us to suggest that the gene specifying β_2-microglobulin evolved directly from an immunoglobulin precursor gene prior to the duplication event (Peterson *et al.*, 1972). This hypothesis was strengthened by the fact that the two half-cystinyl residues are separated by about 60 residues and probably form a disulfide loop in β_2-microglobulin which is similar in size to the intrachain loops found in the immunoglobulin domains (Edelman *et al.*, 1969). On the basis of their limited sequence data, Smithies and Poulik (1972a) proposed a common evolutionary origin for β_2-microglobulin and immunoglobulins. In contrast to our hypothesis, they suggested that the gene for β_2-microglobulin arose as the result of a large deletion in an IgG-like gene.

We now report the determination of the complete amino acid sequence of the protein. The completed sequence supports the previous conclusions about the homology of this protein with portions of IgG and provides a framework for comparison with similar proteins from other species.

Materials and Methods

β_2-Microglobulin was purified from the urine of patients with chronic cadmium poisoning (Berggård and Bearn, 1968). Prior to proteolytic digestion for sequence analysis, the disulfide bond in the protein was reduced with dithiothreitol and the half-cystinyl residues alkylated with iodoacetamide (Waxdal *et al.*, 1968). The reduced and alkylated protein was separated from excess reagents by gel filtration on Sephadex G-50 or Sephadex G-75 in 1% NH_4HCO_3 or in 0.015 M NH_4OH which was 10% in 1-propanol. Direct sequence analysis of the intact polypeptide chain was performed on reduced and alkylated protein using the dansyl-Edman technique and with the Beckman 890C automatic sequencer using the Quadrol-Double Cleavage program (Beckman Instruments, Palo Alto, Calif.). After conversion, the phenylthiohydantoin (PhNCS) amino acids obtained from the sequencer were identified by thin-layer chromatography on Chromagram silica gel sheets with fluorescent indicator (Eastman Kodak Co., Rochester, N. Y.) using solvent systems V and IV described by Jeppson and Sjöquist (1967). For dis-

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TABLE I: Amino Acid Composition of Tryptic Peptides from β_2-Microglobulin.[a]

	TN 1	TN 2	TN 3	TN 3a	TN 3b	TN 4	TN 5	TN 6	TN 7	TN 8
Lys		1.1 (1)				0.9 (1)	1.0 (1)		1.0 (1)	1.0 (1)
His						1.0 (1)	0.9 (1)			1.0 (1)
NH_3										
Arg	1.2 (1)		1.0 (1)		1.0 (1)			1.0 (1)		
Trp[e]										
CMCys[f]							0.7 (1)			
Asp						1.0 (1)	3.5 (4)	0.9 (1)		1.0 (1)
Thr		1.0 (1)								
Ser			1.0 (1)		1.0 (1)		2.3 (3)			3.0 (3)
Glu	1.0 (1)		1.0 (1)	1.1 (1)		0.8 (1)	1.2 (1)	0.9 (1)	0.9 (1)	1.0 (1)
Pro		1.0 (1)				0.9 (1)	1.1 (1)			
Gly						1.0 (1)	1.0 (1)	1.0 (1)		
Ala						0.9 (1)				
Val			1.0 (1)	1.0 (1)			1.8 (2)			1.0 (1)
Met										
Ile	0.9 (1)		1.0 (1)	0.9 (1)			0.9 (1)		0.9 (1)	
Leu							2.5 (3)			1.0 (1)
Tyr			0.9 (1)	0.8 (1)			0.6 (1)			
Phe							1.6 (2)			1.1 (1)
Total residues	3	3	6	4	2	7	22	4	3	10
Yield (%)[g]	26	25	14	18	16	9	25	14	15	16

[a] Values reported are amino acid residues. Amino acids present at a level of 0.2 residue or less are omitted. The assumed integral numbers of residues are given in parentheses. [b] Amino acid analysis of peptide TN 8 after enzymatic digestion. [c] From Berggård and Bearn (1968). [d] Based on final sequence data (see Figure 9). [e] Presence of tryptophan was determined spectrophotometrically

tinguishing Asp>PhNCS, Asn>PhNCS, Glu>PhNCS, and Gln>PhNCS, a solvent system of benzene–acetic acid (9:1 v/v) was used for the thin-layer chromatography.

Digestion with trypsin (trypsin, treated with L-1-tosylamido-2-phenylethyl chloromethyl ketone; Worthington Biochemical Corp., Freehold, N. J.) was performed on samples (5–50 mg) of the reduced and alkylated protein in 1% NH_4HCO_3 using protein:trypsin ratios of 100:1 or 50:1. Digestion was carried out at 37° for 4–6 hr and was stopped by lyophilization or by adding acid to bring the solution to pH 4.0. Digestions with thermolysin (A grade, Calbiochem, Los Angeles, Calif.), α-chymotrypsin (Worthington, CDI-8LK), and subtilisin (Protease, bacterial type VII, Sigma Chemical Co., St. Louis, Mo.) were performed similarly. Digestion with pepsin (Worthington, PM 707) was performed in 5% formic acid at a protein:enzyme ratio of 100:1 and was carried out at 37° for 6 hr.

Digestion with carboxypeptidases A and B (Worthington) was performed as described by Gottlieb *et al.* (1968). For complete enzymatic digestion of peptide TN 8, 50 nmol of the peptide was dissolved in 100 μl of 0.05 M sodium barbital (pH 7.7) and 10 μl of a solution (0.5 mg/ml) of aminopeptidase M (Rohm and Haas, Philadelphia, Pa.) added. The reaction mixture was maintained at 37° for 24 hr and digestion terminated by the addition of 1.0 ml of 0.2 M sodium citrate (pH 2.2).

Separation of peptides by gel filtration was performed on columns of Sephadex G-25, fine, or Sephadex G-50, fine (Pharmacia). The absorbance of the effluent at 230 nm was monitored in a Gilford Model 240 spectrophotometer equipped with flow cells. The output from the spectrophotometer was logged by a PDP-12 computer and the data were recorded graphically on a *X–Y* plotter. Ion-exchange chromatography on DEAE-cellulose (DE-52, Whatman, W. and R. Balston, England) was carried out as described previously (Cunningham *et al.*, 1970). Ion-exchange chromatography on Dowex 50 (Bio-Rad AG 50W-X4) was performed on columns at 37°. Columns were equilibrated in 0.05 M pyridine adjusted to pH 3.1 with acetic acid and loaded in the same solvent. Elution was performed with 50 ml of the same buffer followed by a series of linear gradient systems: I, 50 ml of 0.05 M pyridine–acetate (pH 3.1) and 50 ml of 1.0 M pyridine–acetate (pH 3.1); II, 150 ml each of 0.2 M pyridine–acetate (pH 3.1) and 1.0 M pyridine–acetate (pH 5.5); III, 50 ml each of 1.0 M pyridine–acetate (pH 5.5) and 2.0 M pyridine–acetate (pH 5.5); IV, 50 ml each of 2.0 M pyridine–acetate (pH 5.5) and 50% (v/v) pyridine–H_2O. All solvents were 5% in 1-propanol. Peptides were detected by manual ninhydrin analyses (Moore and Stein, 1954) of 0.1-ml aliquots from alternate tubes.

Automatic parallel cascade chromatography (Edelman and Gall, 1971) was performed using an initial column (2.5 × 100 cm) of Sephadex G-50 equilibrated with 0.015 M NH_4OH which was 10% in 1-propanol and three DEAE-cellulose columns (1.3 × 23 cm) equilibrated with 0.01 M Tris (pH 8.0). DEAE-cellulose columns were automatically eluted with a linear gradient of 275 ml each of 0.01 M Tris (pH 8.0) and the same buffer made 0.3 M in KCl; after the gradient was complete, each column was washed with 75 ml of 0.01 M Tris (pH 8.0) which was 0.5 M in KCl. All column effluents were automatically monitored by measuring the absorbance at 280 nm in a Gilford Model 240 spectrophotometer equipped with flow cells.

Peptides were also fractionated by two-dimensional high voltage paper electrophoresis. Electrophoresis in the first dimension was performed at pH 4.7 (Schwartz and Edelman, 1963) or pH 6.5 (Offord, 1966) and 4500 V; electrophoresis in the second dimension was performed at pH 1.9 (Schwartz

TN 8[b]	TN 7,8	TN 9	TN 10	TN 11	TN 12	TN 13	TN 14	Total	β_2-Microglobulin[c]
1.1 (1)	2.0 (2)	1.3 (1)		0.9 (1)	1.0 (1)			8	8
0.9 (1)	0.9 (1)			0.8 (1)				4	4
								(7)[d]	9
		0.4	0.9 (1)			1.0 (1)		5	5
		(1)				(1)		2	2
		0.4	1.0 (1)					2	2
1.1 (1)	1.1 (1)	2.2 (1)	1.0 (1)	1.0 (1)		1.1 (1)	1.0 (1)	12	12
		2.1 (3)		0.8 (1)				5	5
2.6 (3)	3.0 (3)	1.5 (2)		1.0 (1)				10	10
1.1 (1)	2.1 (2)	2.4 (2)	1.0 (1)	1.0 (1)				11	11
		0.9 (1)		1.0 (1)				5	5
		0.4						3	3
		0.5	1.0 (1)					2	2
1.0 (1)	1.0 (1)			2.0 (2)	0.7 (1)			7	7
							1.0 (1)	1	1
	1.1 (1)				0.7 (1)			5	5
1.1 (1)	1.0 (1)	2.1 (2)		1.0 (1)				7	7
		2.5 (3)	0.8 (1)					6	6
1.0 (1)	1.0 (1)	2.1 (2)						5	5
10	13	18	6	10	3	3	2	100	100
	24		13	32	12	27	15		

and by staining with *p*-dimethylaminobenzaldehyde. [f] CMCys = carboxymethylcysteine. [g] Yields are based on micromoles of peptides isolated compared with micromoles of protein originally digested with trypsin.

and Edelman, 1963) and 3500–4500 V. Peptides were detected by lightly spraying the paper with 0.05% (w/v) ninhydrin in a solvent that was one part 2 N acetic acid and two parts 95% ethanol and were eluted from the paper with 50% (v/v) pyridine in H_2O.

The methods used for amino acid analysis (Edelman *et al.*, 1968), determination of the amino acid sequence of peptides by the dansyl-Edman technique (Cunningham *et al.*, 1968; Gottlieb *et al.*, 1970), and for locating disulfide-bonded peptides by the diagonal electrophoresis technique (Brown and Hartley, 1966; Gall and Edelman, 1970) have been described. The presence of tryptophan in peptides was determined by treating the appropriate peptides with carboxypeptidase A, by staining with *p*-dimethylaminobenzaldehyde (Easley, 1965), or by spectrophotometric methods. The determination of the electrophoretic mobilities of peptides was performed as described by Offord (1966).

Results

Direct sequence analysis of reduced and alkylated β_2-microglobulin both by the dansyl-Edman technique and by use of the automatic sequencer gave the amino acid sequence of the amino-terminal 19 residues: Ile-Gln-Arg-Thr-Pro-Lys-Ile-Gln-Val-Tyr-Ser-Arg-His-Pro-Ala-Glu-Asn-Gly-Lys-. Treatment of the reduced and alkylated protein with carboxypeptidase A, carboxypeptidase B, or both failed to release any amino acid in sufficient amounts above background to unequivocally assign the C-terminal residue. However, a small amount of methionine was released with carboxypeptidase A suggesting that the single methionine (Table I) is the C-terminal residue. Consistent with this result, no peptide was released on treatment of the protein with CNBr. The remainder of the sequence was established by isolating and characterizing peptides after digestion of the reduced and alkylated protein with trypsin, chymotrypsin, thermolysin, pepsin, and subtilisin. The basic strategy was to fractionate peptides from many enzymatic digests starting with 0.5–5 μmol of protein and to characterize only those peptides obtained after a few fractionation steps, rather than to attempt to isolate all peptides from digests of large amounts (10 μmol or greater) of material. For this reason, many of the column profiles presented here contain fractions which are not discussed. These fractions contained peptides not easily purified or contained peptides which were more readily obtained by other techniques.

Isolation and Partial Sequences of Tryptic Peptides. The amino acid composition of β_2-microglobulin (Table I) indicates that the protein contains eight lysyl and five arginyl residues. The expected 14 peptides (Table I) were isolated after tryptic digestion. Three additional peptides, TN 3a, TN 3b, and TN 7,8, were also isolated. Peptides TN 3a and TN 3b apparently resulted from chymotryptic cleavage of peptide TN 3 (Table II). On the other hand, peptide TN 7,8 appears to result from a failure of trypsin to cleave the lysyl–valyl bond that links TN 7 and TN 8.

Peptides TN 1, TN 2, TN 3, TN 3a, TN 3b, TN 4, TN 6, TN 11, TN 12, and TN 14 (Table I) were all isolated directly from tryptic digests of the protein by two-dimensional high voltage paper electrophoresis at pH 4.7 and pH 1.9. Peptides TN 7 and TN 8 (Table I) were also purified by paper electrophoresis but only after the digest had been fractionated by other techniques. The separation of the tryptic peptides soluble at pH 4 by gel filtration on Sephadex G-25 is shown in Figure 1. The mixture of peptides in fraction A was further fractionated by ion-exchange chromatography on a column

TABLE II: Amino Acid Sequences of Tryptic Peptides from β_2-Microglobulin.[a]

TN 1	Ile-Glx-Arg
TN 2	Thr-Pro-Lys
TN 3	Ile-Glx-Val-Tyr-Ser-Arg
TN 3a	Ile-Glx-Val-Tyr
TN 3b	Ser-Arg
TN 4	His-Pro-Ala-Glx-Asx-Gly-Lys
TN 5	Ser-Asx-Phe-Leu-Asx-CMCys-Tyr-Val-Ser-Gly-Phe-(His,Pro,Ser,Asx,Ile,Glx,Val,Asx,Leu,Leu)-Lys
TN 6	Asx-Gly-Glx-Arg
TN 7	Ile-Glx-Lys
TN 7,8	Ile-Glx-Lys-Val-Glx-(His)-(Ser)-Asx-Leu-(Ser,Phe,Ser)-Lys
TN 8	Val-Glx-His-Ser-Asx-Leu-Ser-Phe-Ser-Lys
TN 9	Asx-(Trp)-Ser-Phe-Tyr-Leu-Leu-Tyr-Ser-(Tyr,Thr,Glx,Phe,Thr,Pro,Thr,Glx)-Lys
TN 10	Asx-Glx-Tyr-Ala-CMCys-Arg
TN 11	Val-Asx-His-Val-Thr-Leu-Ser-Glx-(Pro)-Lys
TN 12	Ile-Val-Lys
TN 13	(Trp)-Asx-Arg
TN 14	Asx-Met

[a] Sequence determined by the dansyl-Edman technique. Individual residues in parentheses denote positions where positive identification of the dansyl derivative was not made. Multiple residues in parentheses indicate that the sequences in these regions were not determined but were assumed to be present on the basis of the amino acid composition.

of DEAE-cellulose. Peptide TN 8 was obtained from the major fraction eluted from this column by paper electrophoresis at pH 6.5 followed by electrophoresis at pH 1.9. Peptide TN 7 was obtained directly from material in fraction B (Figure 1) by two-dimensional paper electrophoresis at pH 6.5 and pH 1.9.

Peptides TN 7,8, TN 10, TN 13, and TN 14 (Table I) were obtained directly from digests of the protein by ion-exchange chromatography on Dowex 50 (Figure 2). Peptide TN 14 was obtained from material in fraction 1, peptide TN 10 from material in fraction 2, and TN 13 from material in fraction 4. Peptide TN 7,8 was one of the mixture of peptides in fraction 3, and was obtained by gel filtration of this mixture on Sephadex G-25.

The large peptides TN 5 and TN 9 (Table I) proved difficult to separate from each other and both were only sparingly soluble in acidic solvents. In addition, the bond linking peptides TN 9 and TN 10 appeared to have a limited susceptibility to cleavage by trypsin so that peptide TN 9 was always contaminated with a peptide equivalent to TN 9 plus TN 10. The isolation of peptides TN 5 and TN 9, therefore, required special techniques.

Peptide TN 5 was isolated by making use of a computer-controlled automatic fractionation system (Edelman and Gall, 1971). A tryptic digest of β_2-microglobulin was fractionated using a cascade system in which the digest was first subjected to gel filtration on a column of Sephadex G-50 (Figure 3).

The absorbance at 280 nm of the effluent was monitored continuously by the computer which was programmed to direct the effluent representing each of the first three fractions to separate DEAE-cellulose columns and to store the remaining fractions in a fraction collector. The absorbance at 280 nm of the effluents from each DEAE-cellulose column was also monitored by the computer, and the resulting fractions were collected in a fraction collector.

Fraction I (Figure 3A) represents that portion of the effluent

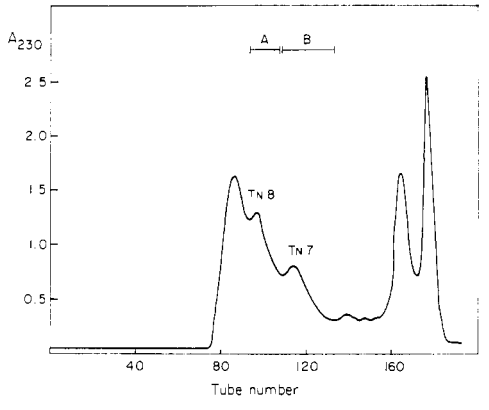

FIGURE 1: Gel filtration of peptides soluble at pH 4 after digestion of β_2-microglobulin (50 mg) with trypsin on a column (2.2 × 100 cm) of Sephadex G-25 in 0.015 M NH_4OH which was 10% in 1-propanol. The solid line denotes the absorbance of effluent fractions at 230 nm as monitored by the computer system. Each tube contained 2.0 ml of effluent. The bars designated A and B denote fractions that were combined for the isolation of peptides TN 7 and TN 8.

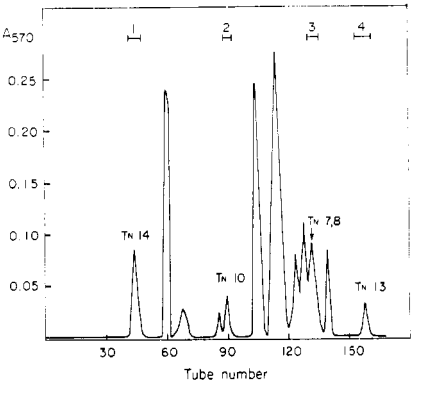

FIGURE 2: Ion-exchange chromatography of a tryptic digest of β_2-microglobulin (40 mg) on a column (0.9 × 10 cm) of Bio-Rad AG 50W-X4 in pyridine–acetate buffers that were 5% in 1-propanol. The solid line indicates the absorbance at 570 nm following manual ninhydrin analyses on 0.1-ml aliquots of alternate fractions. Each tube contained 2.0 ml of effluent. Tryptic peptides isolated from material in fractions 1–4 are indicated.

TABLE III: Amino Acid Composition of Chymotryptic Peptides.[a]

	C 1	C 2	C 3	C 4	C 4a	C 4b	C 5	C 6	C 7	C 8	TN 9 + C	TN 9 + C[c]	TN 11 + C_1
Lys	1.0 (1)	1.0 (1)	0.3	1.0 (1)	0.9 (1)				2.2 (2)				
His		0.8 (1)	0.9 (1)										1.0 (1)
Arg	0.8 (1)	0.9 (1)						1.0 (1)		0.9 (1)			
Trp[b]				(1)*	(1)*				(1)*		(1)	1.0 (1)	
CMCys								0.7 (1)					
Asp		2.0 (2)	1.9 (2)	1.0 (1)	1.1 (1)			1.2 (1)		2.1 (2)	0.9 (1)	0.9 (1)	1.1 (1)
Thr	1.1 (1)		0.7										1.0 (1)
Ser		1.8 (2)	1.5 (2)	1.7 (2)	1.0 (1)	0.7 (1)			0.8 (1)		0.8 (1)	0.9 (1)	
Glu	2.0 (2)	1.0 (1)	1.9 (1)						1.1 (1)				
Pro	1.2 (1)	0.9 (1)	1.1 (1)						1.1 (1)				
Gly		1.1 (1)	1.1 (1)										
Ala		1.2 (1)						0.9 (1)					
Val	1.2 (1)		1.9 (2)					1.2 (1)	1.0 (1)				1.9 (2)
Met										0.9 (1)			
Ile	1.9 (2)		1.0 (1)						0.8 (1)				
Leu			2.0 (2)				2.0 (2)						1.0 (1)
Tyr	0.8 (1)					1.0 (1)	0.9 (1)				1.3 (1)	1.0 (1)	
Phe		0.9 (1)	1.2 (1)	1.0 (1)		1.0 (1)					0.9 (1)	1.1 (1)	
Total residues	10	12	14	6	4	3	3	5	8	4	5	5	6
Yield (%)[d]	9	7	52	19	9	7	20	7	7	12	24		71

[a] Values reported are amino acid residues. Amino acids present at a level of 0.2 residue are omitted. The assumed integral numbers of residues are given in parentheses. [b] Tryptophan was determined by staining with *p*-dimethylaminobenzaldehyde and * by digestion of peptides with carboxypeptidase A followed by amino acid analysis. [c] Amino acid analysis of peptide TN 9 + C after enzymatic hydrolysis. [d] Yields are based on micromoles of peptides isolated compared with micromoles of β_2-microglobulin (or peptide) digested with chymotrypsin.

from the Sephadex G-50 column that was directed to the third DEAE-cellulose column and fraction TN 5 (Figure 3B) represents the portion of the effluent from the DEAE-cellulose column which was deposited as a single fraction in the fraction collector. Gel filtration of this material on Sephadex G-50 in 0.015 M NH_4OH which was 10% in 1-propanol yielded peptide TN 5 free of salts.

The relative insolubility of peptide TN 9 in acidic solvents served as the basis for its purification. Tryptic digests of the reduced and alkylated protein were lyophilized and then extracted repeatedly with 2% acetic acid. The remaining precipitate represented peptide TN 9 (Table I). Similar results were obtained if the digest was first fractionated by gel filtration in basic solvents (*e.g.*, Figure 3A) and the high molecular weight fractions lyophilized and extracted in a similar fashion. While this peptide was never obtained completely free of peptides TN 5 and TN 10, it was sufficiently homogeneous to give an unambiguous sequence by the dansyl-Edman technique.

The amino acid sequences of tryptic peptides TN 1, TN 2, TN 3, TN 3a, TN 3b, TN 4, TN 6, TN 7, TN 8, TN 10, TN 12, TN 13, and TN 14 were determined directly by the dansyl-Edman technique (Table II). The partial amino acid sequences of peptides TN 5, TN 7,8, TN 9, and TN 11 were also determined by this method. The complete sequences of these peptides as well as the order of all the tryptic peptides were established by isolating and characterizing peptides obtained by digestion of β_2-microglobulin with other enzymes.

Isolation and Characterization of Chymotryptic Peptides. Chymotryptic digests of β_2-microglobulin were first fractionated by gel filtration on Sephadex G-25 (Figure 4). Peptide C 3 (Table III) was obtained by gel filtration of material in fraction A on the same column. The remaining chymotryptic peptides were isolated by high voltage paper electrophoresis at pH 4.7 and pH 1.9 of the mixture of peptides in the various fractions. Peptides C 2 and C 8 were obtained from material in fraction B, peptides C 4, C 4a, and C 5 from the mixture of peptides in fraction C, peptide C 1 from ma-

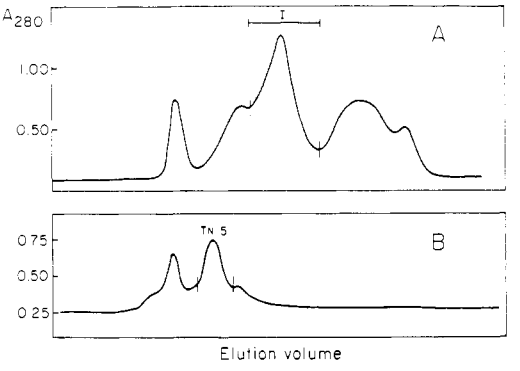

FIGURE 3: Automatic cascade fractionation of a tryptic digest of β_2-microglobulin (40 mg). The solid lines represent the absorbance at 280 nm of column effluents. A: absorbance of effluent from a column (2.5 × 100 cm) of Sephadex G-50 in 0.015 M NH_4OH which was 10% in 1-propanol. The vertical bars denote that segment of the effluent (I) directed automatically to one of three ion-exchange columns. B: absorbance of the effluent of the column (1.3 × 23 cm) on which material in fraction I was automatically chromatographed on DEAE-cellulose in 0.01 M Tris (pH 8.0) using a linear gradient from 0 to 0.3 M KCl. The vertical bars indicate that portion of the effluent automatically combined as a single fraction from which peptide TN 5 was obtained.

TABLE IV: Amino Acid Composition of Thermolysin Peptides.[a]

	TL 1	TL 2	TL 3	TL 4	TL 5	TL 6	TL 7	TL 8
Lys	0.9 (1)		0.9 (1)	1.1 (1)		1.0 (1)	1.1 (1)	
His	0.9 (1)							
Arg	0.9 (1)		0.9 (1)			0.9 (1)		1.0 (1)
Trp[b]				(1)				(1)
CMCys		0.8 (1)				1.0 (1)		
Asp	2.1 (1)	1.0 (1)	1.1 (1)	0.8 (1)		1.1 (1)		2.2 (2)
Thr					1.0 (1)	1.9 (2)		
Ser	1.7 (2)			2.0 (2)			1.0 (1)	
Glu	1.0 (1)		1.0 (1)		1.1 (1)	2.0 (2)	1.2 (1)	
Pro	1.0 (1)					0.9 (1)	0.9 (1)	
Gly	1.1 (1)		1.0 (1)					
Ala	1.0 (1)					1.1 (1)		
Met								0.9 (1)
Leu		0.8 (1)	1.1 (1)				0.7 (1)	
Tyr		1.0 (1)			0.8 (1)	1.0 (1)		
Phe	0.6 (1)			1.2 (1)		1.2 (1)		
Total residues	12	4	6	6	3	12	5	5
Yield (%)[c]	28	10	16	16	26	7	24	24

[a] Values reported are amino acid residues. Amino acids present at a level of 0.2 residue are omitted. The assumed integral numbers of residues are given in parentheses. [b] Presence of tryptophan in peptides was determined spectrophotometrically. [c] Yields are based on micromoles of peptides isolated compared with micromoles of protein digested with thermolysin.

terial in fraction D, and peptides C 4b, C 6, and C 7 from material in fraction E.

The amino acid sequences of peptides C 4, C 4a, C 4b, C 6, C 7, and C 8, and the partial sequences of peptides C 2, C 3, and C 5 were determined by the dansyl-Edman technique (Figure 5). The presence of a tyrosyl residue at the COOH-terminus of peptide C 5 and the presence of tryptophanyl residues at the COOH-termini of peptides C 4 and C 4a were determined by treating each of these peptides with carboxypeptidase A; followed by amino acid analysis. The sequence Lys-Trp at the COOH-terminus of peptide C 7 was established by digestion of this peptide with carboxypeptidase A plus carboxypeptidase B.

Chymotryptic digestions were also performed on the tryptic peptides TN 9 and TN 11 (Table I). Gel filtration of the digest of peptide TN 11 on Sephadex G-25 in 0.015 M NH_4OH which was 10% in 1-propanol gave peptide TN 11 + C_1 (Table III) directly. The amino acid sequence of this peptide was determined by the dansyl-Edman technique (Figure 5).

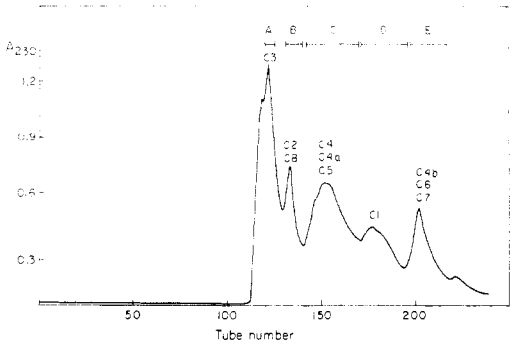

FIGURE 4: Gel filtration of a chymotryptic digest of β_2-microglobulin (21 mg) on a column (2.2 × 100 cm) of Sephadex G-25 in 0.015 M NH_4OH which was 10% in 1-propanol. The solid line denotes the absorbance of effluent fractions at 230 nm monitored and plotted by the computer system. Each tube contained 1.6 ml of effluent.

Treatment with carboxypeptidase A immediately released Leu, followed by a slower release of Thr and Val and a much slower release of His.

Peptide TN 9 + C (Table III) was isolated from a chymotryptic digest of peptide TN 9 (Table I) by gel filtration on Sephadex G-25, followed by high voltage paper electrophoresis of the tryptophan-containing fraction. Peptide TN 9 + C was acidic and contained tryptophan as determined by staining with *p*-dimethylaminobenzaldehyde (Easley, 1965). Complete enzymatic digestion of this peptide indicated that it had the amino acid composition (Table III) Asp, Trp, Phe, Ser, Tyr. The amino terminal residue (Asp) was determined by the dansyl technique. Treatment of peptide TN 9 + C with carboxypeptidase A rapidly released Tyr and Phe with a very slow release of Ser. No Asp or Trp was released after 40 min of digestion.

Isolation and Characterization of Thermolysin Peptides. The mixtures of peptides obtained after digestion of β_2-microglobulin with thermolysin were fractionated in the same manner as the chymotryptic peptides. Separation of the thermolysin peptides by gel filtration on Sephadex G-25 is shown in Figure 6. Peptides (Table IV) were purified from material in each of the designated fractions by two-dimensional high voltage electrophoresis (pH 4.7 and pH 1.9). Peptide TL 6 was isolated from material in fraction A. Peptides TL 1, TL 3, TL 5, and TL 8 and peptides TL 2, TL 4, and TL 7 were isolated from the mixtures of peptides in fractions B and C, respectively. The amino acid sequences of peptides TL 2, TL 3, TL 4, TL 5, TL 7, and TL 8 and the partial sequences of peptides TL 1 and TL 6 were determined by the dansyl-Edman technique (Figure 5).

Isolation and Characterization of Peptic and Subtilisin Peptides. Peptic peptides (Table V) were initially fractionated by ion-exchange chromatography on Dowex 50 (Figure 7). Peptide P 1 was obtained directly from material in fraction 1. The remaining peptides were isolated from the mixtures in the various fractions by high voltage paper electrophoresis

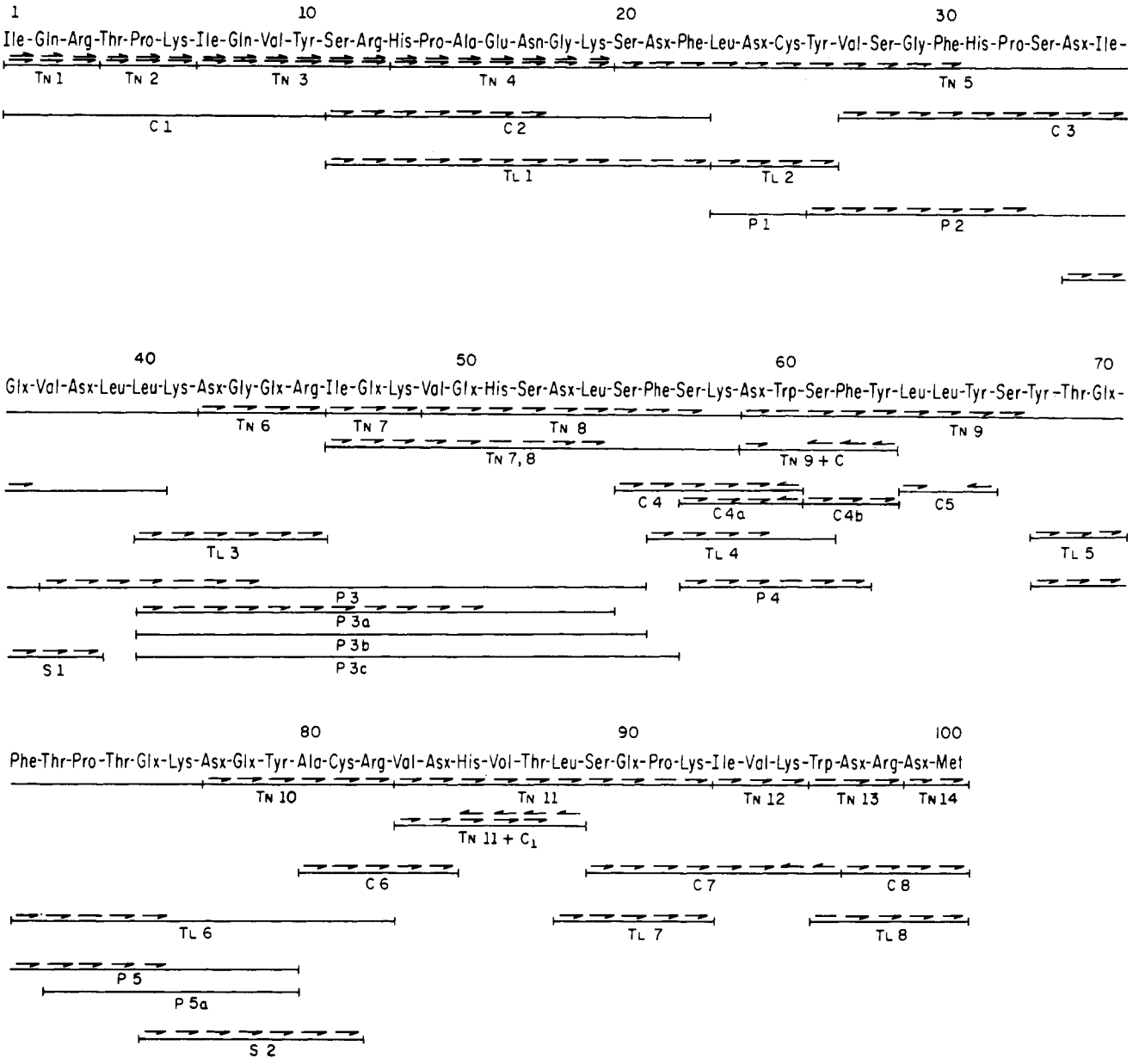

FIGURE 5: Amino acid sequence of peptides used to determine the complete sequence of β_2-microglobulin: TN designates tryptic peptides; C, chymotryptic peptides; TL, thermolysin peptides; P, peptic peptides; and S, subtilisin peptides; (→) the results of direct sequence of the intact polypeptide chain by the dansyl-Edman technique and by use of the automatic sequencer; (—) the results of sequence determination by the dansyl-Edman method on the various peptides; (—) the residue at this position was not unequivocally identified; (←) residues released by treatment of the peptide with carboxypeptidase A and B.

(pH 4.7 and pH 1.9). Material in fraction 2 yielded peptides P 2, P 5, and P 5a while that in fractions 3, 4, and 5 gave peptides P 3, P 2, and P 3b, respectively. The amino acid sequence of peptide P 4 and the partial sequences of peptides P 2, P 3, P 3a, and P 5 were determined (Figure 5).

The subtilisin peptides S 1 and S 2 (Table V) were obtained directly from digests of the protein by paper electrophoresis at pH 4.7 and pH 1.9 and the amino acid sequence of each was determined by the dansyl-Edman technique (Figure 5).

Order and Sequence of Tryptic Peptides. The amino acid sequences of all peptides isolated are summarized in Figure 5. The order of tryptic peptides TN 1 through TN 4 was established by direct sequence analyses of the intact polypeptide chain. The compositions and partial sequences of peptides C 1, C 2, and TL 1 (Tables III and IV) agree with this assignment. The location of peptide TN 5 in the polypeptide chain was established by direct sequence of this peptide and by the amino acid compositions and partial sequences of peptides C 2 and TL 1 (Tables III and IV). The amino acid sequence of peptide TN 5 was completed by determining the sequences of peptides C 3, P 2, P 3, and S 1 (Tables III and V). The compositions and partial amino acid sequences of peptides TL 3, P 3, P 3a, P 3b, and P 3c (Tables III and V) established the location of peptides TN 6, TN 7, and TN 8. The isolation and characterization of peptide TN 7,8 (Table I) agrees with this assignment. The determination of the amino acid sequence of peptide TN 8 was confirmed by characterizing peptides C 4, C 4a, TL 4, and P 4 (Tables III–V). The sequences of these peptides also place peptide TN 9. Sequence analysis of peptides C 4b, TN 9 + C, C 5, TL 5, TL 6, P 5, and S 2 (Tables

TABLE V: Amino Acid Composition of Peptic and Subtilisin Peptides.[a]

	P 1	P 2	P 3	P 3a	P 3b	P 3c	P 4	P 5	P 5a	S1	S 2
Lys			1.8 (2)	1.8 (2)	1.9 (2)	2.0 (2)	1.0 (1)	0.8 (1)	0.8 (1)		1.0 (1)
His		0.7 (1)	0.8 (1)	0.8 (1)	0.8 (1)	0.8 (1)					
Arg			1.0 (1)	1.0 (1)	1.4 (1)	1.2 (1)					
Trp[b]							(1)				
CMCys	1.0 (1)										0.8 (1)
Asp	1.1 (1)	1.1 (1)	3.1 (3)	1.8 (2)	2.2 (2)	2.0 (2)	0.9 (1)	1.3 (1)	1.1 (1)	1.9 (2)	1.2 (1)
Thr								2.8 (3)	1.9 (2)		
Ser		1.8 (2)	1.6 (2)	0.8 (1)	1.9 (2)	1.7 (2)	1.9 (2)				
Glu		1.1 (1)	3.2 (3)	2.6 (3)	2.8 (3)	2.9 (3)		3.0 (3)	2.1 (2)	1.1 (1)	2.1 (2)
Pro		1.0 (1)						1.0 (1)	1.0 (1)		
Gly		1.1 (1)	1.4 (1)	0.9 (1)	1.2 (1)	1.1 (1)					
Ala											1.0 (1)
Val		1.1 (1)	2.1 (2)	0.9 (1)	1.1 (1)	1.2 (1)				1.0 (1)	
Ile		1.0 (1)	1.0 (1)	0.9 (1)	0.9 (1)	1.0 (1)				1.0 (1)	
Leu	0.9 (1)		3.0 (3)	1.7 (2)	1.7 (2)	2.0 (2)					
Tyr		1.0 (1)						1.9 (2)	1.1 (1)		0.9 (1)
Phe		1.1 (1)				1.1 (1)	1.2 (1)	1.2 (1)			
Total residues	3	11	19	15	16	17	6	12	8	5	7
Yield (%)[c]	46	13	4	6	3	4	12	10	10	17	16

[a] Values reported are amino acid residues. Amino acids present at a level of 0.2 residue are omitted. The assumed integral numbers of residues are given in parentheses. [b] Presence of tryptophan in peptides was determined by staining with *p*-dimethylaminobenzaldehyde. [c] Yields are based on micromoles of peptides isolated compared with micromoles of β_2-microglobulin originally digested with pepsin (P) or subtilisin (S).

III, IV and V) confirmed the partial sequence of peptide TN 9 as determined by the dansyl-Edman technique and completed the determination of the amino acid sequence of this peptide. These results also located the position of peptide TN 10. Peptide TN 11 was placed on the basis of the sequence of peptide C 6 (Table III) and the sequences of peptides TN 11 + C_1 and TL 7 (Tables III and IV) confirmed the sequence of the amino terminal portion of peptide TN 11 and completed the determination of the amino acid sequence of this tryptic peptide. The order of peptides TN 11, TN 12, TN 13, and TN 14 was established by analysis of peptides C 7, C 8, and TL 8 (Tables III and IV).

Assignment of Asparaginyl and Glutaminyl Residues. Because the major portion of the amino acid sequence was determined by the dansyl-Edman technique which requires acid hydrolysis, it was necessary to establish the positions of asparaginyl and glutaminyl residues by other methods. The assignments of Gln to positions 2 and 8, Glu to position 16, and Asn to position 17 were made on the basis of identification of the PhNCS derivatives obtained from direct sequence analysis of the intact polypeptide chain using the automatic sequencer. The assignments of the positions of most of the remaining asparaginyl and glutaminyl residues were made on the basis of the electrophoretic mobilities of the appropriate peptides; the data are summarized in Table VI.

The peptide Ser-Asx-Phe (Table VI) used to establish Asn 21 was obtained directly from a tryptic digest of peptide C 2 (Table III, Figure 5) by electrophoresis at pH 6.5. The assign-

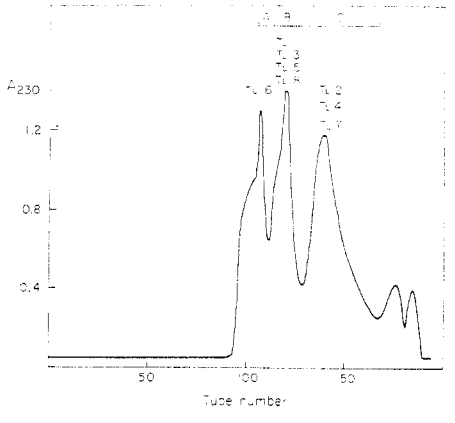

FIGURE 6: Gel filtration of peptides obtained after digestion of β_2-microglobulin (13 mg) with thermolysin on a column (2.2 × 100 cm) of Sephadex G-25 in 0.015 M NH_4OH which was 10% in 1-propanol. The solid line denotes the absorbance at 230 nm of the effluent in each fraction as recorded by the computer system; each tube contained 2.0 ml of effluent.

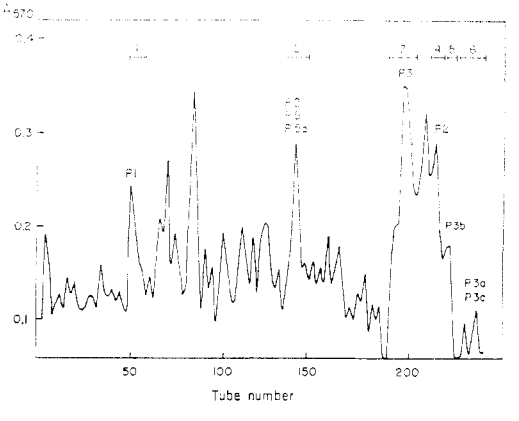

FIGURE 7: Ion-exchange chromatography of a peptic digest of β_2-microglobulin (31 mg) on a column (0.9 × 15 cm) of Bio-Rad AG 50W-X4 in pyridine–acetate buffers that were 5% in 1-propanol. The solid line indicates the absorbance at 570 nm after manual ninhydrin analyses of 0.1-ml aliquots from alternate tubes; each tube contained 2.0 ml of effluent.

TABLE VI: Electrophoretic Mobilities of Peptides Used to Determine the Positions of Asn and Gln Residues.[a]

Residue	Peptide	Ref Table	Mol Wt	Mobility	Net Charge
Gln_2	Gln>PhNCS;[b] TN 1 (Ile-Glx-Arg)	I	416.5	0.53	+1
Gln_8	Gln>PhNCS;[b] TN 3a (Ile-Glx-Val-Tyr)	I	522.6	0.08	0
Glu_{16}	Glu>PhNCS[b]				
Asn_{17}	Asn>PhNCS[b]				
Asn_{21}	C 2 + TN (Ser-Asx-Phe)	III	367.4	0	0
Asn_{24}	P 1 (Leu-Asx-CAMCys)[c]	V	406.3	0	0
Asp_{34}, Glu_{36}, Asp_{38}	S 1 (Asx-Ile-Glx-Val-Asx)	V	589.6	0.92	−3
Asp_{42}, Glu_{44}	TN 6 (Asx-Gly-Glx-Arg)	I	475.4	0.44	−1
Glu_{47}	TN 7 (Ile-Glx-Lys)	I	388.5	0	0
Glu_{50}, Asp_{53}	TN 8 (Val-Glx-His-Ser-Asx-Leu-Ser-Phe-Lys)[d]	I			
Asp_{59}	C 4 (Ser-Phe-Ser-Lys-Asx-Trp)	III	768.9	0.07	0
	TN 9 + C (Asx-Trp-Ser-Phe-Tyr)[d]	III			
Glu_{70}	TL 5 (Tyr-Thr-Glx)	IV	441.4	0.44	−1
Glu_{70}, Glu_{75}	TN 9 + P (Tyr-Thr-Glx-Phe-Thr-Pro-Thr-Glx-Lys)	I	1115	0.23	−1
Asp_{77}, Glu_{78}	TN 10 (Asx-Glx-Tyr-Ala-CAMCys-Arg)	I	812.7	0.30	−1
Glu_{75}, Asp_{77}, Glu_{78}	P 5a (Thr-Pro-Thr-Glx-Lys-Asx-Glx-Tyr)	V	982.0	0.46	−2
Asn_{84}	C 6 (Ala-CAMCys-Arg-Val-Asx)	III	619.5	0.42	+1
Gln_{90}	TL 7 (Leu-Ser-Glx-Pro-Lys)	IV	572.7	0.44	+1
	TN 11 + C_2 (Ser-Glx-Pro-Lys)	I	459.5	0.46	+1
Asp_{97}	TN 13 (Trp-Asx-Arg)	I	475.4	0.03	0
Asp_{99}	TN 14 (Asx-Met)	I	264.3	0.66	−1

[a] Mobilities relative to aspartic acid after electrophoresis at pH 6.5; net charge calculated by the method of Offord (1966). [b] PhNCS derivatives identified from direct sequence of the intact protein with automatic sequencer. [c] CAMCys = carboxamidomethylcysteine. [d] TN 8 and TN 9 + C were digested with aminopeptidase M followed by amino acid analysis (see Tables I and III).

ments of Asp 34 and Glu 36 were confirmed by treatment of peptide S 1 (Table V) with aminopeptidase M which released equal molar amounts of Asp, Ile, and Glu. The valyl residue and the second aspartyl residue were not released by this enzyme and no amino acids were released after treatment of peptide S 1 with carboxypeptidase A at pH 8.5. Glu 50 and Asp 53 were assigned on the basis of amino acid analysis of a complete enzymatic digest of peptide TN 8 (Table I) with aminopeptidase M. The assignment of Asp 59 was made on the basis of the electrophoretic mobility of peptide C 4 (Table III) and was confirmed by enzymatic digestion of peptide TN 9 + C (Table III). The assignment of Glu 70 was made on the basis of the electrophoretic mobility of peptide TL 5 and Glu 75 was assigned on the basis of the electrophoretic mobility of a peptide obtained after digestion of peptide TN 9 (Table I) with pepsin. The peptic peptide was isolated by gel filtration on Sephadex G-25 followed by high voltage paper electrophoresis. Asp 77 and Glu 78 were assigned on the basis of the electrophoretic mobility of peptide TN 10 (Table I) and the assignments of Glu 75, Asp 77, and Glu 78 were confirmed by the electrophoretic mobility of peptide P 5a (Table V). The peptide TN 11 + C_2 (Ser-Glx-Pro-Lys) used to assign Gln 90 was obtained directly from a chymotryptic digest of peptide TN 11 (Table I) by paper electrophoresis at pH 6.5.

Location of Peptides Linked by Disulfide Bonds. To show that half-cystinyl residues 25 and 81 are linked by a disulfide bond, 4.0 mg of the native protein was digested for 12 hr at 37° with pepsin (40 μg) in 5% formic acid and lyophilized. The lyophilized material was redissolved in 1% NH_4HCO_3 and digested for an additional 4 hr with trypsin (80 μg). The material was then lyophilized and the half-cystine-containing peptides were located by the diagonal electrophoretic technique (Brown and Hartley, 1966) at pH 6.5 (Figure 8). Two peptides, I and II (Table VII), were isolated from the same vertical column off the diagonal and therefore are presumed to be linked in the native protein by a disulfide bond. The composition of peptide I suggests that it is Leu-Asn-Cys-Tyr-Val-Ser-Gly (Figure 5) and that it contains half-cystinyl residue 25. The amino acid composition of peptide II indicates that it is Tyr-Ala-Cys-Arg; it therefore contains half-cystinyl residue 81.

Discussion

The complete amino acid sequence of β_2-microglobulin is presented in Figure 9. The sequence is in excellent agreement with the amino acid composition (Table I) and differs from the tentative sequence we reported previously in the transpositions of Glu 50 and Asp 53 and of Trp 60 and Ser 61, and in the assignment of residue 59 as Asp instead of Asn. This study also completes the assignment of the positions of all aspara-

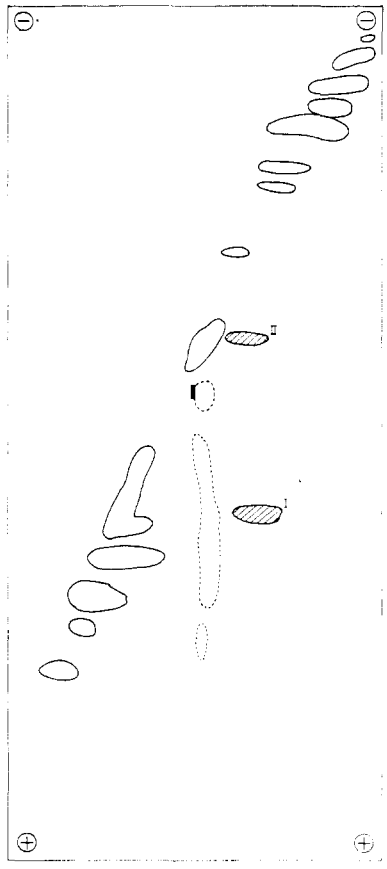

FIGURE 8: Trace of pattern obtained by diagonal electrophoresis (pH 6.5) of peptides obtained after digestion of β_2-microglobulin with pepsin and trypsin. The two peptides (I and II) off the diagonal were eluted from the paper with 50% pyridine. The dashed line denotes a streak of peptides that was off the diagonal, but stained weakly with ninhydrin; no attempt was made to isolate peptides from this region; ▌ indicates the position where the digest was originally spotted on the paper.

TABLE VII: Amino Acid Composition of Peptides from Diagonal Electrophoresis.[a]

	I	II
Arg		1.0 (1)
CysA[b]	0.8 (1)	0.9 (1)
Asp	1.1 (1)	0.3
Ser	1.0 (1)	
Gly	1.3 (1)	0.4
Ala		1.1 (1)
Val	0.8 (1)	
Leu	0.8 (1)	
Tyr	0.5 (1)	0.6 (1)
Total residues	7	4
Yield (%)[c]	3	10

[a] Peptides obtained by diagonal electrophoresis at pH 6.5 of β_2-microglobulin after digestion with pepsin and trypsin (Figure 8). Values reported are amino acid residues. Amino acids present at a level of 0.2 residue are omitted. The assumed integral numbers of residues are given in parentheses. [b] CysA = cysteic acid. [c] Yields are based on nanomoles of peptides isolated compared with nanomoles of β_2-microglobulin digested.

ginyl and glutaminyl residues. Our studies confirm the sequence reported for 44 of the first 46 residues (Smithies and Poulik, 1972a) except that we assign residue 21 as Asn instead of Asp.

The strategy employed in this sequence determination emphasized the isolation of the easily obtainable peptides from many enzymatic digests of the protein. The program chosen for the use of the computer-controlled fractionation system (Edelman and Gall, 1971) was aimed at obtaining the large tryptic peptides TN 5 and TN 9 because the small peptides had already been obtained by other techniques (Figure 3). The low molecular weight fraction (Figure 3A), however, served as a valuable reserve of the smaller peptides. The computer system proved equally valuable in logging the absorbance of the effluents of single columns such as those shown in Figures 1, 4, and 6 which were under gravity flow on remote units. Extensive use was made of paper electrophoresis which accounts for the relatively low overall yields of purified peptides (Tables I–VI). The major advantages of this approach were increased speed in obtaining data and the need for a relatively small amount of starting material. The studies reported here required about 250 mg of protein; much of this was used in resolving problems associated with peptides TN 7, TN 8, and TN 9 (Tables I and II). The major difficulties associated with these peptides were due to the resistance of the bonds between Lys 48 and Val 49 and between Lys 76 and Asp 77 to proteolytic cleavage by trypsin. We assume that this resistance to tryptic digestion is the result of the presence of negatively charged residues adjacent to these bonds (Figure 9). The problems associated with peptides TN 7 and TN 8 were readily resolved by the isolation of these peptides from a digest of larger amounts of protein (4 μmol, Figure 1). Difficulties with peptide TN 9 were never fully resolved; however, any potential ambiguities in the sequence should be confined to the area of residues 64–67 (Figure 9) inasmuch as the remaining sequence is adequately represented by peptides obtained from digests of the protein with other enzymes (Figure 5).

The demonstration (Figure 8, Table VII) that half-cystinyl residues 25 and 81 are linked by a disulfide bond confirms the previous conclusion that β_2-microglobulin contains no free half-cystinyl residues, but contains one intrachain disulfide bond forming a loop of 57 residues in the polypeptide chain. Although peptide I (Table VII) was obtained in only 3% overall yield and peptide II in 10% yield, the compositions of both peptides (I and II) located off the diagonal (Figure 8) are consistent with the specificities of the enzymes (pepsin and trypsin) used to digest the protein. The failure to obtain cleavage by pepsin at the tyrosyl residue in peptide I is probably the result of steric hindrance imposed by the disulfide bond inasmuch as pepsin did cleave this bond in the reduced and alkylated protein (Figure 5). We assume that the lower yield of peptide I as opposed to peptide II is due to the larger size of this peptide and that the generally low yield of both peptides is the result of the fact that pepsin cleaves in many portions of the peptide chain, particularly in the region around Cys 25. The ability of pepsin to cleave a variety of bonds in the region of Cys 25 is also probably responsible for the streak of weakly ninhydrin positive material off the diagonal in the center of the map (Figure 8). We found it necessary to use pepsin as well as trypsin, for two reasons: (1) because Cys 25 is contained in tryptic peptide TN 5, which is too large to move a significant distance on paper electro-

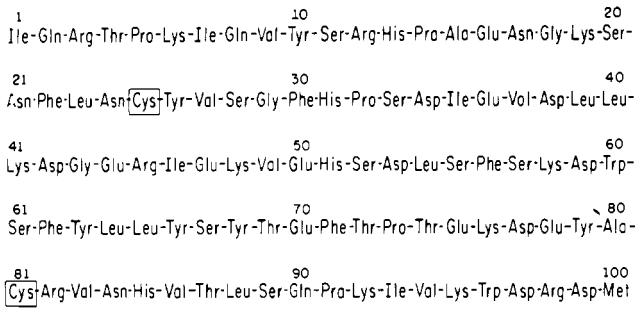

FIGURE 9: Complete amino acid sequence of β_2-microglobulin. The half-cystinyl residues (enclosed in boxes) form an intrachain disulfide bond.

phoresis, and (2) because the unreduced protein proved particularly resistant to digestion with trypsin even after denaturation.

In our previous report (Peterson *et al.*, 1972) of the tentative amino acid sequence of β_2-microglobulin, we found that the sequence of this protein is homologous with the amino acid sequence of the homology regions, C_L, C_H1, C_H2, and C_H3 of the myeloma protein Eu. The few changes introduced by the completion of the sequence do not alter this conclusion. In addition to the sequence homologies, β_2-microglobulin and each homology region of the γG1 immunoglobulin contain a single disulfide bond that forms a loop of about 60 residues in the polypeptide chains. These findings have raised a number of questions about the possible relationship between β_2-microglobulin and immunoglobulins.

β_2-Microglobulin has been found on the surface of human lymphocytes (Peterson *et al.*, 1972; Hütteroth *et al.*, 1973). It is synthesized by lymphoid cells (Bernier and Fanger, 1972; Hütteroth *et al.*, 1973; Evrin and Nilsson, 1973) and its production can be increased by stimulation with mitogenic lectins (Bernier and Fanger, 1972). On the basis of the extensive homologies of β_2-microglobulin with the homology regions of γG1 immunoglobulin, we proposed that the gene specifying β_2-microglobulin evolved directly from an immunoglobulin precursor gene prior to the duplication event (Peterson *et al.*, 1972). In addition, we suggested that β_2-microglobulin might serve a function similar to that of the C_H3 domain of γG1 immunoglobulin. In contrast, Smithies and Poulik (1972) suggested that the gene coding for β_2-microglobulin arose as the result of a large deletion in an IgG-like gene.

The synthesis of β_2-microglobulin, however, does not parallel the synthesis of immunoglobulins (Hütteroth *et al.*, 1973; Evrin and Nilsson, 1973). More importantly, it has been recently demonstrated (Hütteroth *et al.*, 1973; Evrin and Nilsson, 1973) that the production of β_2-microglobulin is not restricted to hematopoietic cells. This finding provides additional support to our precursor gene hypothesis, and suggests the possibility that β_2-microglobulin serves a function at the surface of eukaryotic cells. It should be stressed, however, that the function of this low molecular weight protein remains unknown.

Partial sequence data suggest that a similar protein exists in dogs (Smithies and Poulik, 1972b) and probably in other species, although no antigenic cross-reactivity has been demonstrated between β_2-microglobulin and proteins from other species. In spite of the fact that cells of mesenchymal and epithelial origin also produce β_2-microglobulin (Hütteroth *et al.*, 1973; Evrin and Nilsson, 1973), this protein may still be a descendant of a precursor for the immunoglobulins. The complete sequence of β_2-microglobulin reported here provides a reference structure for evolutionary comparison and should be a valuable aid in defining similar proteins in species such as the mouse where detailed functional and genetic studies can be carried out.

Acknowledgment

We are grateful to Professor Gerald M. Edelman for his advice and criticism throughout these studies. We also thank Dr. W. E. Gall for help in the use of the automatic fractionation unit and acknowledge the excellent technical assistance of Mrs. Martha O'Connor, Miss Joan Beck, and Mrs. Helvi Hjelt.

Added in Proof

After submission of this manuscript, it had been reported that β_2-microglobulin constitutes one of the two polypeptide chains of highly purified papain-solubilized human transplantation antigens (HL-A antigens) (Peterson *et al.*, 1973).

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Equilibrium and Kinetics of the Denaturation of a Homogeneous Human Immunoglobulin Light Chain†

Elizabeth S. Rowe‡ and Charles Tanford*,§

ABSTRACT: A study of the denaturation equilibrium and the kinetics of the approach to equilibrium as a function of guanidine hydrochloride (Gdn·HCl) concentration of a homogeneous human κ light (L) chain (Wes) is described. The results of this study are analyzed quantitatively in terms of the present understanding of Gdn·HCl perturbations of denaturation processes. In terms of these principles it is shown that the denaturation of monomeric Wes L chain is quantitatively consistent with a domain model in which the two halves of Wes L chain denature independently, giving rise to stable half-denatured intermediates in the transition. This result confirms the domain hypothesis and demonstrates that the domains are essentially independent in the intact L chain in solution. Analysis of the kinetic and equilibrium data in terms of this model gives the result that the two domains of Wes L chain have similar relatively low intrinsic stabilities of 5.5 kcal/mol in aqueous solution at pH 7.0 and 25°.

Amino acid sequence studies of homogeneous light (L) chains of immunoglobulins[1] have shown that L chains are composed of two homologous regions of sequence, one which is essentially constant within a given class and type and one which contains a large proportion of variable residues (for a review, see Edelman and Gall, 1969). This finding and the similar finding that IgG heavy (H) chains are composed of four such homology regions have led to the hypothesis that each of these homologous regions of sequence is independently folded into discrete globular entities called "domains" which contribute to some particular function of the IgG molecule (Edelman *et al.*, 1969). Circumstantial evidence in favor of this hypothesis for isolated L chains is provided by recent demonstrations (Solomans and McLaughlin, 1969; Karlsson *et al.*, 1969) that isolated L chains can be cleaved by a variety of proteolytic enzymes into compact mol wt 11,000 fragments corresponding to these homology regions which retain their original gross conformation (Björk *et al.*, 1971; Karlsson *et al.*, 1972). Preliminary 6-Å resolution X-ray crystallographic data on an L-chain dimer are consistent with this hypothesis (Edmundson *et al.*, 1972). However, the relationship between these homology regions in the intact L chain in solution has not been established.

Denaturation studies are capable of yielding information about the native state in terms of its cooperativity, intrinsic stability, and the nature of the forces responsible for maintaining its tertiary structure (see Tanford, 1968, 1970). Denaturation studies of model proteins such as lysozyme (Aune and Tanford, 1969a,b) and ribonuclease (Salahuddin and Tanford, 1970) in the presence of Gdn·HCl[2] have provided some general principles of Gdn·HCl induced denaturations which are useful in the analysis of such studies of more complicated proteins of biological importance. Many model proteins undergo a two-state transition from the native state N to the cross-linked random coil state D; that is, during the transition $N \rightleftharpoons D$ no states other than N and D are significantly present. This finding has been interpreted to mean that the native state is highly cooperative, such that disruption of any portion of its noncovalent interactions destabilizes the entire structure. In the case of the hypothetical domain structure of L chains, it would be expected that its denaturation would not be two state; if the two homologous halves of L chain are independently folded into discrete globular entities which do not interact, then stable partially denatured intermediates would be expected to appear during unfolding.

In the present article is reported the results of a study of the Gdn·HCl denaturation equilibrium and the kinetics of the approach to equilibrium of a κ-type L chain derived from the hyman myeloma protein Wes. A manuscript is currently in preparation which describes a similar study of the whole Wes Fab fragment.

† From the Department of Biochemistry, Duke University Medical Center, Durham, North Carolina 27710. *Received June 25, 1973.* Supported in part by Research Grant AM-04576 from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service.

‡ National Institutes of Health Predoctoral Fellow (1968–1971); supported by Grant 5-F01-GM-39,557-03 from the U. S. Public Health Service. This material was submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biochemistry, Duke University. Present address: Department of Chemistry, Georgetown University, Washington, D. C. 20007.

§ Research career awardee, National Institutes of Health, U. S. Public Health Service.

[1] The nomenclature and the abbreviations for the immunoglobulins and their subunits produced by reduction and proteolysis are those recommended by the World Health Organization (1964).

[2] Abbreviation used is: Gdn·HCl, guanidine hydrochloride.