Biochemistry

 Volume 17, Number 6 March 21, 1978

Guinea Pig β_2-Microglobulin. Purification, Properties, and Partial Structure†

Ragnhild Cigén, Jack A. Ziffer, Barbro Berggård, Bruce A. Cunningham, and Ingemar Berggård*

ABSTRACT: β_2-Microglobulin has been purified from urine of guinea pigs treated with sodium chromate by a combination of ultrafiltration, gel chromatography, ion-exchange chromatography, and zone electrophoresis. Two forms of the protein, differing in net charge, were isolated. On Ouchterlony immunodiffusion analyses, the two forms appeared immunologically identical and cross-reacted with rabbit β_2-microglobulin. At pH 8 to 9, however, the net charge of the major type was more positive than that of the minor type, and the amino acid composition of the major form contained 8 lysyl residues whereas that of the minor form contained only 7. The two proteins therefore may represent genetically distinct forms of β_2-microglobulin. Alternatively, the minor component may be derived from the major protein by proteolysis or deamidation. The results of amino acid analyses and of experiments with starch gel electrophoresis in urea indicate that both guinea pig proteins have a disulfide loop analogous to those characteristic of human β_2-microglobulin and the immunoglobulin domains. The major form of the protein constitutes about three-fourths of the total urinary β_2-microglobulin, and the amino acid sequence of the first 18 residues of this form is closely homologous to corresponding sequences in β_2-microglobulins from other species, particularly the rabbit. Physicochemical characterization of the protein revealed a molecular weight of about 11 500, a sedimentation coefficient of 1.6 S, a Stokes' radius of 1.6 nm, and an isoelectric point of 6.6. The molecule has a low frictional ratio (1.12) indicating a compact, globular shape. The protein has a tendency to self-associate at high concentrations.

Human β_2-microglobulin is a small protein produced by various types of cells (Nilsson et al., 1973). It occurs both bound to cell surfaces (Peterson et al., 1972; Poulik, 1973) and in free form in various body fluids (Berggård & Bearn, 1968; Berggård, 1965). The protein is of special interest because it is structurally related to the immunoglobulins (Smithies & Poulik, 1972a; Peterson et al., 1972) and is homologous to the constant domains of these proteins (Peterson et al., 1972; Cunningham et al., 1973; Cunningham, 1976). Furthermore, recent work has shown that is occurs associated with human HLA histocompatibility antigens (Nakamuro et al., 1973; Grey et al., 1973; Peterson et al., 1974).

Studies of β_2-microglobulin from other species have become important in order to gain more insight into the structural, evolutionary, and functional relations of this protein to histocompatibility antigens, to other cell surface structures, and to immunoglobulins. During recent years, β_2-microglobulins have been isolated from dog (Smithies & Poulik, 1972b), rabbit (Berggård, 1974; Cunningham & Berggård, 1975), mouse (Natori et al., 1975; Appella et al., 1976), rat (Poulik et al., 1975), chicken (Winkler & Sanders, 1977), and cow (Groves & Greenberg, 1977). The mouse homologue of β_2-microglobulin is associated not only with the major H-2 histocompatibility antigens (Rask et al., 1974; Silver & Hood, 1974) but also with the closely related thymus leukemia (TL) antigens (Östberg et al., 1975; Vitetta et al., 1975) and Qa-2 antigens (Michaelson et al., 1977).

Structural and genetic variants of β_2-microglobulin from any species could be particularly valuable in determining its biological activity, its interactions with specific cell surface molecules, and the organization of β_2-microglobulin genes in mammalian chromosomes. No genetic variant of β_2-microglobulin has been unambiguously identified. Variant forms of the protein, however, have been described. For example, lactollin, a crystalline protein of molecular weight 43 000, isolated from milk (Groves et al., 1963), is composed of subunits of molecular weight 12 000 that represent the bovine homologue of β_2-microglobulin (Groves & Greenberg, 1977), and two forms of human β_2-microglobulins, differing in isoelectric point, have been detected (Hall et al., 1977).

This report describes the preparation of guinea pig β_2-microglobulin. The protein was obtained in two forms that differ in isoelectric point and amino acid composition. A partial physicochemical and structural characterization has been carried out, particularly of the major form which resembles the human protein in many properties, but differs from human β_2-microglobulin in that it aggregates at high concentrations.

† From the Department of Physiological Chemistry, University of Lund, S-220 07 Lund, Sweden (R.C., B.B., and I.B.), and the Rockefeller University, New York, New York 10021 (J.A.Z. and B.A.C.). *Received October 11, 1977.* This work was supported by grants from the Swedish Medical Research Council (Project No. 512), the Medical Faculty, University of Lund, and from the U.S. Public Health Service (AI 09273 and AI 11378). B.A.C. is the recipient of a Career Scientist award from the Irma T. Hirschl Trust.

Some preliminary results of this investigation have been reported in a previous communication (Berggård, 1976).

Materials and Methods

Urines and Sera. Concentrates of urinary proteins were prepared from five to seven 24-h urine specimens collected from each of 10 to 21 outbred guinea pigs treated with a single subcutaneous dose of sodium chromate (15 mg/kg of body weight of a solution containing 20 mg/mL). Only male guinea pigs were used and they were kept in metabolic cages with one animal per cage. In most experiments, some animals died before the end of a collection period. The surviving animals were given repeated injections after periods of rest. To each collecting vessel 0.5 mL of 5% sodium azide was added as a preservative. When a 24-h collection was terminated, the sodium azide concentration was adjusted to 0.1%. The urine specimens were pooled, adjusted to pH 6.5, centrifuged, tested for protein with Uristix strips (Ames Co., Slough, England), and then stored at −20 °C until the end of the collection period. Tests for protein were usually negative in normal urine but positive after sodium chromate administration with maximum proteinuria (2+ to 3+) during the first 3 to 4 days after injection.

Sera were obtained from healthy guinea pigs which were bled from the carotid artery. After removal of the clot, the sera were stored at −20 °C until used.

Antisera. Antisera were raised in one goat and two rabbits against the major form of guinea pig β_2-microglobulin and in two rabbits against the minor form of this protein (see below). The immunization procedures have been described (Berggård & Bearn, 1968; Björck et al., 1977). The goat antiserum appeared specific, and one of the rabbit antisera practically specific for the isolated proteins when tested on immunoelectrophoresis against varying amounts of concentrated urinary proteins from guinea pigs treated with sodium chromate. Antisera against rabbit β_2-microglobulin were prepared as described (Björck et al., 1977).

Other Materials. DEAE-cellulose 23-SH was obtained from Serva Feinbiochemica, Heidelberg, Germany; Pevikon C-870 was from Kema Nord AB, Stockholm, Sweden; partially hydrolyzed starch was from Connaught Laboratories, Toronto, Canada; polyamide thin layers were from Schleicher and Schuell, Keene, N.H.; aminopeptidase M was from Rohm & Haas, Darmstadt, Germany; carrier ampholytes (Ampholine) were from LKB-Produkter AB, Bromma, Sweden; and $^{23}/_{32}$-in. dialysis tubing was from Union Carbide Corp., Chicago, Ill. Human and rabbit β_2-microglobulins were prepared as described (Berggård & Bearn, 1968; Berggård, 1974). Sephadex G-100 and Sepharose CL-6B were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden, and prepared according to the instructions supplied. All other chemicals were reagent grade or of the best quality available.

Protein Determinations. Protein concentrations were measured by the modified Folin method of Lowry et al. (1951) or by reading the absorbance at 280 nm. The major form of guinea pig β_2-microglobulin was used as the standard in both procedures. In the Folin method, guinea pig β_2-microglobulin gave about the same absorbancy as an equal amount of human IgG. The absorption coefficient of guinea pig β_2-microglobulin at 280 nm and its light-absorption spectrum were determined in 0.1 M sodium phosphate buffer (pH 7.0) on a Gilford Model 222 spectrophotometer, after correction for ash and moisture content. Moisture was measured by drying to constant weight at 105 °C and 0.05 mmHg.

Concentration of Proteins. Urine, sera, and protein solutions were concentrated by ultrafiltration with $^{23}/_{32}$-in. dialysis tubing from Union Carbide (Berggård, 1962). This tubing retains β_2-microglobulin completely under the conditions used (Berggård & Bearn, 1968). Urine was concentrated 40- to 90-fold and centrifuged, before purification of β_2-microglobulin.

Electrophoretic Methods. Preparative zone electrophoresis in blocks of Pevikon C-870, polyacrylamide gel electrophoresis in a discontinous buffer system, and starch gel electrophoresis in 8 M urea and formate buffer (pH 3.0) were performed with previously reported techniques (Peterson & Berggård, 1971). A 0.1 M sodium borate buffer (pH 8.9) was used for preparative zone electrophoresis (Berggård & Bearn, 1968). Protein samples analyzed by starch gel electrophoresis were dissolved in 0.2 M Tris-HCl buffer (pH 8.0) or in the same buffer containing 8 M urea. Some of these samples were reduced with 0.1 M 2-mercaptoethanol for 60 min at room temperature and thereafter alkylated with 0.12 M iodoacetamide for 30 min.

Agarose gel electrophoresis was carried out in 75 mM sodium barbital buffer (pH 8.6) containing 2 mM calcium lactate, essentially as described by Johansson (1972).

Polyacrylamide gel electrophoresis in slabs with sodium dodecyl sulfate was performed according to Neville (1971) using the GE-4 apparatus from Pharmacia Fine Chemicals, Uppsala, Sweden. The running gel had a pH of 9.2 and an acrylamide concentration of 16% with an acrylamide/bisacrylamide ratio of 97:3. Human IgG heavy chain, chicken egg albumin, human IgG light chain, bovine ribonuclease, and human β_2-microglobulin were used as molecular weight markers, assuming values of 50 000, 43 000, 23 500, 13 700, and 11 800, respectively.

Isoelectric Focusing. Analytical isoelectric focusing was conducted at 5 °C in a 110-mL column (LKB-Produkter AB, Bromma, Sweden) essentially as described by Vesterberg (1971). Carrier ampholyte solutions of pH range 4 to 8 were used. The pH gradient was stabilized by a sucrose gradient from 0 to 50%. Focusing proceeded for 24 h at a constant power of 5.6 W and a current from 4 to 2 mA. The effluent from the column was analyzed for pH, for protein by reading the absorbance at 280 nm, and for β_2-microglobulin by single radial immunodiffusion.

Immunochemical Techniques. Immunodiffusion in gel according to Ouchterlony, immunoelectrophoresis, and single radial immunodiffusion were performed as previously reported (Berggård & Bearn, 1968).

Ultracentrifugation. Sedimentation velocity and equilibrium experiments were conducted at 20 °C in a Beckman Model E analytical ultracentrifuge, equipped with an RTIC temperature control unit and an electronic speed control device (Beckman Instruments, Palo Alto, Calif.). The protein samples were examined in 0.02 M Tris-HCl buffer (pH 8.0) containing 0.15 M NaCl. Standard 12-mm double sector cells with sapphire windows were used. The centrifuge was operated at 60 000 or 48 000 rpm for the sedimentation velocity determinations. The sedimenting boundary was recorded every 4 min with the phase plate schlieren optics. Calculations were done as described by Schachman (1957).

Sedimentation equilibrium experiments were carried out with the meniscus depletion technique of Yphantis (1964). Recordings were made with Rayleigh interference optics.

Determination of Molecular Weight by Gel Chromatography. Analyses were made on a Sepharose CL-6B column (120 × 1.5 cm), equilibrated with 0.05 M sodium acetate buffer (pH 4.8) containing 6 M guanidine hydrochloride, Gdn·HCl[1] (Mann and Fish, 1972). Heavy and light chains of

[1] Abbreviations used: Gdn·HCl, guanidine hydrochloride; Pth, phenylthiohydantoin.

human IgG, bovine albumin, chicken egg albumin, equine myoglobin, and human β_2-microglobulin were used as reference proteins. Guinea pig β_2-microglobulin and the reference proteins were reduced for 2 h in 0.2 M Tris-HCl buffer (pH 8.0) containing 6 M guanidine hydrochloride and 0.02 M dithioerythritol. Thereafter, iodoacetamide was added to a concentration of 0.05 M and the samples were left to alkylate for 30 min in the dark.

Estimation of Stokes' Radius. Stokes' radius (r_s) was measured at room temperature by chromatography on a Sephadex G-100 column (107 × 0.9 cm) equilibrated with 0.02 M Tris-HCl buffer (pH 8.0) containing 0.15 M NaCl. The details of the experimental procedure have been described (Karlsson et al., 1972). Bovine albumin (r_s = 3.6 nm), bovine chymotrypsinogen (r_s = 2.2 nm) and human β_2-microglobulin (r_s = 1.6 nm) were utilized to calibrate the column. The equation given by Laurent & Killander (1964) was used for calculation of Stokes' radius from the gel chromatography data.

Calculations of the Apparent Diffusion Coefficient, of the Molecular Weight by the Svedberg Equation, and of the Frictional Ratio. Stokes–Einstein's equation (Gosting, 1956) was used to calculate the apparent diffusion coefficient ($D_{20,w}$) from Stokes' radius. The molecular weight was determined from the diffusion coefficient and the sedimentation coefficient by Svedberg's equation. The frictional ratio (f/f_0) was computed from the sedimentation coefficient and the molecular weight derived from the amino acid composition of the protein. A partial specific volume for the calculations was estimated from the amino acid composition as described by Cohn & Edsall (1943). The formulas used have been reported by Svedberg & Pedersen (1940).

Amino Acid Analysis. Quantitative amino acid analyses were carried out on a Durrum Model D-500 amino acid analyzer (Durrum, Palo Alto, Calif.). Protein samples of about 0.5 mg were hydrolyzed in 6 M HCl at 110 °C for 24 and 72 h (von Hofsten et al., 1965). Half-cystine was measured as cysteic acid after performic acid oxidation (Hirs, 1967). Half-cystine was also determined as carboxymethylcysteine. For these analyses, samples of protein (5 mg/mL) were reduced for 60 min in 0.2 M Tris-HCl buffer (pH 8.0) containing 6 M Gdn·HCl and 0.01 M dithioerythritol. The samples were alkylated at room temperature for 15 min in the dark by addition of a 15% molar excess (over reagent − SH) of iodoacetamide. Amino acid analyses were performed after exhaustive dialysis against distilled water at 4 °C, followed by lyophilization and hydrolysis. Tryptophan was estimated spectrophotometrically as described by Edelhoch (1967).

Analysis of Free Sulfhydryl Groups. These were determined by carboxymethylation followed by analyses for carboxymethylcysteine on the amino acid analyzer. Protein samples (2.5 mg/mL) were treated for 15 min in the dark with 2 mM iodoacetamide in 0.2 M Tris-HCl buffer (pH 8.0) containing 6 M guanidine hydrochloride. Thereafter the samples were dialyzed exhaustively against distilled water at 4 °C, lyophilized, and submitted to amino acid analysis.

Sequence Analyses. Half-cystines were reduced with dithiothreitol in the presence of 6 M guanidine hydrochloride and alkylated with iodoacetamide (Waxdal et al., 1968). Excess reagents were removed by exhaustive dialysis against distilled water. Amino-terminal sequence analysis on 100 nmol of the reduced and alkylated protein was performed using the automated method of Edman & Begg (1967) in a Beckman Model 890 C sequencer with the Quadrol double cleavage program (Beckman Instruments, Palo Alto, Calif.). The thiazolanone amino acids were converted to their phenylthiohydantoin (Pth) derivatives using 1 M HCl and identified qualitatively using polyamide thin layers according to the method of Summers et al. (1973). Arginine (Yamada & Itano, 1966) and histidine were detected calorimetrically. Leucine was resolved from isoleucine by 18-h hydrolysis of the Pth-amino acid in 6 M HCl at 150 °C, followed by amino acid analysis using a Beckman Model 121 M amino acid analyzer (Beckman Instruments). Manual sequence analysis was carried out on 100 nmol of the reduced and alkylated protein using the dansyl-Edman technique of Gottlieb et al. (1970); identification was carried out using the thin-layer chromatography system of Weiner et al. (1972). Aminopeptidase M digestion was used to quantitate the results.

TABLE I: Purification of the Major Form of Guinea Pig β_2-Microglobulin.

Purification step	Total protein[a] (mg)	β_2-Microglobulin[b] (mg)	Yield (%)	Purification (fold)
Concentrated urinary proteins[c]	5900	73	100	1
First Sephadex G-100 chromatography	190	43	59	18
DEAE-cellulose chromatography	33	19	26	46
Zone electrophoresis	13	12	16	74
Second Sephadex G-100 chromatography	9	9	12	81

[a] Determined by the Folin method in the first four steps and then spectrophotometrically from the optical density at 280 nm. [b] Measured by single radial immunodiffusion. [c] The urinary proteins were obtained from four to six 24-h specimens of urine from each of 21 guinea pigs treated with sodium chromate.

Results

Purification of Two Forms of Guinea Pig β_2-Microglobulin. Two homologues of β_2-microglobulin were obtained. One of the forms was present in the urine concentrates in about three times higher quantities than the other form (see below). Antisera were raised against the first preparations of β_2-microglobulin and were used to trace and measure the protein during the following purifications.

The various isolation steps are described below and the protocol of a typical purification of the major form of the protein is summarized in Table I. All operations were carried out at 4 °C starting from concentrates of pooled urine. The yields of the major form varied between 9 and 16% of the total amount of β_2-microglobulin found in concentrates of urine.

First Gel Chromatography on Sephadex G-100. Concentrated urinary proteins from guinea pigs treated with sodium chromate were chromatographed on Sephadex G-100 columns (113 × 8 or 104 × 5 cm) equilibrated with 0.02 M Tris-HCl buffer (pH 8.0) containing 0.15 M NaCl and 0.02% NaN_3. In early purifications, protein with the elution volume of human β_2-microglobulin was taken to the next step (DEAE-cellulose chromatography). When antisera became available, the effluents from the columns were analyzed both for total protein by reading the absorbance at 280 nm and for guinea pig β_2-microglobulin by single radial immunodiffusion. β_2-Microglobulin was eluted from the Sephadex G-100 columns after most of the other urinary proteins (Figure 1) but did not appear as a peak in the absorbance at 280 nm.

Chromatography on DEAE-Cellulose. The pooled fractions from one or two Sephadex G-100 chromatographies were dialyzed against 0.04 M Tris-HCl buffer (pH 8.0) and applied

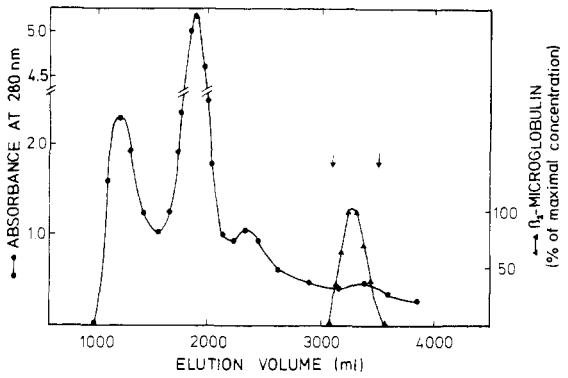

FIGURE 1: Chromatography on a Sephadex G-100 column (113 × 8 cm) of concentrated urinary proteins from 20 guinea pigs with renal damage induced by sodium chromate. The sample (2.6 g of protein in 18 mL) was eluted with a 0.02 M Tris-HCl buffer (pH 8.0) containing 0.15 M NaCl and 0.02% NaN_3. Fractions of 14.8 mL were collected at a flow rate of 44.4 mL per h. The distributions in the effluent of protein (●) and β_2-microglobulin (▲) are shown. Fractions containing β_2-microglobulin were combined as indicated by the arrows.

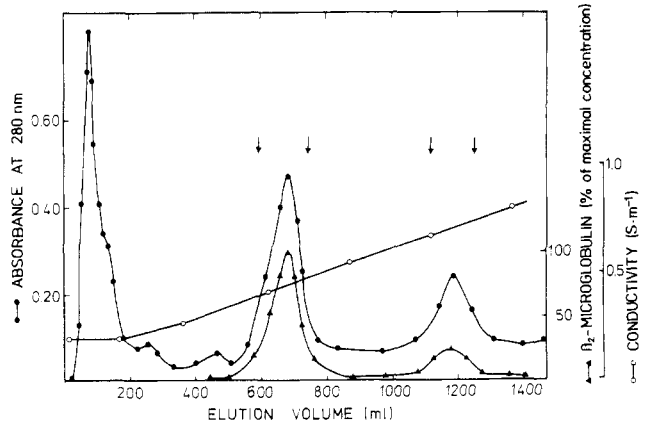

FIGURE 2: DEAE-cellulose chromatography of the β_2-microglobulin-containing material from gel chromatography on Sephadex G-100. The sample (0.40 g of protein) was dialyzed against 0.04 M Tris-HCl buffer (pH 8.0) and applied to a column (13.0 × 3.5 cm) of DEAE-cellulose 23-SH equilibrated with the same buffer. The column was eluted in 0.04 M Tris-HCl buffer (pH 8.0) with a 2000-mL linear gradient of sodium chloride from 0 M to 0.1 M. Fractions of 6.2 mL were collected at a flow rate of 18.6 mL per h. The effluent was analyzed for protein (●), β_2-microglobulin (▲), and conductivity (○). Eluates corresponding to a large and a small peak of β_2-microglobulin were pooled as marked by the arrows.

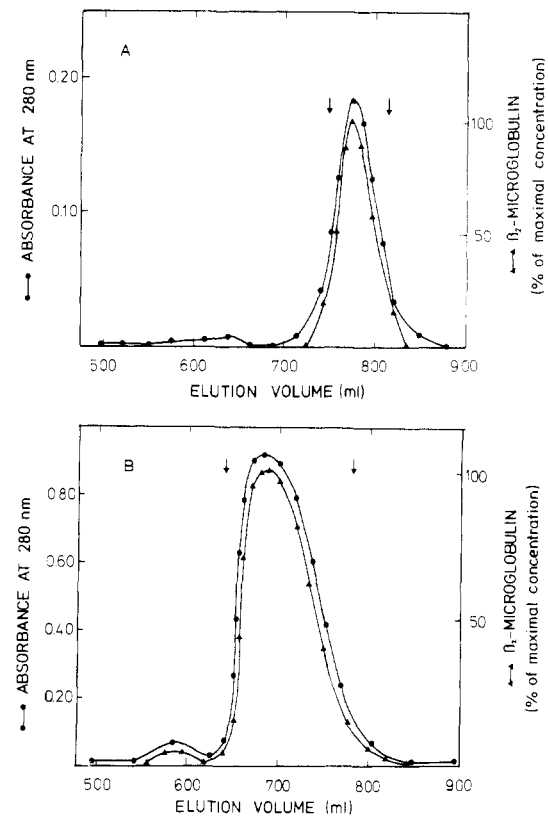

FIGURE 3: Gel chromatography on Sephadex G-100 of the major form of guinea pig β_2-microglobulin previously purified by zone electrophoresis in borate buffer. The column (145 × 3 cm) was equilibrated with 0.02 M Tris-HCl buffer (pH 8.0) containing 0.15 M NaCl and 0.02% NaN_3. The distributions in the effluent of total protein (●) and β_2-microglobulin (▲) are shown. The arrows indicate how the fractions were combined. (A) Thirteen milligrams of protein in 5.1 mL was applied. Fractions of 6.0 mL were collected at 20-min intervals. (B) Seventy milligrams of protein in 8.4 mL was applied. Fractions of 3.3 mL were collected at 20-min intervals.

to a DEAE-cellulose column that was equilibrated with the same buffer. Elution was performed with a linear gradient of sodium chloride (0 to 0.1 M). Figure 2 shows that three main protein fractions were obtained. The last material to be eluted emerged at about the same sodium chloride concentration as human β_2-microglobulin. Therefore, only the protein present in this fraction was used for further purification in the initial work. The first antisera were prepared against a homologue of β_2-microglobulin obtained from this material. With these antisera, we found that β_2-microglobulin was present also in the intermediate fraction and in higher quantities. The ratio of the amounts of β_2-microglobulin in the two fractions was about 1 to 3.

Zone Electrophoresis in Borate Buffer (pH 8.9). The two β_2-microglobulin-containing fractions from DEAE-cellulose chromatography were concentrated separately and subjected to preparative zone electrophoresis in 0.1 M sodium borate buffer, pH 8.9. Material from one to four columns was used for each separation. Usually, only one distinct protein component containing β_2-microglobulin was observed after electrophoresis of the major or the minor fraction from DEAE cellulose. Some contaminants were removed, especially from the minor fraction. Most contaminants were found in the anodal direction from the main protein component. The minor fraction has a higher mobility than the major fraction; both fractions migrated toward the anode.

Second Gel Chromatography on Sephadex G-100. After concentration, the two β_2-microglobulin fractions were separately chromatographed on a Sephadex G-100 column (145 × 3 cm). Moderate amounts of the major fraction (5–15 mg) gave symmetrical peaks with the elution volume of human β_2-microglobulin (Figure 3A). In contrast, large amounts of this fraction (50–70 mg) gave asymmetrical peaks with significantly smaller elution volumes (Figure 3B). Only a slight purification was achieved by this final step (Figure 3A and Table I). A small amount of material containing β_2-microglobulin appeared ahead of the main peak when relatively large amounts of the major fraction were chromatographed (Figure 3B).

On Sephadex G-100 chromatography, the minor β_2-microglobulin fraction resolved into two protein components that were not completely separated from each other. The first component was a contaminant, whereas the second consisted of β_2-microglobulin, as shown by single radial immunodiffusion analyses.

After each chromatography, the eluate corresponding to the

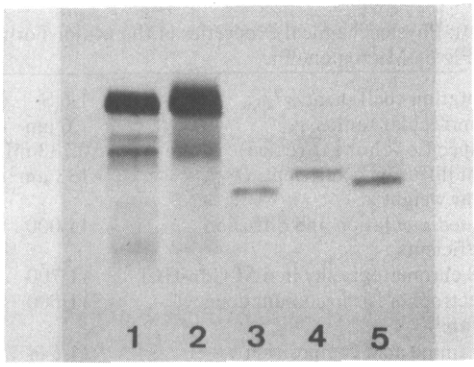

FIGURE 4: Agarose gel electrophoresis at pH 8.6 of serum from a normal guinea pig (1), concentrated urinary proteins from a guinea pig treated with sodium chromate (2), the major form of guinea pig β_2-microglobulin (3), the minor form of guinea pig β_2-microglobulin (4), and human β_2-microglobulin (5). The anode is at the top.

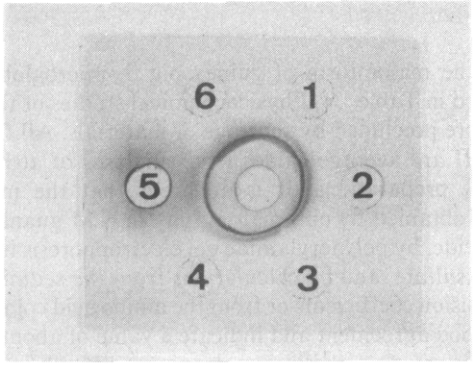

FIGURE 5: Ouchterlony immunodiffusion analysis of the major form of guinea pig β_2-microglobulin (1 and 4), the minor form of the protein (3 and 6), concentrated urine form a guinea pig treated with sodium chromate (2), and concentrated serum from an untreated guinea pig (5). The center well contained goat antiserum against the major form of guinea pig β_2-microglobulin.

main part of the β_2-microglobulin peak was dialyzed exhaustively against distilled, deionized water, and then lyophilized.

Purity and Charge Difference of the Two Forms of the Protein. The purity of the isolated protein was assessed by agarose gel electrophoresis and by polyacrylamide gel electrophoresis in the presence or absence of sodium dodecyl sulfate. Usually, only single protein bands were seen. The results of amino acid analyses and isoelectric focusing experiments also indicated purity (see below).

The charge difference between the two forms of guinea pig β_2-microglobulin observed during the purification was confirmed by agarose gel electrophoresis and by polyacrylamide gel electrophoresis. The major form had somewhat lower mobility than human β_2-microglobulin whereas the minor form migrated somewhat faster (Figure 4).

Immunodiffusion Analyses. On Ouchterlony immunodiffusion analyses using goat antiserum against rabbit β_2-microglobulin, the two purified guinea pig proteins gave relatively weak precipitin lines that fused completely with each other and gave a reaction of partial identity with the strong precipitin line of rabbit β_2-microglobulin. This observation indicates that both proteins are homologues of β_2-microglobulin and rather closely related to the rabbit homologue of this protein.

Figure 5 shows that guinea pig serum and urine contain β_2-microglobulin that gives reactions of identity with the purified major and minor forms of this protein when an antiserum against the major form is used. Reactions of identity between the two forms were found with all antisera directed against either form. One strong and one weak precipitin line were always seen (Figure 4). Two parallel arcs, one strong and one weak, were observed in immunoelectrophoretic experiments. We have observed this phenomenon with two precipitin lines or arcs also when other purified β_2-microglobulins (human, rabbit, and rat) have reacted with their corresponding antisera. Therefore, and because both forms of guinea pig β_2-microglobulin gave the same two lines, it is highly probable that both lines are given by β_2-microglobulin.

TABLE II: Amino Acid Composition[a] of the Major and the Minor Form of Guinea Pig β_2-Microglobulin.

Amino acid	The major form of the protein	The minor form of the protein
Asp	13.0 ± 0.4 (13)	13.1 ± 0.3 (13)
Thr[b]	3.1 ± 0.1 (3)	3.2 ± 0.1 (3)
Ser[b]	8.8 ± 0.3 (9)	9.1 ± 0.1 (9)
Glu	9.8 ± 0.2 (10)	10.2 ± 0.4 (10)
Pro	7.1 ± 0.2 (7)	7.0 ± 0.2 (7)
Gly	3.4 ± 0.2 (3)	3.7 ± 0.2 (4)
Ala	4.2 ± 0.1 (4)	4.4 ± 0.1 (4)
½-cystine[c]	1.9 ± 0.3 (2)	2.0 ± 0.2 (2)
Val[d]	8.7 ± 0.3 (9)	8.7 ± 0.3 (9)
Met	0.8 ± 0.1 (1)	0.9 ± 0.1 (1)
Ile[d]	4.9 ± 0.1 (5)	4.9 ± 0.2 (5)
Leu	7.1 ± 0.3 (7)	7.1 ± 0.2 (7)
Tyr[b]	4.0 ± 0.4 (4)	3.6 ± 0.5 (4)
Phe	5.0 ± 0.1 (5)	5.0 ± 0.2 (5)
His	4.9 ± 0.1 (5)	5.0 ± 0.3 (5)
Lys	8.1 ± 0.3 (8)	7.0 ± 0.3 (7)
Arg	3.1 ± 0.1 (3)	3.2 ± 0.1 (3)
Trp[e]	1.9 (2)	(2)[f] (2)
Total	99.8 (100)	100.1 (100)

[a] Calculated on the basis of 100 amino acid residues per molecule. Except where noted, all figures are means ± SD obtained from analyses of four different preparations of each form of the protein, and each analysis is an average of one 24-h and one 72-h hydrolysis. The nearest integer values are given in parentheses. [b] Values obtained by extrapolation to zero-hour hydrolysis. [c] Measured as cysteic acid on three preparations of each form. [d] Seventy-two-hour hydrolysis value only. [e] Determined spectrophotometrically on two preparations of the major form. The average value is given. [f] Not determined.

Amino Acid Composition. The amino acid compositions of the two types of guinea pig β_2-microglobulin are given in Table II. The recovery was about 95% (w/w). The calculations were based on the assumption that the two guinea pig proteins contain 100 residues per molecule, like human β_2-microglobulin (Berggård & Bearn, 1968; Cunningham et al., 1973). The two forms seem to have the same composition except that the minor form contains one residue less of lysine. Close to integral numbers of residues were obtained for all amino acids with the exception of glycine, suggesting that the protein samples are homogeneous. The number of half-cystine residues in the major form was 1.9 per molecule when measured as cysteic acid after performic acid oxidation and 1.8 when measured as carboxymethylcysteine after reduction and alkylation in 6 M guanidine hydrochloride. No evidence for free sulfhydryl groups in this protein was obtained by analysis for carboxymethylcysteine after carboxymethylation in 6 M guanidine hydrochloride.

Evidence for Similar Disulfide-Linked Loops in Guinea Pig β_2-Microglobulin and Its Human Counterpart. On starch gel electrophoresis in 8 M urea and formate buffer, the mobilities of both guinea pig proteins and human β_2-microglobulin de-

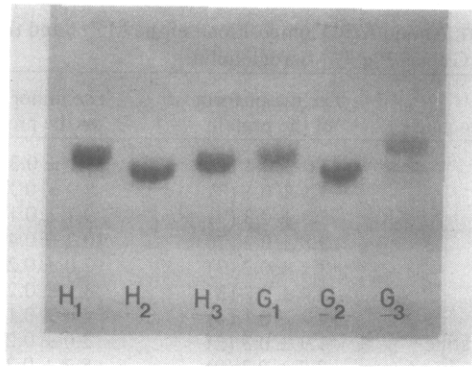

FIGURE 6: Starch gel electrophoresis in 8 M urea and formate buffer (pH 8.0) of human β_2-microglobulin (H_1–H_3) and the minor form of guinea pig β_2-microglobulin (G_1–G_3). (H_1, G_1) Untreated protein; (H_2, G_2) protein reduced and alkylated in the presence of 8 M urea; (H_3, G_3) protein reduced and alkylated in the absence of urea. Electrophoresis proceeded in cathodal direction from the bottom to the top.

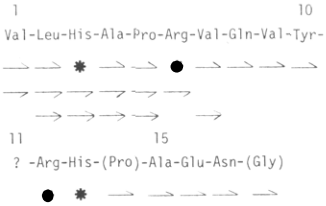

FIGURE 7: Amino acid sequence of residues 1 to 18 of the major form of guinea pig β_2-microglobulin. (→, ⇢, and ✱) denote sequence analysis of intact protein in the automatic sequencer with phenylthiohydantoins identified: (→) by thin-layer chromatography; (⇢) by hydrolysis to free amino acid followed by amino acid analysis; (✱) by colorimetric test. (↛) Indicates sequence determination of the protein by the dansyl–Edman technique. Residues in parentheses were detected on a single analysis and, therefore, are less certain.

creased by about the same extent after reduction and alkylation in the presence of urea. The minor component, reduced and alkylated in the absence of urea, showed a weak additional band with the same mobility as the minor component that was treated in the presence of urea. The comparisons of the minor component and human β_2-microglobulin are illustrated in Figure 6. These observations suggest that the two guinea pig proteins and human β_2-microglobulin have disulfide-linked loops of similar size, and that only the intrachain disulfide bridge of the minor guinea pig component is sensitive to reduction in the absence of a denaturing agent.

Evidence for Self-Association. As described above and illustrated in Figure 3, the major form of guinea pig β_2-microglobulin in moderate amounts (5–15 mg) emerged from the Sephadex G-100 column (145 × 3 cm) as a symmetrical peak, whereas relatively large amounts (50–70 mg) of material appeared as asymmetric peaks with smaller elution volumes. This difference suggests that the protein self-associates at increasing concentration. Further evidence for self-association was obtained by analyses of the major β_2-microglobulin species in the ultracentrifuge. The sedimentation coefficient showed a marked protein concentration dependence with an increasing coefficient at increasing concentration. The concentration dependence appeared linear in the range examined (0.7 to 8 mg/mL) and can be described by the equation $s_{20,w} = 1.605\ S + 0.139c$, where c is the protein concentration in mg per mL. On sedimentation equilibrium analysis the plot of ln c vs. r^2 was curved with increasing slope toward the bottom of the cell.

Other Physicochemical Properties. Some physical properties of the major form of guinea pig β_2-microglobulin are presented in Table III. Physicochemical studies of the minor form were precluded by shortage of materials. All figures in Table III are average values from analyses of at least two different preparations. It is apparent that the molecular weights obtained by chromatography in 6 M guanidine hydrochloride, by polyacrylamide gel electrophoresis in sodium dodecyl sulfate, and by calculations from the sedimentation and diffusion coefficients or from the amino acid composition, are in good agreement and indicate a value of about 11 500. The sedimentation equilibrium data were difficult to interpret because of the tendency of the protein to self-associate (see above). On polyacrylamide gel electrophoresis in sodium dodecyl sulfate, the major and the minor forms seemed to have the same molecular weight.

TABLE III: Physicochemical Properties of the Major Form of Guinea Pig β_2-Microglobulin.

Sedimentation coefficient, $s^0_{20,w}$	1.6 S
Stokes' molecular radius, r_s	1.6 nm
Partial specific volume, $\bar{V}$(calcd)[a]	0.733 mL/g
Apparent diffusion coefficient, $D_{20,w}$	133 $\mu m^2/s$
Molecular weight	
From sedimentation and diffusion coefficients	11 000
By gel chromatography in 6 M Gdn·HCl	11 700
By electrophoresis in sodium dodecyl sulfate	11 500
From amino acid composition	11 544
Frictional ratio	1.12
Absorption coefficient at 280 nm (pH 7.0)	$1.52 \times 10^4\ M^{-1}\ cm^{-1}$
Isoelectric point at 5 °C	6.7
at 20 °C	6.6

[a] From amino acid composition.

The light-absorption spectrum in the ultraviolet range was that of a typical protein. The absorption coefficient at 278 nm and pH 7.0 was estimated to be $1.53 \times 10^4\ M^{-1}\ cm^{-1}$ with a value of 11 500 for the molecular weight. A similar absorption coefficient ($1.66 \times 10^4\ M^{-1}\ cm^{-1}$) was obtained by calculation according to Wetlaufer (1962).

Isoelectric focusing experiments on two preparations gave single, symmetrical peaks with the pI value given in Table III.

Partial Amino Acid Sequence. The amino terminal sequence of the first 18 residues is shown in Figure 7, as well as the methods used for identification of each residue. In preliminary studies Pth-amino acids were detected only weakly at positions 6, 11, 12, and 14. Position 6 was tentatively identified as glutamic acid or glutamine (Cunningham et al., 1977) by the dansyl–Edman technique. Subsequent studies by us and by others (Wolfe & Cebra, 1978) have shown that residue 6 is arginine. We have also been able to provide a more definitive assignment of the arginyl residue at position 12. In all of our experiments, after position 18, identification was ambiguous, probably due to incomplete Edman degradation of the asparaginyl-glycine sequence.

Discussion

β_2-Microglobulin is apparently eliminated from the blood mainly in the kidneys (Bernier & Conrad, 1969), and urine from patients with renal tubular disease has been the best source for the isolation of human β_2-microglobulin (Berggård, 1965; Berggård & Bearn, 1968). We have previously used sodium chromate to induce renal damage and β_2-microglob-

TABLE IV: Comparison of the Amino Acid Composition of Human, Rabbit, Mouse, and Cow, β_2-Microglobulins with that of Guinea Pig β_2-Microglobulin.[a]

Amino acid	Guinea pig β_2-microglobulin	Difference between Human & guinea pig	Rabbit & guinea pig	Mouse & guinea pig	Cow & guinea pig
Asp	13	−1	+2	−3	−2
Thr	3	+2	+1	+4	−1
Ser	9	+1	−3	−2	−1
Glu	10	+1	+1	+1	+2
Pro	7	−2	+1	+1	+2
Gly	3	0	0	+1	0
Ala	4	−2	−2	+1	−3
½-cystine	2	0	0	0	0
Val	9	−2	+1	−4	−4
Met	1	0	0	+3	−1
Ile	5	0	−2	+1	+1
Leu	7	0	0	−3	+1
Tyr	4	+2	+1	0	+2
Phe	5	0	0	−1	−1
Lys	8	0	0	+1	+1
His	5	−1	−1	−1	−1
Arg	3	+2	+1	+1	+2
Trp	2	0	0	0	0
Total	100	0	0	0	−3

[a] Given as deviation from the composition of the major form of guinea pig β_2-microglobulin. Data for the human protein are from Berggård & Bearn (1968), for the rabbit protein from Berggård (1974), for the mouse protein from Natori et al. (1975), and for the cow protein from Groves & Greenberg (1977).

ulinuria in rabbits (Berggård, 1974). In this investigation the same agent was chosen for treatment of guinea pigs and yielded two forms of the protein, differing in isoelectric point and amino acid composition (Table II).

The immunological cross-reactions between rabbit β_2-microglobulin and the two proteins isolated from guinea pig urine indicated that the latter proteins are homologues of β_2-microglobulin. This was established by the finding that the two forms have almost identical amino acid compositions (Table II) and by comparison of the partial amino acid sequence of the major form of the guinea pig protein with corresponding sequences of β_2-microglobulin from other species (Figure 8). The sequence of residues 1–18 of the guinea pig protein resembles closely those of β_2-microglobulins from all other species. As is seen among the other β_2-microglobulins, most variations are seen in residues 1 to 7, whereas residues 8–18 are more conserved.

Comparisons of amino acid compositions of four β_2-microglobulins suggest that the entire primary structures of β_2-microglobulins from various species are similar (Table IV). The β_2-microglobulins from all four animals contain two half-cystinyl residues that could form disulfide-loops similar to those characteristic of human β_2-microglobulin and the immunoglobulin domains (Cunningham et al., 1973). The results of our experiments with starch gel electrophoresis in urea and the apparent absence of free sulfhydryl groups in guinea pig β_2-microglobulin suggest that the guinea pig and human proteins do have analogous disulfide loops.

Guinea pig and human β_2-microglobulins also have very similar physical-chemical properties. The molecular sizes, sedimentation coefficients, molecular weights, and frictional ratios are almost the same for the two proteins, and also for isolated immunoglobulin domains (Karlsson et al., 1972; Karlsson, 1974; Berggård, 1976). The low frictional ratios of these molecules (1.1 to 1.2) indicate that they have compact, globular shapes.

Like its human counterpart (Berggård & Bearn, 1968), guinea pig β_2-microglobulin occurs in free form in serum and

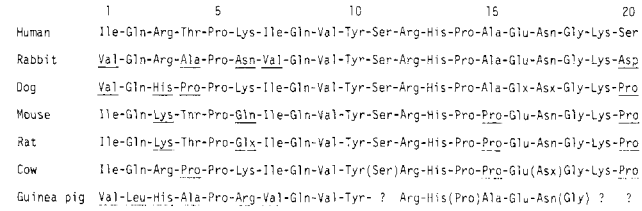

FIGURE 8: Comparison of the NH_2-terminal amino acid sequences of β_2-microglobulins from different species. The data for the human protein are from Cunningham et al. (1973); for the rabbit protein from Cunningham & Berggård (1975); for the dog protein from Smithies & Poulik (1972b); for the mouse protein from Appella et al. (1976); for the rat protein from Uhr et al. (1977); and for the cow protein from Groves & Greenberg (1977) and Becker et al. (1977). Residues differing from the human are underlined.

urine and appears on cell surfaces. Schwartz et al. (1976) have demonstrated that GPLA histocompatibility antigens, solubilized from guinea pig lymphocytes, are 40 000 dalton glycoproteins that are noncovalently associated with a 12 000 dalton molecule. The latter molecule is guinea pig β_2-microglobulin (Schwartz, B. D., Cigén, R., Berggård, I., and Shevach, E. M., in preparation). Solubilized guinea pig I region-associated (Ia) antigens do not contain any subunit of the size of β_2-microglobulin (Schwartz et al., 1976). In agreement with these observations, we have found that β_2-microglobulin is associated with GPLA antigens, but not with Ia antigens, on the surfaces of intact guinea pig lymphocytes (Björck et al., 1977). Analogous results have previously been obtained for the human HLA antigens, the murine H-2 antigens, and the human and murine Ia (or Ia-like) antigens, see Björck et al. (1977).

The histocompatibility antigens with which β_2-microglobulin is associated on cell surfaces are characterized by their extensive genetic polymorphism (Klein, 1975). In contrast, no genetic variant of β_2-microglobulin has been unambiguously identified in any species. Such variants could be particularly valuable because they would allow more definitive determi-

nation of the locations of β_2-microglobulin genes in mammalian chromosomes (Goodfellow et al., 1975) and their possible relationship to the genes for histocompatibility antigens and immunoglobulins. Variant forms of the protein, such as degradation products or polymers of defined size, would also be valuable in that they might provide clues to the function of the protein and its interaction with histocompatibility antigens.

The two forms of guinea pig β_2-microglobulin gave reactions of immunological identity on immunodiffusion analyses, but differed in charge and lysine content. As shown by ion-exchange chromatography and electrophoresis, the net charge of the minor form was more negative than that of the major form in the pH range of 8 to 9 consistent with difference in amino acid composition. Two forms of human β_2-microglobulin have also been detected by their difference in isoelectric point (Hall et al., 1977; Berggård, unpublished observations). These two forms have not been isolated so the nature of the difference is not known. In both species, the minor form of the protein might be synthesized separately or be a degradation product of the major form. Definitive evidence for either of these hypotheses will depend on the isolation of larger amounts of the minor form for peptide mapping studies and amino acid sequence determination.

Some evidence that the minor form may arise by degradation of the major form in the guinea pig is suggested by the fact that the intrachain disulfide bond of the minor form is susceptible to reduction even in the absence of a denaturing agent and hence the minor form may be a modified form of the protein. In contrast, the disulfide bonds of the major form of the protein and of human β_2-microglobulin could be reduced only after denaturation. Although the charge difference between the two β_2-microglobulin species is consistent with our finding of 7 lysyl residues in preparations of the minor species vs. 8 in the major, we cannot rigorously exclude the possibility that the difference in lysyl residues is due to an impurity that is copurified with one or the other of the forms. If the difference in lysyl residues is due to an impurity, the minor β_2-microglobulin species may be derived from the major species by deamidation of labile glutaminyl or asparaginyl residues. Modification of proteins in vivo by loss of amide groups is a well-known phenomenon (Flatmark & Sletten, 1968).

The domains of different immunoglobulin chains occur in pairs (Edelman, 1970). As human β_2-microglobulin is homologous to such domains (Peterson et al., 1972; Cunningham et al., 1973) and apparently is a subunit of the HLA histocompatibility antigens (Nakamuro et al., 1973; Grey et al., 1973; Peterson et al., 1974), it may be bound to a similar domain in the larger subunit of these antigens. Some fragments corresponding to single domains of immunoglobulin chains appear in solution as monomers (Karlsson et al., 1972), whereas other such fragments appear as dimers (Epp et al., 1974). Human β_2-microglobulin occurs in monomeric form at pH 8 (Berggård & Bearn, 1968). At the same pH, the major form of guinea pig β_2-microglobulin was found to self-associate when its concentration was increased. This difference in the behavior of the human and guinea pig proteins could, at least partly, be due to the fact that the self-association was recorded at pH 8, which is closer to the isoelectric point of guinea pig protein (6.7) than to the isoelectric point of the human protein (5.7) (Hall et al., 1977). The self-association of the guinea pig protein might be promoted by the β_2-microglobulin region that, on the cell surface, interacts with a region on the larger subunit of histocompatibility antigens.

A correlation may exist between the tendencies of a β_2-microglobulin to self-associate and to crystallize. Bovine β_2-microglobulin (Groves & Greenberg, 1977) was originally reported as a crystalline, 43 000 dalton molecule (Groves et al., 1963) isolated from milk and colostrum. Studies of the molecule in dilute solution and in the crystal indicated that the predominant form was the monomer (Becker et al., 1977). The protein, however, did aggregate at higher concentrations, suggesting that the aggregation may reflect the ability of the molecule to crystallize.

Acknowledgment

We thank Ms. Boel Wieslander and Ms. Britt-Marie Palmblad for excellent technical assistance and Dr. Håkan Pertoft for performing the ultracentrifugations.

References

Appella, E., Tanigaki, N., Natori, T., & Pressman, D. (1976) *Biochem. Biophys. Res. Commun. 70,* 425.

Becker, J. W., Ziffer, J. A., Edelman, G. M., & Cunningham, B. A. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74,* 3345.

Berggård, I. (1962) *Ark. Kemi 18,* 291.

Berggård, I. (1965) in *Protides of the Biological Fluids,* Proceedings of the 12th Colloquium, Bruges, 1964 (Peeters, H., Ed.) p 285, Elsevier, Amsterdam.

Berggård, I. (1974) *Biochem. Biophys. Res. Commun. 57,* 1159.

Berggård, I. (1976) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 35,* 1167.

Berggård, I., & Bearn, A. G. (1968) *J. Biol. Chem. 243,* 4095.

Bernier, G. M., & Conrad, M. E. (1969) *Am. J. Physiol. 217,* 1359.

Björck, L., Cigén, R., Berggård, B., Löw, B., & Berggård, I. (1977) *Scand. J. Immunol. 6,* 1063.

Cohn, E. J., & Edsall, J. T., Ed. (1943) *Proteins, Amino Acids and Peptides,* p 370, Reinhold, New York, N.Y.

Cunningham, B. A. (1976) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 35,* 1171.

Cunningham, B. A., & Berggård, I. (1975) *Science 187,* 1079.

Cunningham, B. A., Wang, J. L., Berggård, I., & Peterson, P. A. (1973) *Biochemistry 12,* 4811.

Cunningham, B. A., Henning, R., Milner, R. J., Reske, K., Ziffer, J. A., & Edelman, G. M. (1977) *Cold Spring Harbor Symp. Quant. Biol. 41,* 351.

Edelhoch, H. (1967) *Biochemistry 6,* 1948.

Edelman, G. M. (1970) *Biochemistry 9,* 3197.

Edman, P., & Begg, G. (1967) *Eur. J. Biochem. 1,* 80.

Epp, O., Colman, P., Fehlhammer, H., Bode, W., Schiffer, M., & Huber, R. (1974) *Eur. J. Biochem. 45,* 513.

Flatmark, T., & Sletten, K. (1968) *J. Biol. Chem. 243,* 1623.

Goodfellow, P. N., Jones, E. A., van Heyningen, V., Solomon, E., Bobrow, M., Miggiano, V., & Bodmer, W. F. (1975) *Nature (London) 254,* 267.

Gosting, L. J. (1956), *Adv. Protein Chem. 11,* 449.

Gottlieb, P. D., Cunningham, B. A., Rutishauser, U., & Edelman, G. M. (1970) *Biochemistry 9,* 3155.

Grey, H. M., Kubo, R. T., Colon, S. M., Poulik, M. D., Cresswell, P., Springer, T., Turner, M., & Strominger, J. L. (1973) *J. Exp. Med. 138,* 1608.

Groves, M. L., & Greenberg, R. (1977) *Biochem. Biophys. Res. Commun. 77,* 320.

Groves, M. L., Basch, J. J., & Gordon, W. G. (1963) *Biochemistry 2,* 814.

Hall, P. W., Ricanati, E. S., & Vacca, C. V. (1977) *Clin.*

Chim. Acta 77, 37.
Hirs, C. H. W. (1967) *Methods Enzymol. 11,* 59.
Johansson, B. G. (1972) *Scand. J. Clin. Lab. Invest. 29, Suppl. 124,* 7.
Karlsson, F. A. (1974) *Immunochemistry 11,* 111.
Karlsson, F. A., Peterson, P. A., & Berggård, I. (1972) *J. Biol. Chem. 247,* 1065.
Klein, J. (1975) *Biology of the Mouse Histocompatibility-2 Complex,* Springer-Verlag, New York, N.Y.
Laurent, T. C., & Killander, J. (1964) *J. Chromatogr. 14,* 317.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193,* 265.
Mann, K. G., & Fisch, W. W. (1972) *Methods Enzymol. 26,* 28.
Michaelson, J., Flaherty, L., Vitetta, E., & Poulik, M. D. (1977) *J. Exp. Med. 145,* 1066.
Nakamuro, K., Tanigaki, N., & Pressman, D. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70,* 2863.
Natori, T., Tanigaki, N., Appella, E., & Pressman, D. (1975) *Biochem. Biophys. Res. Commun. 65,* 611.
Neville, D. M. (1971) *J. Biol. Chem. 246,* 6328.
Nilsson, K., Evrin, P. E., Berggård, I., & Pontén, J. (1973) *Nature (London) 244,* 44.
Östberg, L., Rask, L., Wigzell, H., & Peterson, P. A. (1975) *Nature (London) 253,* 735.
Peterson, P. A., & Berggård, I. (1971) *J. Biol. Chem. 246,* 25.
Peterson, P. A., Cunningham, B. A., Berggård, I., & Edelman, G. M. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69,* 1697.
Peterson, P. A., Rask, L., & Lindblom, J. B. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71,* 35.
Poulik, M. D. (1973) *Immunol. Commun. 2,* 403.
Poulik, M. D., Shinnick, E. S., & Smithies, O. (1975) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 34,* 945 (Abstract).
Rask, L., Lindblom, J. B., & Peterson, P. A. (1974) *Nature (London) 249,* 833.
Schachman, H. K. (1957) *Methods Enzymol. 4,* 32.
Schwartz, B. D., Kask, A. M., Paul, W. E., & Shevach, E. M. (1976) *J. Exp. Med. 143,* 541.
Silver, J., & Hood, L. (1974) *Nature (London) 249,* 764.
Smithies, O., & Poulik, M. D. (1972a) *Science 175,* 187.
Smithies, O., & Poulik, M. D. (1972b) *Proc. Natl. Acad. Sci. U.S.A. 69,* 2914.
Summers, M. R., Smythers, G. W., & Oroszlan, S. (1973) *Anal. Biochem. 53,* 624.
Svedberg, T., & Pedersen, K. O. (1940), *Die Ultrazentrifuge,* p 35, Leipzig, Verlag von Theodor Steinkopff.
Uhr, J. W., Vitetta, E. S., Klein, J., Poulik, M. D., Klapper, D. G., & Capra, J. D. (1977) *Cold Spring Harbor Symp. Quant. Biol. 41,* 363.
Vesterberg, O. (1971) *Methods Enzymol. 22,* 389.
Vitetta, E. S., Uhr, J. W., & Boyse, E. A. (1975) *J. Immunol. 114,* 252.
von Hofsten, B., van Kley, H., & Eaker, D. (1965) *Biochim. Biophys. Acta 110,* 585.
Waxdal, M. J., Konigsberg, W. H., Henley, W. L., & Edelman, G. M. (1968) *Biochemistry 7,* 1959.
Weiner, A. M., Platt, T., & Weber, K. (1972) *J. Biol. Chem. 247,* 3242.
Wetlaufer, D. B. (1962) *Adv. Protein Chem. 17,* 303.
Winkler, M. A., & Sanders, B. G. (1977) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 36,* 1199 (Abstract).
Wolfe, P. B., & Cebra, J. J. (1978) Third International Congress of Immunology (in press).
Yamada, S., & Itano, H. A. (1966) *Biochim. Biophys. Acta 130,* 538.
Yphantis, D. A. (1964) *Biochemistry 3,* 297.