PARTIAL AMINO ACID SEQUENCE OF MOUSE β_2-MICROGLOBULIN

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SUMMARY: A highly purified preparation of mouse β_2-microglobulin has been obtained from sodium thiocyanate extracts of liver cell membranes of A/J strain mice and the amino acid sequence has been partially determined. The first 40 residues have been assigned except for position 34. In the sequence, the mouse protein differs only at 9 positions from human β_2-microglobulin at 8 positions from the dog homologue and at 11 positions from the rabbit homologue. The sequence has also a homology to the constant regions of mouse γG_{2a}, most closely to the C_H3 region. These data support the conclusion that the mouse protein is indeed the mouse homologue of human β_2-microglobulin.

Human β_2-microglobulin is a simple protein of a molecular weight 11,800 and is present in various body fluids and on the cell surface of various cell types as well (1). It was first isolated from urine of patients with kidney malfunction (2). The amino acid sequence has been totally determined and the homology to the constant regions of IgG heavy chain has been pointed out (3). Recent work has demonstrated that human β_2-microglobulin is associated with human HLA histocompatibility antigens (4). The major histocompatibility antigens in other species have been found to contain a human β_2-microglobulin homologue. The homologues in dog and rabbit have been isolated and the partial amino acid sequences have been reported (5,6).

A mouse protein that appears to be the mouse homologue of human β_2-microglobulin in molecular weight, amino acid composition and antigenicity has been purified from cell membranes of spleen and liver of A/J strain mice (7,8). In this communication, we report the partial amino acid sequence of this mouse protein and show it to be similar to human β_2-microglobulin and the homologues in dog and rabbit.

MATERIALS AND METHODS: The mouse β_2-microglobulin was extracted from liver cell membranes of A/J strain mice by sodium thiocyanate (NaSCN) and purified

by gel filtration, ion-exchange chromatography and column electrophoresis as described previously (5). NH_2-terminal amino acid was determined by the dansyl-chloride method according to Gray (9) in the presence of 2% sodium dodecyl sulphate. Automatic sequence analysis of the native protein (1.3 mg) was performed by using dimethylallylamine buffer in the Beckman Model 890 B Sequencer with program 111314 (Beckman Instruments, Palo Alto, Ca.). PTH-amino acids were identified qualitatively by thin-layer chromatography on polyamide layers (10). Quantitative identification was made by gas chromatography on columns of 2% OV-275 in a nuclear Chicago gas chromatograph (11) and by hydrolysis with HI (12).

RESULTS AND DISCUSSION: The amino terminal residue was found to be Ile by the danysl method. Table 1 shows the amounts of the amino acids recovered, in nanomoles, after HI hydrolysis of the phenylthiohydantions obtained by degradation of the purified mouse β_2-microglobulin. These data in conjunction with data obtained from gas-chromatography and thin layer chromatography have permitted the sequence determination of 39 of the first 40 residues. No residue was recovered at position 34. The assignment of cysteine at position 25 is at the moment based on the homology with human β_2-microglobulin. After step 30, yields were low and the identification of PTH-amino acids depended upon qualitative assessment. The partial sequence obtained is compared with the human β_2-microglobulin and the rabbit and dog homologue (Fig. 1). Of the 39 residues compared, there are only nine differences from the human protein, eight differences from the dog homologue, and 11 differences from the rabbit homologue. When aligned for maximum homology, the mouse homologue of β_2-microglobulin appears to more closely resemble the C_H3 region of IgG than any of the other homology regions. The extent of homology between the NH_2-terminal portion of mouse β_2-microglobulin and the homology regions of mouse IgG is similar to that found between the corresponding portions of human and rabbit β_2-microglobulins and the homology regions of human and rabbit IgG (Table 2). The data support the conclusion that the mouse protein is the mouse homologue of human β_2-micro-

Table 1

Automated Amino Terminal

Degradation of Mouse β_2-Microglobulin

Cycle	Residue	Yield[a]	Cycle	Residue	Yield[a]
1	Ile	57.1	26	Tyr	4.0
2	Glu	53.2	27	Val	8.2
3	Lys	43.2	28	Thr	5.8
4	Thr	45.9	29	Glu	4.4
5	Pro	44.5	30	Phe	3.0
6	Glu	40.8			
7	Ile	36.6			
8	Glu	33.0			
9	Val	31.9			
10	Tyr	21.0			
11	Ser	8.3			
12	Arg	c			
13	His	b			
14	Pro	27.2			
15	Pro	26.0			
16	Glu	17.3			
17	Asp	9.6			
18	Gly	12.9			
19	Lys	6.7			
20	Pro	9.5			
21	Asp	6.4			
22	Ile	8.0			
23	Leu	6.5			
24	Asp	5.0			
25	Cys	-			

[a]The yields (nmoles) for the major amino acid phenylthiohydantoins identified at each cycle were determined by HI hydrolysis and were not corrected for either overlap or background.

[b]The phenylthiohydantoin derivative of histidine was identified by the Pauly reaction.

[c]The phenylthiohydantoin derivative of arginine was identified by the phenanthrenequinone reaction.

	1	2	3	4	5	6	7	8	9	10
Mouse	Ile	Gln	Lys	Thr	Pro	Gln	Ile	Gln	Val	Tyr
Human	Ile	Gln	Arg	Thr	Pro	Lys	Ile	Gln	Val	Tyr
Dog	Val	Gln	His	Pro	Pro	Lys	Ile	Gln	Val	Tyr
Rabbit	Val	Gln	Arg	Ala	Pro	Asn	Val	Gln	Val	Tyr
	11	12	13	14	15	16	17	18	19	20
Mouse	Ser	Arg	His	Pro	Pro	Glu	Asn	Gly	Lys	Pro
Human	Ser	Arg	His	Pro	Ala	Glu	Asn	Gly	Lys	Ser
Dog	Ser	Arg	His	Pro	Ala	Glu	Asn	Gly	Lys	Pro
Rabbit	Ser	Arg	His	Pro	Ala	Glu	Asn	Gly	Lys	Asp
	21	22	23	24	25	26	27	28	29	30
Mouse	Asn	Ile	Leu	Asn	Cyr	Tyr	Val	Thr	Glu	Phe
Human	Asn	Phe	Leu	Asn	Cys	Tyr	Val	Ser	Gly	Phe
Dog	Asn	Phe	Leu	Asn	Cys	Tyr	Val	Ser	Gly	Phe
Rabbit	Asn	Phe	Leu	Asn	Cys	Tyr	Val	Ser	Gly	Phe
	31	32	33	34	35	36	37	38	39	40
Mouse	His	Pro	Pro	?	Ile	Glx	Ile	Asx	Leu	Leu
Human	His	Pro	Ser	Asx	Ile	Glx	Val	Asx	Leu	Leu
Dog	His	Pro	?	Glx	Ile	Glx	Ile	Asx	Leu	Leu
Rabbit	His	Pro	Ser	Asp	Ile					

Figure 1 - Comparison of the amino acid sequences of mouse, human, dog and rabbit β_2-microglobulin. The data for the human protein are from Smithies and Poulik (21) and from Peterson et al. (3); for the dog protein from Smithies and Poulik (13); for the rabbit protein from Cunningham and Beggard (6).

Table 2

Identical Residues in the Sequences of

Mouse β_2-Microglobulin and Mouse γG_{2a} [1]

	β_2m	C_H1	C_H2	C_H3	C_L
β_2m	-				
C_H1	11	-			
C_H2	5	8	-		
C_H3	13	13	10	-	
C_L	7	8	10	15	-

[1] Residues used for comparison have been taken from the sequence of mouse γG_{2a} 173 (22).

globulin and indicate that all the four proteins from human, dog, rabbit and mouse are closely related.

Since β_2-microglobulin was found to have a homology to immunoglobulin domains and to be a fundamental component of the major histocompatibility antigens, the genetic and immunobiological significance have been the major problem to be elucidated.

Recent work in mouse has revealed that β_2-microglobulin or a β_2-microglobulin-like substance is present associated with TL antigens (13,14) and Ss protein (15) both coded for by the major histocompatibility complex and with F_9 antigen (16) coded for by the T/t complex, a possible embryonic equivalent of the major histocompatibility complex. Furthermore, Allogeneic Effect Factor, a soluble immune mediator, has been reported to contain β_2-microglobulin determinants (17). In humans, there is a report that anti-human β_2-microglobulin antiserum blocks the capacity of a T-cell factor to stimulate antibody production in in vitro culture (18). Thus, β_2-microglobulin or the analogous substances appear to have an important role in the mechanism of cell to cell interaction.

We have found that Ss protein and F_9 antigen do not react with rabbit antiserum raised against our preparation of mouse β_2-microglobulin that binds well to the 12,000-dalton component of H-2 antigens, i.e. mouse β_2-microglobulin (19,20). Therefore, the small subunits reported for these substances are not identical to mouse β_2-microglobulin. However, they may perhaps still be very similar to mouse β_2-microglobulin in function and structure. Isolation of a 12,000 dalton subunit from TL antigen, Ss protein, F_9 antigen and T-cell factors when accompanied by the analysis of chemical structure may shed light on the evolutional relationship and biological significance of the small subunits found in various immunologically important substances.

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