AMINO ACID COMPOSITION AND PHYSICOCHEMICAL PROPERTIES OF MOUSE β_2-MICROGLOBULIN

Natori, T., Tanigaki, N., Appella, E. and Pressman, D.

Department of Immunology Research, Roswell Park Memorial Institute, Buffalo, New York 14263, Laboratory of Cell Biology, National Cancer Institute, Bethesda, Maryland 20014.

Received June 2,1975

SUMMARY: Highly purified preparations of an 11,000-dalton substance with a pI value of 7.5 have been obtained from 3 M sodium thiocyanate extracts of spleen and liver cell membranes of A/J strain mouse. This substance appears to be the mouse homologue of human β_2-microglobulin since a) it has a homology in amino acid composition, including the presence of two-half cystine residues, to constant domains of mouse immunoglobulins as well as to human β_2-microglobulin, b) it is not distinguishable antigenically from the 11,000-dalton component isolated from H-2 antigen molecules and c) it has a weak but significant cross-reactivity with human β_2-microglobulin.

Previously, we isolated an 11,000-dalton substance from mouse liver cell membranes by extraction with sodium thiocyanate and showed it to be very similar antigenically to the 11,000-dalton component of non-ionic detergent solubilized mouse H-2 antigens (1). It possessed a weak but significant cross-reactivity with human β_2-microglobulin and appeared to be the mouse homologue of human β_2-microglobulin. In the present work, we purified this substance from cell membranes of spleen and liver and determined the amino acid composition and the degree of homology with mouse immunoglobulins and human β_2-microglobulin. Several physicochemical parameters, such as electrophoretic mobility, isoelectric point and molecular size were also determined and compared with those of human β_2-microglobulin.

MATERIALS AND METHODS: Crude membrane materials that were used as source materials for isolation of mouse β_2-microglobulin were prepared from spleen and liver of A/J strain mouse by the methods of Davies (2) and of Schneider and Hogeboom (3), respectively. The mouse β_2-microglobulin was extracted

directly from the membrane materials by sodium thiocyanate (NaSCN) and purified by gel filtration, ion-exchange chromatography and column electrophoresis as described previously (1). The yield in the total number of A_{280} units was 0.75 in the isolation experiment that started with 150 g of spleen and 1.91 and 1.41 in the two isolation experiments that each started with 450 g of liver. The yields represented 50%, 35% and 30%, respectively, of mouse β_2-microglobulin in the 3 M NaSCN extracts as estimated by the antigenic activity.

For the determination of the amino acid composition, the samples were hydrolyzed in vacuo in 6 N HCl at 105 C for 24, 48 and 72 hrs. Two analysis of each hydrolysate were performed on a Durrum D500 amino acid analyzer. The total half-cystine content was determined as S-carboxymethylcysteine after fully reduction and alkylation. Samples were evacuated to less than 10 μm of Hg and hydrolyzed for 24, 72 and 96 hrs. The tryptophan content was determined by hydrolysis with 3 M mercaptoethanesulfonic acid in vacuo at 105 C for 24 hrs.

RESULTS: Three preparations of mouse β_2-microglobulin, one from spleen cell membranes and the others from liver cell membranes were subjected to analytical gel electrophoresis (4,5) and gel isoelectrofocusing (6) to determine the purity as well as the physicochemical parameters. The staining patterns are shown in Fig. 1 for one of the mouse preparations along with a human β_2-microglobulin preparation isolated from spent culture media of a human lymphoid cell line (7). The mouse β_2-microglobulin preparations had been shown to have a γ_1-globulin mobility on a column of Bio-Gel P-2 at pH 8.6 during the isolation experiments (1).

When subjected to electrophoresis on 7% acrylamide gel a pH 9.5, all the mouse preparations showed a single band of the same mobility. The Rf value was 0.28. The human preparation gave a value of 0.48. Upon electrophoresis on 10% acrylamide gel in presence of 0.1% SDS and 0.5 M urea, the mouse preparations showed also a single band which corresponds in migra-

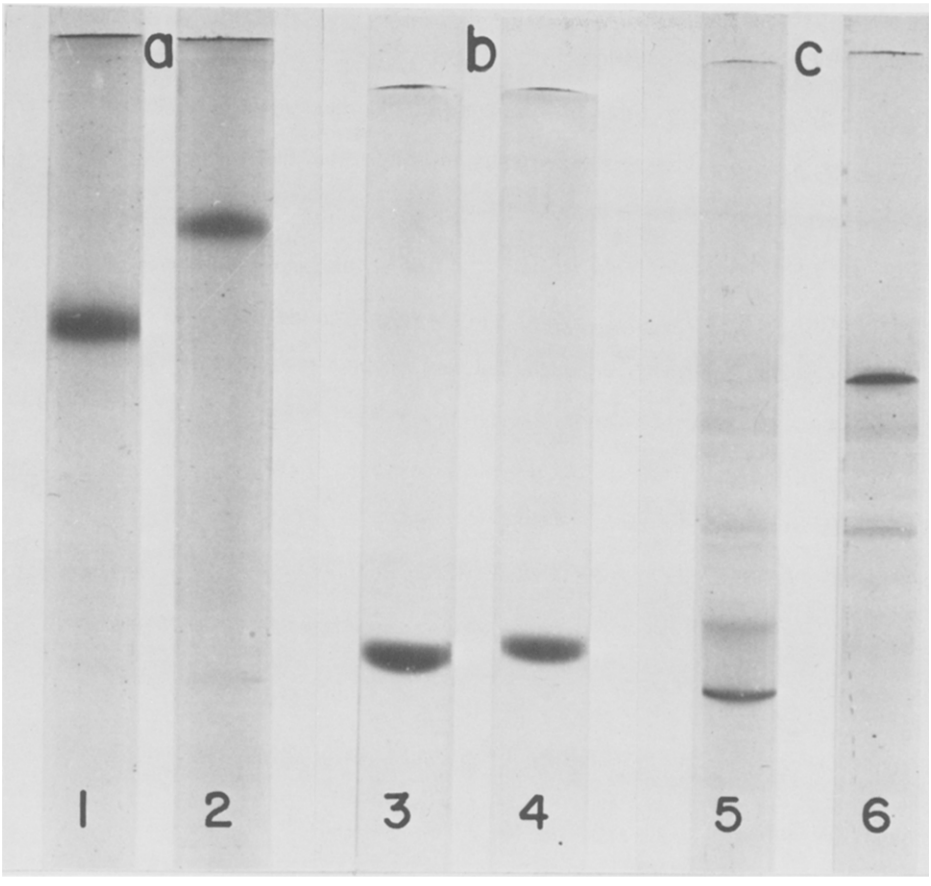

Fig. 1. Staining patterns of a preparation of mouse β_2-microglobulin on gel electrophoresis and gel isoelectric focusing. A preparation of mouse β_2-microglobulin from liver was subjected to (a) acrylamide gel electrophoresis on 7% gel at pH 9.5, (b) sodium dodecyl sulfate-acrylamide gel electrophoresis on 10% gel containing 0.1% SDS and 0.5 M urea and (c) acrylamide gel isoelectric focusing in a pH range of 3.5 to 10 on 10% gel containing 6 M urea. The amount applied was about 10 μg, 5 μg and 26 μg, respectively. (A value of 14.5 that was calculated from the amino acid composition was used as $E^{1\%}_{280}$.) Coomassie blue was used for staining. The patterns are shown in tubes 2, 4 and 6. A preparation of human β_2-microglobulin was examined in parallel runs. The patterns are shown in tubes 1, 3 and 5. In each case, the anode is at the bottom.

tion distance to a molecular size of 11,000 to 12,000 daltons. Isoelectrofocusing on 7.5% acrylamide gel in a pH range of 3.5 to 10 gave a single sharp band for all the mouse preparations. The band was located at higher pH range on the gel than that of human β_2-microglobulin. In order to determine the isoelectric point gels in a separate run were divided into 33

fractions by an autogeldivider. The fractions were assayed for the pH and for the β_2-microglobulin activity by radioimmunoassay (1,7). The pI value obtained was 7.5 for the mouse preparations and 6.1 for the human preparation.

These analytical methods did not indicate any differences in molecular size or ionic charge among the mouse preparations, but did reveal a clear difference in ionic charge between the mouse preparations and the human preparations of mouse β_2-microglobulin isolated by the current method.

The amino acid composition of mouse β_2-microglobulin is given in Table 1 with that of human β_2-microglobulin. Two different preparations of mouse β_2-microglobulin, one from spleen and another from liver were assayed.

Table 1

Amino Acid Composition of Mouse β_2-Microglobulin

Amino acid	Mouse β_2-microglobulin[a]	Human β_2-microglobulin[b]	Difference
	residues/mol		
Aspartic acid	10.3 (10)	12	-2
Threonine	7.1 (7)	5	+2
Serine	6.8 (7)	10	-3
Glutamic acid	11.1 (11)	11	0
Proline	8.1 (8)	5	+3
Glycine	4.5 (4)	3	+1
Alanine	4.7 (5)	2	+3
Half-cystine	1.6 (2)	2	0
Valine	5.2 (5)	7	-2
Methionine	3.8 (4)	1	+3
Isoleucine	5.9 (6)	5	+1
Leucine	4.4 (4)	7	-3
Tyrosine	4.1 (4)	6	-2
Phenylalanine	3.7 (4)	5	-1
Lysine	9.1 (9)	8	+1
Histidine	4.3 (4)	4	0
Arginine	3.5 (4)	5	-1
Tryptophan	2.0 (2)	2	0

[a] The figures for all amino acids are average values from analysis of two different preparations, one from spleen and another from liver. The figures in parenthesis are the nearest integer values.

[b] Data from Berggård and Bearn (13).

The assay did not reveal significant difference in the mouse preparations. The figures shown in Table 1 are the average values obtained from analysis of the two preparations. The comparison with human β_2-microglobulin revealed compositional differences involving 1, 2 or 3 residues of the particular amino acid affected. Such differences were seen in 14 amino acids. The data showed two half-cystine residues per mole which suggests that mouse β_2-microglobulin has one intrachain disulfide bond, possibly of the same position as human β_2-microglobulin (8).

DISCUSSION: The isolated mouse substance has a homology in amino acid composition, including the presence of two half-cystine residues, to constant domains of mouse immunoglobulins as well as to human β_2-microglobulin. When the amino acid composition of the mouse substance is compared with that of the constant parts of heavy and light chains of mouse immunoglobulins by calculating the difference indices which express the difference in the fractional contents of each amino acid (9), relatively smaller difference indices are obtained with $C_L(\kappa)$, C_H2 and C_H3 domains (see Table 2). The values are 15.9, 17.1 and 14.9, respectively. These values are very close to the values of 15.5 and 14.8, respectively, which are obtained when human β_2-microglobulin is compared with $C_L(\kappa)$ and C_H3 domains of human immunoglobulin in the same manner (10). The same degree of compositional relatedness can be seen between the mouse substance and human β_2-microglobulin. In this case, the difference index is calculated to be 14.0. In the antigenic properties, the mouse substance was not distinguished from the 11,000-dalton component isolated from non-ionic detergent solubilized H-2 antigen molecules (1). It has also shown to have a weak but significant cross-reactivity with human β_2-microglobulin (1). In the present work, we raised in rabbits antisera against one preparation of the mouse substance and found them to bind equally well with the mouse substance and the 11,000-dalton component that was isolated from specifically purified, papain-solubilized H-2 antigen molecules by acid treatment as described previously

Table 2

Assessment of Compositional Relatedness of Mouse β_2-Microglobulin to Human β_2-Microglobulin and to Constant Parts of Mouse Immunoglobulins by Comparison of Different Indices[a]

	Difference index with respect to mouse β_2-microglobulin
Human β_2-microglobulin[b]	14.0
$C_L(\kappa)$ of MOPC 21[c]	15.9
$C_L(\lambda)$ of MOPC 104E[c]	22.4
C_H1 of MOPC 173[d]	25.4
C_H2 of MOPC 173[d]	17.1
C_H3 of MOPC 173[d]	14.9

[a] Two proteins are compared by determining the difference in the fractional contents of each amino acid, obtaining the sum of the absolute value of those differences, and dividing the sum by two.

[b] The amino acid composition of human β_2-microglobulin used is that shown in Table 1.

[c] These are the constant parts of the light chain of mouse immunoglobulins; residues 107-218 of mouse Bence Jones protein MOPC 21 for $C_L(\kappa)$ (14) and residues 105-215 of mouse myeloma protein MOPC 104E for $C_L(\lambda)$ (15).

[d] These are the constant parts of the heavy chain of mouse myeloma protein MOPC 173 (IgG_{2a}) (16,17,18); residues 116-220 for C_H1, residues 234-341 for C_H2 and residues 342-446 for C_H3.

(11). In addition, the substance or very similar substances is present in plasma and urine of normal mice and in tumor ascites fluid of lymphoma (L1210) bearing mice (unpublished data). These findings are highly analogous to those described for human β_2-microglobulin and appear to satisfy the essential requirements for regarding this substance as the mouse homologue of human β_2-microglobulin. Recently, Rask et al. (12) isolated a 12,000-dalton component from a papain-solubilized H-2 preparation and showed it to have a high degree of similarity in the chemical structure to human β_2-microglobulin. They used autoradiographic peptide

mapping of the chymotrypsin-digested radioiodinated samples. They also reported that the 12,000-dalton component has a cross-reactivity with rabbit antibodies to human β_2-microglobulin.

Acknowledgements. We wish to thank Mr. A.J. Trott, Mr. R. Maturski, Mrs. J. Shaver and Miss P. Overturf for the excellent assistance.

References

1. Natori, T., Katagiri, M., Tanigaki, N. and Pressman, D., Transplantation 18: 550 (1974).
2. Davies, D.A.L., Immunology 11: 115 (1966).
3. Schneider, W.S. and Hogeboom, G.H., J. Biol. Chem. 183: 123 (1950).
4. Davis, B.J., Ann. N.Y. Acad. Sci. 121: 404 (1964).
5. Shapiro, A.L., Vinuela, E. and Maizel, J.V., Jr., Biochem. Biophys. Res. Comm. 28: 815 (1967).
6. Hoffman, D.R., Grossberg, A.L. and Pressman, D., J. Immunol. 108: 18 (1972).
7. Nakamuro, K., Tanigaki, N., Kreiter, V.P. and Pressman, D., Immunology 27: 1127 (1974).
8. Peterson, P.A., Cunningham, B.A., Berggård, I. and Edelman, G.M., Proc. Nat. Acad. Sci. 69: 1697 (1972).
9. Metzger, H., Shapiro, M.B., Mosimann, J.E. and Vinton, J.E., Nature 219: 1166 (1968).
10. Nakamuro, K., Tanigaki, N. and Pressman, D., Proc. Nat. Acad. Sci. 70: 2863 (1973).
11. Appella, E., Henrikson, O., Natori, T., Tanigaki, N., Law, L.W. and Pressman, D., Transplant. Proc., in press.
12. Rask, L., Ostberg, L., Lindblom, J.B., Fernstedt, Y. and Peterson, P.A., Transplant. Rev. 21: 85 (1974).
13. Berggård, I. and Bearn, A.G., J. Biol. Chem. 243: 4095 (1968).
14. Gray, W.R., Dreyer, W.J. and Hood, L., Science 155: 465 (1967).
15. Appella, E., Proc. Nat. Acad. Sci. 68: 590 (1971).
16. Bourngois, A., Fougereau, M. and Rocca-Sera, J., Eur. J. Biochem. 43: 423 (1974).
17. De Preval, C. and Fougereau, M., Eur. J. Biochem. 30: 452 (1972).
18. De Preval, C., Pink, J.R.L. and Milstein, C., Nature 228: 30 (1970).