BIP: A LOW MOLECULAR WEIGHT PROTEIN COISOLATED WITH BETA-2 MICROGLOBULIN

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SUMMARY

A protein (bip) with molecular weight 11,000 and 6-8 half cysteine residues was coisolated from a rabbit's urine along with beta-2 microglobulin. Compositional and N-terminal sequence analyses of the separated proteins revealed no similarities between beta-2 microglobulin and bip. Furthermore, bip shows no significant sequence homology to any protein for which data are currently available.

INTRODUCTION

Low molecular weight serum proteins are filtered through the renal glomeruli, and under normal conditions are reabsorbed by the tubules. Disturbance of renal tubular function induces proteinuria which has in experimental situations provided an enriched source of low molecular weight proteins. Prominent among the examples of biologically significant proteins first isolated from urine are Bence-Jones proteins (1) and beta-2 microglobulin (2).

The present report describes a protein of molecular weight 11,000 that was fortuitously coisolated with $\beta_2\mu$ from the urine of a single rabbit. The protein, which we call bip (*b*eta-2 microglobulin co*i*solated *p*rotein), was detected by amino acid sequence analysis of a preparation thought to contain pure $\beta_2\mu$. Bip was not detected during preliminary isolation steps because it has no absorbance at 280 nm, it does not stain with Coomassie blue or amido black, and it cannot be radioiodinated in its undenatured state. Partial characterization of this protein, and comparison of its properties to other described proteins provide no clues as to its origin.

Abbreviations: $\beta_2\mu$, beta-2 microglobulin

MATERIALS AND METHODS

Bip and $\beta_2\mu$ were isolated from rabbit urine by methods similar to those previously described (3,4). Rabbit 4555 was given a single subcutaneous injection of 70 mg of sodium chromate, and urine was collected from the third through the 14th day following the injection. The pooled sample was centrifuged at 5,000 x g for 15 min, dialyzed against running tap water in Spectrapor 1 dialysis tubing (Spectrum Medical Industries, Los Angeles), and lyophilized. The lyophilized material was chromatographed on Sephadex G-75 as described previously (4). After recycling through the same column, fractions of the appropriate molecular size were next subjected to three stages of anion exchange chromatography on DEAE-cellulose at pHs 7.8, 6.8 and 7.2 in 0.01 M Tris-HCl buffer. At each stage, a linear gradient from 0 to 0.2 M NaCl was used for protein elution. Separation of $\beta_2\mu$ from bip was carried out by chromatography on Sephadex G-50 in 0.1 M Tris-HCl buffer, pH 8 with 5 M guanidine-HCl following complete reduction and radioacylation of the protein mixture. Proteins were radioiodinated by the ICl method (5). Radioimmune binding and inhibition of binding assays were described previously (4).

Carbohydrate was determined by the phenol-sulfuric acid method, using a glucose reference standard (6).

Amino acid analyses were performed on a Durrum D500 analyzer on samples hydrolyzed by the procedure of Moore and Stein (7).

Sequence analysis was carried out with the Beckman 890B sequencer using program no. 111374. Phenylthiohydantoin derivatives of amino acids were identified by gas chromatography (8), by thin layer chromatography on polyamide sheets (9), and in some cases, by amino acid analysis following acid hydrolysis of the derivative to the free amino acid. Cysteine was identified as [^{14}C]-carboxamidomethyl cysteine

RESULTS

Urine collected from rabbit 4555 yielded 10 g of nondialyzable material. This was divided into 10 aliquots for chromatography on Sephadex G-75. The fractions of appropriate molecular size were pooled, concentrated through an Amicon UM2 membrane, and twice rechromatographed on the same column. The material obtained in this peak, 320 units of absorbance at 280 nm, was concentrated, taken up in 0.01 M Tris-HCl, pH 7.8, and chromatographed on DEAE-cellulose in this buffer. Fractions containing $\beta_2\mu$ were further purified on DEAE-cellulose in 0.01 M Tris-HCl buffer at pH 6.8 and then at pH 8.2, using identical NaCl gradients.

The resulting material, containing 21 units of absorbance at 280 nm, gave a single stained band on SDS and alkaline polyacrylamide gel electrophoresis (10). The protein concentration of the isolate was estimated by absorbance at 280 nm. On the basis of this estimate, it inhibited the binding of [^{125}I]-pooled $\beta_2\mu$ to goat anti-rabbit $\beta_2\mu$ to the same extent as a rabbit $\beta_2\mu$ sample which was isolated from pooled rabbit urine by the same procedure and which was demonstrated to be pure by automated amino acid sequence analysis. Fur-

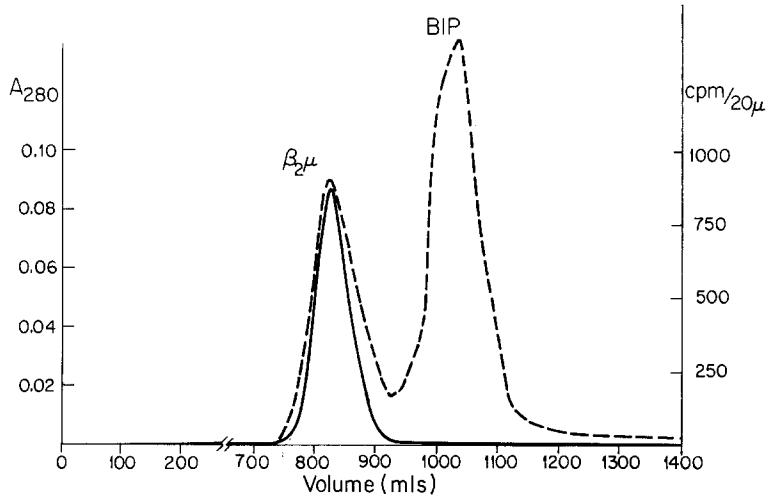

Figure 1: Elution profile of completely reduced and radioalkylated 4555 isolate. Gel chromatography was carried out in a column (3 cm x 135 cm) of Sephadex G-50 equilibrated with 0.1 Tris-HCl buffer, pH 8.0, with 5 M guanidine hydrochloride. Solid line indicates A_{280}. Broken line indicates cpm/20 μl.

N-TERMINAL SEQUENCE OF BIP AND BETA-2 MICROGLOBULIN

								5								10								
BIP	X	–	PRO	–	VAL	–	GLN	–	VAL	–	MET	–	CYS	–	PRO	–	GLU	–	PRO	–	SER	–	SER	–	SER	–
$\beta_2\mu$	VAL	–	GLN	–	ARG	–	ALA	–	PRO	–	ASN	–	VAL	–	GLN	–	VAL	–	TYR	–	SER	–	ARG	–	HIS	–
								15								20								25		
BIP	GLX	–	GLU	–	THR	–	LEU	–	CYS	–	LEU	–	SER	–	ASP	–	ASN	–	ASX	–	CYS	–	LEU	–	GLY	–
$\beta_2\mu$	PRO	–	ALA	–	GLU	–	ASN	–	GLY	–	LYS	–	PRO	–	ASN	–	PHE	–	LEU	–	ASN	–	CYS	–	TYR	

Figure 2: Amino terminal sequences of bip and $\beta_2\mu$.

thermore, when material isolated from rabbit 4555 was radioiodinated, its binding to goat anti-$\beta_2\mu$ was inhibited equally well by the pooled $\beta_2\mu$ and the unlabeled 4555 isolate. By all these criteria, it was assumed that a pure sample of $\beta_2\mu$ had been obtained.

The fallacy of this assumption was immediately evident upon examination of the results obtained by automated sequence analysis. At each step in the Edman degradation two residues were detected in approximately equal amounts.

The 4555 isolate was next completely reduced and carboxamidomethylated with [^{14}C]-iodoacetamide (New England Nuclear, Boston). Chromatography on Sephadex G-50 in the presence of 5 M guanidine-HCl (Fig. 1) gave two peaks of [^{14}C] activity, but only the first had optical density at 280 nm. The material from each peak was subjected to automated sequence analysis. The fist peak was shown to be identical to rabbit $\beta_{2\mu}$ (11); the second was designated bip. The amino terminal sequences of bip and $\beta_{2\mu}$ are shown in Fig. 2 and their compositions are presented in Table I. As estimated from chromatographic and

TABLE I

AMINO ACID COMPOSITIONS OF BIP AND $\beta_{2\mu}$

Amino acid	Bip	$\beta_{2\mu}$
Cys[a]	6.6	1.6
Asp	10.0	13.4
Thr	7.8	4.4
Ser	16.4	6.2
Glu	15.0	10.6
Pro	7.7	9.7
Gly	12.8	5.2
Ala	8.8	3.6
Val	6.5	10.6
Met	1.7	1.0
Ile	3.6	3.2
Leu	5.9	7.2
Try	0.9	6.4
Phe	1.6	4.8
His	2.1	3.1
Lys	2.0	6.2
Arg	1.9	4.3
Trp[b]	0	2.0

[a] Determined as S-carboxymethyl cysteine

[b] Not determined

compositional data, the approximate length and molecular weight of bip are 111 amino acids, and 11,000 daltons, respectively. The presence of 6 to 8 half cysteine residues in 111 residues suggest a compact structure for bip. Bip contains about 5% carbohdyrate by weight; it has previously been determined that $\beta_2\mu$ contains no carbohydrate (2).

In an attempt to identify bip, the amino terminal sequence was compared to protein sequences in the data collection of the National Biomedical Research Foundation. No obvious homologue was found. By a unitary matrix method, which only scores for identity of residues, 12 protein fragments were 32% homologous (8 out of 25 residues) to the amino terminus of bip. These are listed in Table II. When the computer search was carried out using a program weighted

TABLE II

PROTEINS SHOWING HIGHEST SEQUENCE HOMOLOGY (32%) TO BIP N-TERMINAL FRAGMENT[a]

Protein	Species	Residues
Ferredoxin	Taro	1 - 26
Prophospholipase A2	Pig	74 - 99
Lactogen	Human	52 - 77
Growth hormone	Human	52 - 77
IgG kappa chain	Human	14 - 39
IgG kappa chain	Rabbit	11 - 36
IgG kappa chain	Rabbit	59 - 84
IgG gamma-1 chain	Human	344 - 369
IgG gamma-1 chain	Human	346 - 371
IgG gamma-4 chain	Human	225 - 250
Keratin	Sheep	2 - 27
Keratin	Sheep	59 - 84

[a] Based on direct comparison of residues

for single base changes, charge preservation, as well as other factors (12), the results were similar, with the exception that high scores were also achieved by several snake neurotoxin fragments. The amino terminal sequence of bip has also been compared to the published amino acid sequences of murine and human histocompatability antigens (13,14), and it shows no obvious homology to these proteins.

DISCUSSION

Bip is an unusual protein in that it is undetected by many of the criteria used for protein identification. It does not absorb light at 280 nm; it has a single tyrosine which is not accessible for [^{125}I]-labeling under nondenaturing conditions; in polyacrylamide gels it does not stain with Coomassie blue or Amido black. Thus, in our routine immunological and electrophoretic procedures, bip was not detected. Its presence became apparent only upon amino acid sequence analysis.

The origin and function of bip are thus not yet known. Its coisolation with $\beta_2\mu$ is probably due to similarities of the physicochemical characteristics of the two molecules rather than to a specific interaction, because during the initial steps of the isolation, both eluted from Sephadex G-75 at the position expected for free $\beta_2\mu$; a physical association would have doubled the apparent molecular size. It is also not known whether bip is an intact protein or a fragment of a larger protein. Immunizations are now in progress to prepare antisera against bip to use in experiments to define this unusual protein.

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