Complete Amino Acid Sequence of Rabbit β_2-Microglobulin†

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ABSTRACT: The complete amino acid sequence of rabbit β_2-microglobulin has been determined as follows: Val-Gln-Arg-Ala-Pro-Asn-Val-Gln-Val-Tyr-Ser-Arg-His-Pro-Ala-Glu-Asn-Gly-Lys-Pro-Asn-Phe-Leu-Asn-Cys-Tyr-Val-Ser-Gly-Phe-His-Pro-Pro-Gln-Ile-Asp-Ile-Glu-Leu-Leu-Lys-Asn-Gly-Val-Lys-Ile-Glu-Asn-Val-Glu-Gln-Ser-Asp-Leu-Ser-Phe-Asn-Lys-Asp-Trp-Ser-Phe-Tyr-Leu-Leu-Val-His-Thr-Glu-Phe-Thr-Pro-Asn-Asn-Lys-Asn-Glu-Tyr-Ser-Cys-Arg-Val-Lys-His-Val-Thr-Leu-Lys-Glu-Pro-Met-Thr-Val-Lys-Trp-Asp-Arg-Asp-Tyr. Comparison of this sequence to that reported from human β_2-microglobulin [Cunningham, B. A., Wang, J. L., Berggård, I., & Peterson, P. A. (1973) *Biochemistry 12*, 4811] shows a homology of 71%, with a minimum 13% difference in the nucleotide sequences of the genes encoding the two proteins. A single insertion must be introduced before position 68 of the rabbit protein in order to maintain maximum homology to human β_2-microglobulin. Amino acid substitutions which can be attributed to single base changes are evenly distributed throughout the molecule, whereas the majority of those requiring multiple base changes are restricted to the carboxy-terminal third of the molecule. Although the rabbit β_2-microglobulin analyzed in this study was isolated from the pooled urine of 15 rabbits, no heterogeneity in amino acid sequence was observed.

β_2-Microglobulin (β_2-m)[1] was first described by Berggård & Bearn (1968) as a low molecular weight protein occurring in human biological fluids and has since been found on cell surfaces in association with histocompatibility antigens (Grey et al., 1973; Peterson et al., 1974), tumor antigens (Östberg et al., 1975; Vitetta et al., 1975), and other molecules such as TL (Geib et al., 1976; Vitetta et al., 1976) and Qa-2 (Michaelson et al., 1977).

The amino acid composition and partial amino acid sequence of the β_2-m homologues from rabbits (Berggård, 1974; Cunningham & Berggård, 1974; Poulik & Reisfeld, 1975; Gordon & Kindt, 1976b), dogs (Smithies & Poulik, 1972), mice (Appella et al., 1976), guinea pigs (Berggård, 1976; Cebra et al., 1977), and cows (Groves & Greenberg, 1977) have been reported, as has the entire sequence of human β_2-m (Cunningham et al., 1973). The structural similarity of these β_2-m homologues observed from partial sequence data is also reflected in immunologic cross-reactivity (Gordon & Kindt, 1976a).

The present report describes the determination of the complete amino acid sequence of β_2-m isolated from the urine of rabbits with renal dysfunction induced by sodium chromate. This 99-residue sequence of rabbit β_2-m is compared to the sequences of both human β_2-m and rabbit immunoglobulin constant regions.

Materials and Methods

Enzymes. The "V8-protease" enzyme from *Staphylococcus aureus* strain V8 (Houmard & Drapeau, 1972) was purchased from Miles Laboratories, Inc., Elkhart, IN. Carboxypeptidase Y (CpY) from yeast was purchased from Worthington, Freehold, NJ. Carboxypeptidase C (CpC) was obtained from Boehringer Mannheim Biochemicals, Indianapolis, IN.

Chemicals. Pyridine was refluxed over ninhydrin (2 g/L) and phthalic anhydride (4 g/L) for 1 h and then distilled. Iodoacetic acid was recrystallized from diethyl ether and stored in the dark at −20 °C. [2-^{14}C]Iodoacetic acid (57 mCi/mmol) was purchased from Amersham/Searle, Arlington Heights, IL, and stored at −70 °C as a 50 μCi/mL solution in ethanol. [1,4-^{14}C]Succinic anhydride (7.3 mCi/mmol) was purchased from New England Nuclear Corp., Boston, MA, and stored at −70 °C as a 250 μCi/mL solution in $CHCl_3$. 2-(2-Nitrophenylsulfenyl)-3-methyl-3-bromoindolenine (BNPS skatole) was purchased from Pierce Chemical Co., Rockford, IL, and stored in the dark at −20 °C. Poly(1,5-dimethyl-1,5-diazaundecamethylene methobromide) (hexadimethrine bromide) (Polybrene) was from the Aldrich Chemical Co., Milwaukee, WI.

Isolation of β_2-m. Modifications of the methods of Berggård (1974) and Gordon & Kindt (1976a,b) were used to isolate β_2-m. Renal tubular dysfunction was induced in 15 rabbits by a single subcutaneous injection of sodium chromate (10 mg of Na_2CrO_4 per kg) in saline. Urine was collected daily in the presence of NaN_3 and monitored for β_2-m by radioimmunoassay. Peak output of β_2-m occurred from days 2–5. For a single isolation, 6 L of urine containing significant amounts of β_2-m (>1 μg/mL) were pooled, centrifuged to remove particulate matter, and dialyzed and concentrated in a Model DC2 hollow fiber dialyzer/concentrator equipped with a HIP5 hollow fiber cartridge (Amicon Corp., Lexington, MA). The urine which had been concentrated approximately 10-fold was chromatographed in 100-mL amounts on a Sephadex G-100 column (5 × 145 cm) in 0.1 M Tris-HCl and 1.0 M NaCl, pH 8.0. The initial column effluents were monitored for β_2-m by radioimmunoassay, and the activity peak was pooled. In subsequent column runs, a trace amount of purified ^{125}I-labeled rabbit β_2-m was added to the urine pool prior to chromatography, and the β_2-m elution was determined by monitoring elution of radioactivity. The β_2-m pool was dialyzed into 0.01 M Tris-HCl, pH 8.6, and fractionated on a DEAE-cellulose (Whatman, Balston, England) column (2.5 × 7 cm) by using a 500-mL linear salt gradient from 0.01 M Tris-HCl, pH 8.6, to the same buffer containing 0.2 M NaCl, followed by 3 M NaCl and 0.01 M Tris-HCl, pH 8.6. As determined by radioimmunoassay, a single peak of β_2-m eluted at approximately 0.07 M NaCl. After dialysis against 0.01 M sodium acetate, pH 5.6, the β_2-m was chromatographed on a CM-

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[1] Abbreviations used: β_2-m, β_2-microglobulin; BNPS skatole, 2-(2-nitrophenylsulfenyl)-3-methyl-3-bromoindolenine; Pth, phenylthiohydantoin; W, peptides obtained by tryptophan cleavage; V, peptides obtained by V8-protease digestion; CpC and CpY, carboxypeptidases C and Y, respectively; Gdn·HCl, guanidine hydrochloride; Polybrene, poly(1,5-dimethyl-1,5-diazaundecamethylene methobromide) (hexadimethrine bromide).

cellulose (Whatman) column (2.5 × 10 cm) by using a 200-mL linear salt and pH gradient from 0.01 M sodium acetate, pH 5.6, to 0.11 M sodium acetate, pH 6.5. The β_2-m eluted as a single peak at approximately 0.05 M sodium acetate (pH 6.0) as determined by radioimmunoassay. The purified β_2-m was dialyzed against distilled H_2O or 10% acetic acid and lyophilized.

BNPS Skatole Cleavage of Reduced, [^{14}C]Carboxymethylated, and Succinylated β_2-m. Isolated β_2-m (7 mg) suspended in 1.1 mL of 4 M guanidine hydrochloride (Gdn·HCl), 0.02 M disodium EDTA, and 0.1 M sodium borate, pH 8.7, was reduced completely by addition of 0.4 mL of 0.1 M dithiothreitol. After incubation in the dark at ambient temperature for 1 h under N_2, [2-^{14}C]iodoacetic acid (0.25 μCi) was added to the reaction mixture. Two hours later 17.4 mg of iodoacetic acid was added, and the reaction was stirred for another 30 min (Gurd, 1972). The reaction mixture was passed through a column (28 × 1.5 cm) of Bio-Gel P-2 equilibrated with 2 M Gdn·HCl and 50 mM sodium borate, pH 8.7, and the [^{14}C]carboxymethylated β_2-m appearing in the void volume was pooled, reacted with solid succinic anhydride (58 mg) (Klapper & Klotz, 1972), and then desalted on a column (27 × 1.5 cm) of Bio-Gel P-2 in 1 N NH_4OH and lyophilized.

The lyophilized, [^{14}C]carboxymethylated, succinylated rabbit β_2-m was dissolved in 1 mL of glacial acetic acid and then 0.6 mL of 50% acetic acid was added to a final concentration of 80% acetic acid. Tyrosine (15.0 mg) and BNPS skatole (23.2 mg) were added, and the reaction was stirred at ambient temperature in the dark for 72 h. The reaction mixture was diluted with 1 mL of distilled H_2O and extracted 4 times with 2 mL of ethyl acetate. The aqueous layer was neutralized with Tris base, and solid Gdn·HCl was added to a concentration of 6 M. Insoluble material (tyrosine) was removed by centrifugation, and the cloudy supernatant was loaded on a column (200 × 1.5 cm) of Sephadex G-50 superfine equilibrated in 6 M Gdn·HCl and 25 mM Tris-HCl, pH 8.2. Isolated peptides were desalted on a column (36 × 3 cm) of Sephadex G-10 in 50% redistilled pyridine and 1 N NH_4OH and lyophilized.

Peptide W1 was resuspended in 1 mL of 0.5% NH_4HCO_3, pH 8.0, and 0.04 mL of a 0.5 mg/mL solution of V8-protease was added. After 15 h at 37 °C, the reaction was made 2 N in formic acid and then loaded onto a Sephadex G-50 superfine column (200 × 0.9 cm) equilibrated with 2 N formic acid. Peptides were detected by radioactivity and by a ninhydrin color reaction (Moore & Stein, 1954) after alkaline hydrolysis.

V8-Protease Digestion of β_2-m. β_2-m (17.5 mg) was suspended in 2 M Gdn·HCl and 0.05 M sodium borate, pH 8.7. [1,4-^{14}C]Succinic anhydride (10 μCi) in $CHCl_3$ was added at ambient temperature while stirring over a period of 30 min. After another 30 min, 145 mg of succinic anhydride was added slowly, with the concurrent addition of sufficient 5 N NaOH to keep the pH between 8.0 and 9.0. After 1 h the reaction was desalted on a column of Bio-Gel P-2 (25 × 1.5 cm) in 1 N NH_4OH, and the succinylated β_2-m was lyophilized.

The lyophilized material was solubilized in 2 mL of 0.5% NH_4HCO_3, and the pH was adjusted to 8.0 with NH_4OH. One hundred microliters of a 1 mg/mL solution of V8-protease was added, and the reaction was incubated at 37 °C for 30 h. The reaction was made 6 M in Gdn·HCl and loaded onto a column (145 × 1.5 cm) of Sephadex G-50 superfine equilibrated in 6 M Gdn·HCl and 25 mM Tris-HCl, pH 8.2. Peptides isolated from the column were desalted on a column (36 × 3 cm) of Sephadex G-10 in 1 N NH_4OH and lyophilized.

Compositional and Sequence Analysis of Peptides. Peptides were hydrolyzed in 6 N HCl at 110 °C for 18 h in vacuo (Moore & Stein, 1963) and analyzed on a Durrum D-500 amino acid analyzer.

Automated sequence analysis was carried out on a Beckman 890C sequencer by using a DMAA program (No. 102974). Peptides were usually sequenced both with and without the addition of Polybrene (Klapper et al., 1978) to the sequencer cup. The recovered phenylthiazolinone derivatives were converted to phenylthiohydantoin (Pth) amino acids by heating at 80 °C for 5 min in 1 N HCl under N_2 and identified by high-pressure liquid chromatography and by back hydrolysis of the Pth derivatives with HI (Smithies et al., 1971). Where appropriate, cysteine was determined as the [^{14}C]carboxymethyl derivative and lysine was determined as the [^{14}C]-succinyl derivative by liquid scintillation counting.

Carboxy-terminal residues were determined by amino acid analysis subsequent to digestion at pH 5.5 in 0.1 M ammonium acetate with CpY (0.02 μg/nmol peptide) or with CpC (0.07 μg/nmol peptide) as described by Hayashi (1977) by using norleucine as an internal standard. It was necessary to add pepstatin (0.02 μg/mL) (a gift of M. Peyton, NHLBI, NIH) to CpY digestions in order to inhibit a contaminating endopeptidase activity.

High-Pressure Liquid Chromatography of Pth Amino Acids. Pth amino acids in 3 μL of ethyl acetate (or 3 μL of methanol in the case of Pth-His and Pth-Arg) were analyzed on a Waters Associates Model 204 high-pressure liquid chromatograph equipped with a Dupont Zorbax ODS (25 cm × 4 mm) column with a 55 °C water jacket by using a modification of the method of Zimmerman et al. (1977). Isocratic elution at 2 mL per min by using 32% acetonitrile and 68% 0.02 M sodium acetate, pH 4.52, containing 1% acetonitrile separates in 14 min all the Pth amino acids commonly encountered during sequence analysis (Figure 1). These include Pth-succinyllysine, Pth-(carboxymethyl)cysteine, Pth-dehydroserine, and Pth-dehydrothreonine, as well as Pth-norleucine which is used as an internal standard for repetitive yield and relative retention time calculations. Experience has indicated that optimal resolution of all of the Pth amino acid derivatives requires slight adjustments of temperature, pH, sodium acetate concentration, and acetonitrile concentration for different columns. For example, temperature changes affect the resolution of Pth-Met and Pth-Val, whereas pH variations affect the relative mobility of Pth-Glu, and the sodium acetate concentration affects the relative mobility of Pth-Glu, Pth-His, and Pth-Arg. The use of Polybrene in the sequencer cup results in the contamination of the early sequencer steps with large amounts of UV light absorbing material which obscures the positions of Pth-Asn, Pth-Gln, and Pth-Trp, necessitating a sequencer run without Polybrene in order to positively identify Trp, Gln, and Asn.

Results

Isolation of β_2-m. A low molecular weight fraction from the urine of 15 randomly bred rabbits which had been injected with sodium chromate was purified by gel filtration, DEAE-cellulose chromatography, and CM-cellulose chromatography. Characterization of this material by sodium dodecyl sulfate–polyacrylamide gel electrophoresis as described by Laemmli (1970), by alkaline urea–polyacrylamide gel electrophoresis as described by Maizel (1971), by amino acid composition (Table I), and by NH_2-terminal amino acid sequence analysis (Table II) (see paragraph concerning

Table I: Amino Acid Compositions of Rabbit β_2-m Peptides[a]

		peptides									
AA[b]	β_2-m[c]	W1[d]	W2[d]	W1V3	W1V4	V2	V4	V7	V8	V9	V10
Asp	15.1 (15)[g]	10.5	4.6	2.1	4.4	7.6	5.0	2.9	2.9	1.1	3.4
Thr	3.9 (4)	0.8	3.5	0.2	0.2	1.8	0.2	1.9	0.9		1.0
Ser	5.4 (6)	3.8	2.2	1.2	2.1	2.4	1.5	1.3	2.4		0.3
Glu	11.1 (11)	7.5	3.6	1.4	1.9	6.3	4.8	1.7	2.1	1.2	1.4
Pro	6.5 (7)	5.9	1.4			7.2	5.4				1.1
Gly[e]	3.9 (3)	3.0	0.1	1.2	0.4	3.1	2.2	0.9	0.2	1.0	0.3
Ala[e]	3.2 (2)	2.2			0.1	1.5	2.3		0.1	0.1	0.5
Val	10.0 (10)	4.4	2.9	1.0	0.7	7.4	4.1	3.2	1.1	1.2	0.2
Met	0.8 (1)	0.2	0.5			0.9	0.1	0.9			
Ile	2.9 (3)	2.8	0.6	0.9	0.4	1.9	1.7	0.1	0.1	1.0	0.2
Leu	7.2 (7)	4.5	3.4	1.9	1.4	2.1	1.2	1.4	2.7	2.0	0.3
Tyr[f]	4.5 (5)	0.5	0.5	0.1	0.1	3.7	1.6	2.1	0.9	0.1	0.1
Phe	5.0 (5)	3.7	2.5	0.2	1.4	2.5	1.8	0.4	1.8	0.1	0.8
His	4.0 (4)	3.0	1.8	0.2	0.2	3.0	1.7	1.3	1.0		0.1
Lys	7.8 (8)	3.4	3.5	1.5	0.8	3.9	1.3	2.9	1.0	2.0	1.1
Arg	4.3 (4)	1.9	1.0		0.1	4.0	2.0	1.9	0.1	0.1	0.1
no. of residues[h]	99	60	35	9	13	60	38	22	19	9	8
residue positions	1-99	1-60	61-95	39-47	48-60	1-38 + 78-99	1-38	78-99	51-69	39-47	70-77

[a] Compositions are expressed as residues per molecule. [b] Neither Met nor Cys were oxidized prior to analysis. [c] Average of three determinations on unreduced, nonalkylated β_2-m. [d] W peptides containing [^{14}C](carboxymethyl)cysteine. [e] May contain hydrolysis product of Trp. [f] Tyr is largely destroyed by the BNPS skatole cleavage reaction. [g] Parentheses contain the number of residues calculated from sequence data. [h] The number of residues determined by amino acid sequence analysis.

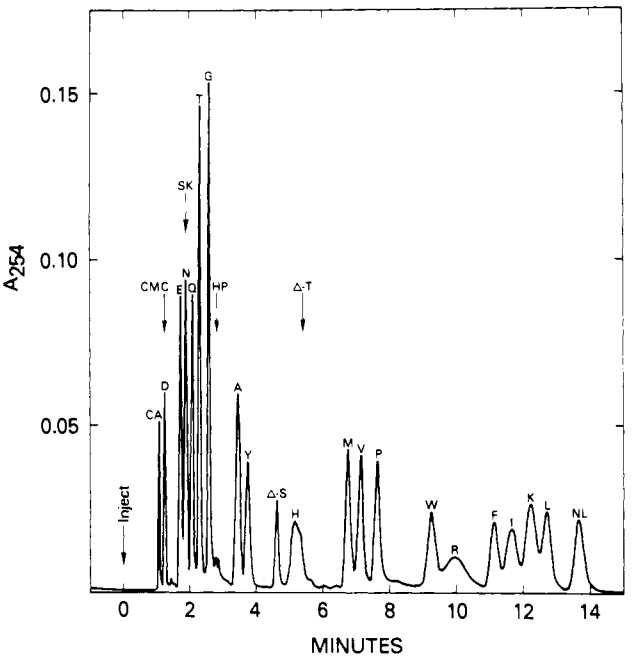

FIGURE 1: High-pressure liquid chromatography of Pth amino acids. A standard containing 1 mg/mL Pth amino acids in 3 μL of ethyl acetate–methanol (1:1) was analyzed on a Waters Associates Model 204 liquid chromatograph equipped with a Dupont Zorbax ODS (25 cm × 4 mm) column with a 55 °C water jacket. The column was eluted isocratically by using 32% acetonitrile and 68% 0.02 M sodium acetate, pH 4.52, containing 1% acetonitrile at 2 mL per min. Arrows show the locations of additional Pth amino acid derivatives. CA, Pth-cysteic acid; CMC, Pth-(carboxymethyl)cysteine; SK, Pth-succinyllysine; HP, Pth-hydroxyproline; Δ-S, Pth-dehydroserine; Δ-T, Pth-dehydrothreonine; NL, Pth-norleucine. Pth-serine elutes slightly behind Pth-glutamine. Pth-Cys elutes in two peaks: slightly behind Pth-Gly and slightly before Pth-Δ-Ser.

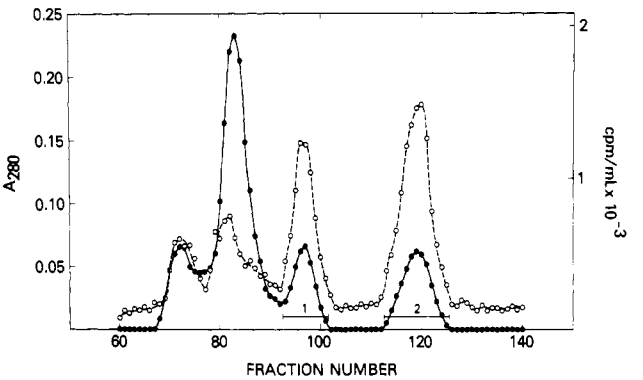

FIGURE 2: Gel filtration of BNPS skatole cleavage products of [^{14}C]carboxymethylated, succinylated rabbit β_2-m on a column (200 × 1.5 cm) of Sephadex G-50 superfine in 6 M Gdn·HCl and 25 mM Tris-HCl, pH 8.2. Fraction size was 2 mL. (●) A_{280}; (○) radioactivity (cpm/mL × 10^{-3}).

supplementary material at the end of this paper) indicated that it was pure β_2-m. The overall yield of purified β_2-m from each 6-L pool of urine containing approximately 100 mg of β_2-m was approximately 25 mg.

The ^{125}I-labeled rabbit β_2-m used to monitor subsequent gel filtration purifications eluted from DEAE-cellulose after the unlabeled material, at 0.13 M and 0.2 M NaCl, indicating that the radioiodination procedure (McFarlane, 1958) probably changed the charge of the β_2-m. Such a modification of β_2-m has also been observed by Natori et al. (1976).

NH_2-Terminal Sequence of β_2-m. Automated sequence analysis of 4 mg of rabbit β_2-m allowed identification of the amino acids at 52 of the first 58 positions (Table II).

Carboxypeptidase Digestion of β_2-m. Rabbit β_2-m was denatured by heating at 95 °C for 2 min at pH 5.5 and then subjected to digestion by CpC or CpY. In both cases only Tyr was released in significant amounts, although small amounts of Asp could be detected in prolonged digestions with CpC.

BNPS Skatole Cleavage of β_2-m. Reduced, [^{14}C]-carboxymethylated, and succinylated rabbit β_2-m was subjected to cleavage by using BNPS skatole, and two [^{14}C]-(carboxymethyl)cysteine-containing peptides, W1 and W2, were isolated by gel filtration (Figure 2). The yield of W1 was 54% and the yield of W2 was 76%, based upon the recovery of radioactivity. The material in fractions 70–90 was presumed to be aggregated and uncleaved material, as well as insoluble tyrosine from the cleavage reaction.

Amino acid compositional analysis (Table I) indicated that peptide W1 was the succinyl-blocked amino-terminal peptide, residues 1–60. A V8-protease digest of W1 gave two un-

Table II: Amino Acid Sequence Analysis of Peptides Obtained from Rabbit β_2-m

peptide	residue positions	positions identified
β_2-m	1–99	V[a] *Q* R A P N V *Q* V Y S R H P A E N G K P N F L N C Y V S G F → H P P *Q* I D I E L L K N G V K I E [b] V Z Z D L F K... Y[c] → → → → → → → → → → → → → → → → → – → → → – → → – → – → ←
V4	1–38	...I D I E ← ← ← ←
W1V3	39–47	L L K N G V K I E → → → → → → → → →
W1V4	48–60	N V E *Q* D L F B → → → → – → → – → → – – –
V8	51–69	*Q* S D L S F N K D W S F Y L L V H T E → → → → → → → → → → → → → → → → → → →
W2	61–95	S F Y L L V H T E F T P N N K N E Y C R V K H V L P V → → → → → → → → → → → → → → → → → → – → → → → → → – → – – → – – → – –
V2	71–99	Y S R V K H V T L K E P M T V K W D R D Y → → – → → → → → → → → → → → → → → → → → →
V7	71–99	Y R V K H V L P V Y → – – → → → → → – → – – → – – → – – – – – – ←

[a] (→) Identified after automated Edman degradation by high-pressure liquid chromatography and/or HI hydrolysis and/or radioactivity as described in the supplementary tables. [b] (–) Not identified. [c] (←) Identified after carboxypeptidase digestion by amino acid analysis as described in the supplementary tables (Supplementary Material).

blocked peptides W1V3 and W1V4, neither of which were UV absorbing nor contained [^{14}C](carboxymethyl)cysteine and which were located by a ninhydrin color reaction (Moore & Stein, 1954). By composition (Table I) and automated sequence analysis (Table II) W1V3 encompassed residues 39–47 and W1V4 spanned residues 48–60. A ^{14}C-labeled peptide was also recovered from the V8-protease digest which by compositional analysis represented residues 1–38.

Automated sequence analysis of peptide W2 for 35 steps identified amino acids at 27 positions, including [^{14}C]-(carboxymethyl)cysteine at position 20 (Table II).

V8-Protease Digest of β_2-m. Having established the positions of most of the Glu residues of rabbit β_2-m from the NH_2-terminal sequence and BNPS skatole cleavage peptides, it was apparent to us that a V8-protease digest of β_2-m might yield the overlapping peptides necessary to complete the sequence determination. Separation of a V8-protease digest of [^{14}C]succinylated rabbit β_2-m by gel filtration on Sephadex G-50 superfine in the presence of 6 M Gdn·HCl (Figure 3) yielded several peptides which were subjected to compositional and sequence analysis.

Peptide V2 contained two peptides in disulfide linkage and gave a single NH_2-terminal sequence by automated Edman degradation. With the exception of the third residue, positive identification of 22 consecutive amino acids was established (Table II), spanning positions 78–99. It was assumed from compositional data (Table I) that the nondegraded peptide is the succinyl-blocked NH_2-terminal peptide, residues 1–38.

Compositional data for peptide V4 (Table I) showed it to be the blocked NH_2-terminal peptide residues 1–38, and this was confirmed by CpY digestion (Table II).

Compositional and automated sequence data revealed peptide V7 to be the 22-residue carboxy-terminal peptide spanning positions 78–99. The partial sequence obtained for peptide V7 corresponded to that obtained for peptide V2 (Table II). Carboxypeptidase analysis of peptide V7 by using CpC in prolonged digestions gave only tyrosine. On the basis of the yields of peptides V4 and V7 relative to V2, more than 80% of the β_2-m used for this digestion had an intact disulfide bond between the cysteines at positions 25 and 80. Whether this disulfide bond is an artifact of the isolation and purification procedures employed remains to be determined.

NH_2-terminal sequence analysis of peptide V8 (Table II) demonstrated that it spanned positions 51–69, which provided

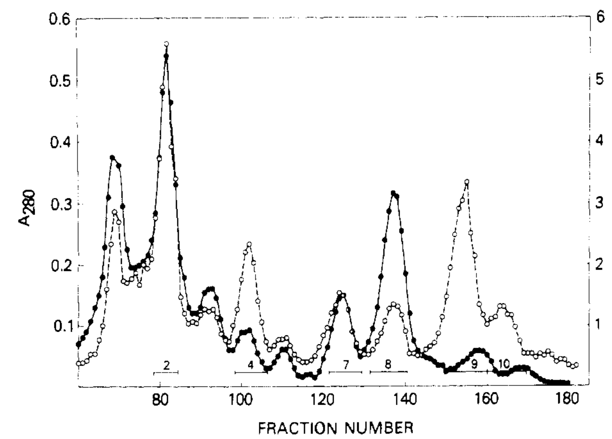

FIGURE 3: Gel filtration of V8-protease digest of [^{14}C]succinylated rabbit β_2-m on a column (200 × 1.5 cm) of Sephadex G-50 superfine in 6 M Gdn·HCl and 25 mM Tris-HCl, pH 8.2. Fraction size was 2 mL. (●) A_{280}; (○) radioactivity (cpm/mL × 10^{-4}).

an overlap for the tryptophan cleavage peptides W1 and W2 (Figure 4). Compositional analysis (Table I) confirmed that the sequence of this peptide had been completely determined.

The amino acid compositions of peptides V9 and V10 (Table I) placed them at residues 39–47 and residues 70–77, respectively (Figure 4).

Discussion

The strategy employed in the present sequence determination has emphasized the production and isolation of relatively large peptides which were amenable to automated sequence analysis. The success of this strategy depended upon the use of Polybrene in the sequencer cup, which helped prevent washout of peptides, and the development of high-pressure liquid chromatography as a method of identification of virtually all of the Pth amino acids. The latter allows the reliable identification of acid and amide residues without data on the electrophoretic mobility of peptides and further allows quantitation of yields of Pth amino acids at each sequencer step. This strategy has the advantage of requiring very little material; the present study required less than 30 mg of protein and two cleavage methods. It must be noted that the choice of this strategy was directed by a foreknowledge of the human β_2-m sequence (Cunningham et al., 1973) and the assumption

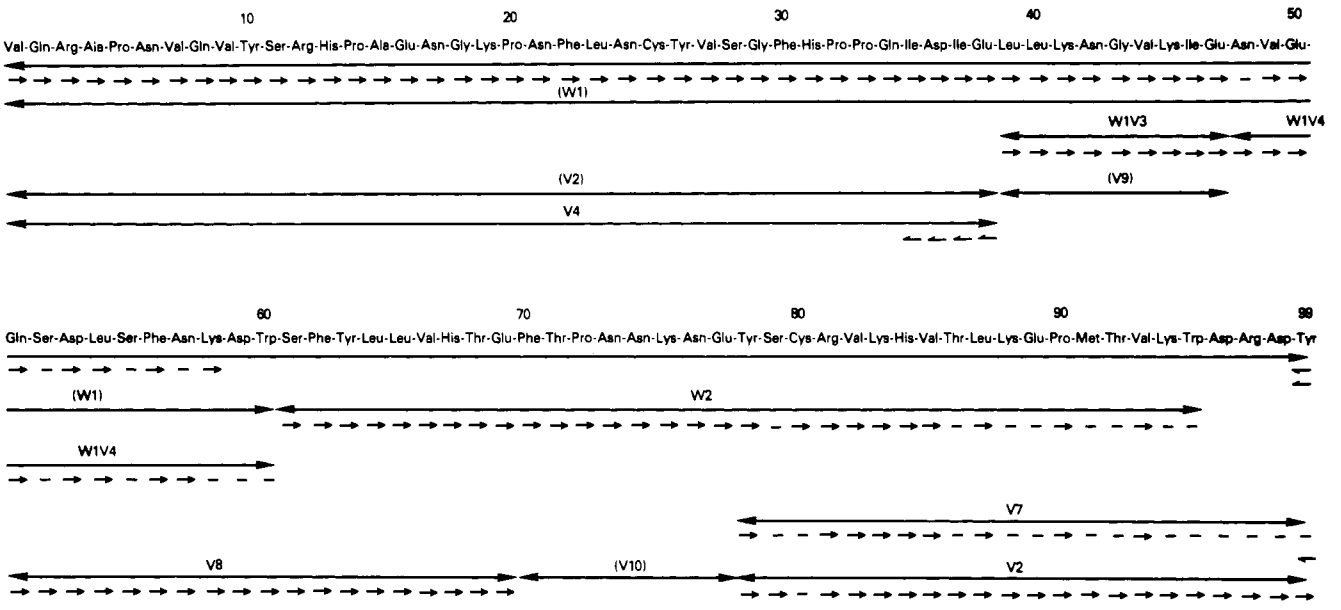

FIGURE 4: Alignment of overlapping peptides obtained for rabbit β_2-m. Peptides are aligned by using sequence and/or compositional data. Parentheses indicate that alignment is only from compositional data. Peptide V2 consists of peptides V4 and V7 in disulfide linkage. (→) Analysis by automated Edman degradation; (←) analysis by CpC digestion; (←) analysis by CpY digestion.

that the rabbit sequence would be similar.

The present data show that the tryptophan at position 60 of both human and rabbit β_2-m has been conserved throughout evolution. It is believed that the tryptophan cleavage employed in the present study will be an efficient tool in the determination of the sequences of β_2-m from other species, many of which have been reported to also contain two tryptophan residues (Groves & Greenberg, 1977; Cebra et al., 1977).

The present study provides additional information on the specificity of the V8-protease. Although the V8-protease has been shown by Houmard & Drapeau (1972) to be able to cleave Glu–Gln, Glu–Asn, and Glu–Pro bonds in a limit digest, these bonds were in some instances refractory to cleavage in rabbit β_2-m. The Glu–Asn bond at positions 16–17 was cleaved only sparingly, as judged by the yield of the 1–38 peptide, whereas the Glu–Asn bond at positions 47–48 appeared to have been cleaved quantitatively. No evidence was obtained for any cleavage of the Glu–Pro bond at positions 89–90, while the Glu–Gln bond at positions 50–51 was more sensitive to cleavage in the V8-protease digestion of the whole molecule than in the digestion of peptide W1 (1–60) obtained by tryptophan cleavage. The specificity of the V8-protease for glutamoyl bonds when the digestion is performed in ammonium bicarbonate buffer is supported by our results.

Three amino-terminal sequences of rabbit β_2-m have been previously reported (Cunningham & Berggård, 1974; Poulik & Reisfeld, 1975; Gordon & Kindt, 1976b) which disagree in assignments for positions 20, 33, and 34. The present sequence determination agrees with the findings of Poulik & Reisfeld (1975) and Gordon & Kindt (1976b) in finding Pro at 20, Pro at 33, and Gln at 34 and agrees with the three previous reports at all other positions of the amino terminus. Although the β_2-m isolated from the urine of a single rabbit has recently been observed to have Pro instead of Asn at position 6 (M. D. Poulik and O. Smithies, personal communication), no heterogeneity was detected in our pooled rabbit β_2-m at this or any other position. In addition, rabbit β_2-m has been isolated from a rabbit lymphoid tumor cell line intrinsically radiolabeled by culturing in the presence of radioactive amino acids, and the amino-terminal sequence has been partially determined by radiosequencing techniques (Coligan et al., 1978). This rabbit β_2-m, radiolabeled in either

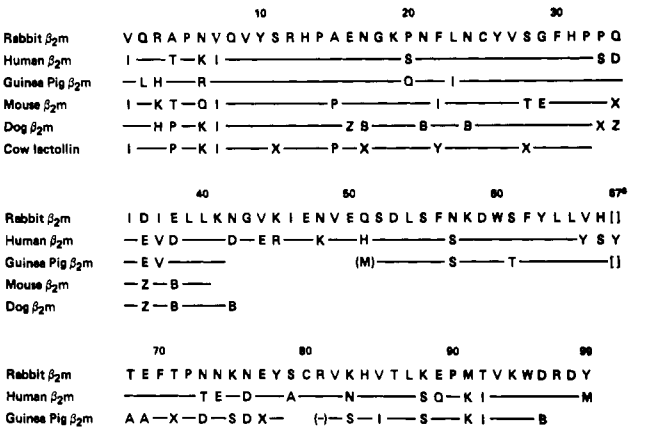

FIGURE 5: Comparison of β_2-m sequence data for various mammals. Brackets at position 67a of the rabbit sequence represent an insertion introduced to maximize homology to the human sequence. Rabbit β_2-m: this paper. Human β_2-m: Cunningham et al. (1973). Guinea pig β_2-m: Cebra et al. (1977). Mouse β_2-m: Appella et al. (1976). Dog β_2-m: Smithies et al. (1972). Cow lactollin: Groves & Greenberg (1977). Lines indicate homology to the rabbit sequence.

Arg, Pro, Ser, Tyr, Phe, or Val, gave results identical with those reported in this paper (E. S. Kimball, personal communication).

Comparison of human β_2-m with the rabbit protein (Figure 5) shows a homology of 71%, requiring a minimum of 38 base changes between the two nucleotide sequences of the genes encoding the two proteins. An insertion must be introduced before position 68 of the rabbit (and guinea pig) protein in order to align the β_2-m sequences for maximum homology. Although amino acid differences attributable to single base changes are distributed evenly throughout the molecule, the majority of those requiring multiple base changes are restricted to the carboxy-terminal third of the molecule. The most extensive region of homology between rabbit and human β_2-m lies between residues 8 and 32, in which there is a single Pro-Ser interchange at position 20. On the basis of NH_2-terminal sequence comparison, the rabbit β_2-m is more homologous to the dog and guinea pig proteins than to the human, with six and seven interchanges, respectively, in the first 42 residues (Figure 5).

Human β_2-m has been reported to bear some homology to human immunoglobulin domains (Peterson et al., 1974), and a comparison of a partial sequence of rabbit β_2-m with the domains of the constant regions of rabbit immunoglobulins gave support to this homology (Cunningham & Berggård, 1974). Comparison of the entire rabbit β_2-m sequence with rabbit immunoglobulin domains reveals a maximum of 29 identities (with the C_3H domain), the most significant stretches of identity spanning the cysteine-containing regions and residues 38–45 of rabbit β_2-m. Although this limited homology indicates a common evolutionary origin of β_2-m and immunoglobulins, it is doubtful that this homology reflects a relationship at the level of gene expression or synthesis. On the other hand, it is possible that common structural features are preserved in globular proteins with disulfide bonds.

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Supplementary Material Available

Tables giving residue by residue basis for sequence assignment (14 pages). Ordering information is given on any current masthead page.

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