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Two allelic forms of mouse β_2-microglobulin

(major histocompatibility antigens/genetic polymorphism)

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ABSTRACT **Two allelic forms of mouse β_2-microglobulin (β_2m), the small polypeptide chain of H-2 histocompatibility antigens, have been identified. The two forms can be distinguished by NaDodSO$_4$/polyacrylamide gel electrophoresis. All inbred mouse strains express a single β_2m isotype. Mice heterozygous for β_2m synthesize both forms, showing codominant expression of β_2m alleles. In mice heterozygous for both H-2 and β_2m, individual H-2 histocompatibility antigens associate with both β_2m forms. Preliminary structural studies indicate differences in peptide composition between the two forms.**

β_2-Microglobulin (β_2m) is a small protein (M_r, 12,000) present in the serum of most mammals. It is also found on the surfaces of most mammalian cells and has been shown to be a component of the classical histocompatibility antigens (1, 2). These antigens, like the mouse H-2 antigens, are the principal targets for allograft rejection and are directly involved in cellular communication within the immune system (3). H-2 molecules are composed of two noncovalently linked polypeptide chains, a transmembrane glycopolypeptide of $M_r \approx 46{,}000$ and β_2m which is not glycosylated and does not itself associate with the cell membrane (4). β_2m has a remarkably conserved structure, in contrast to the larger chain which is genetically polymorphic and is encoded by genes in the *K*, *D*, and *L* regions of the mouse major histocompatibility complex on chromosome 17 (5). In the case of human (HLA, A and B) histocompatibility antigens, both the larger polymorphic chain and β_2m have large sections that are similar in amino acid sequence to immunoglobulin constant region domains (6). This suggests that immunoglobulin domains and β_2m may have evolved from a common ancestral gene. Despite numerous attempts to detect genetic polymorphism of human β_2m (P. A. Peterson, personal communication), no such differences have been demonstrated in any species. In this paper we present biochemical and genetic evidence for the existence of two forms of mouse β_2m.

MATERIAL AND METHODS

Mice and Antisera. All mice were bred and maintained in our own colony. Rabbit anti H-2^d antiserum was prepared and characterized by Kvist *et al.* (7). Alloantisera were obtained from the Research Resources Branch, National Institutes of Health, Bethesda, MD. Strain combinations of the alloantisera were as follows: anti-K^k, (A.TL × 129) anti-A.AL; anti-K^b, (B10.D2 × A) anti-B10.A(5R); anti-D^k, [B10.A(2R) × C3H.SW] anti-C3H; anti-D^b, [B10.A(5R) × LPRIII] anti-B10.

Biosynthetic Labeling of Mouse Spleen Cells. Spleen cells from healthy mice were teased into RPMI-1640 medium lacking methionine but supplemented with 2 mM glutamine. Cells were washed three times in this medium and resuspended at a concentration of 10^8 cells per ml. Cells (10^7) were labeled for 1 hr at 37°C with 100 μCi (1 Ci = 3.7×10^{10} becquerels) of [^{35}S-]methionine (Amersham, England). Cells were then solubilized in cold 20 mM, Tris·HCl, pH 7.4/0.1 M NaCl/1% Triton X-100/1 mM phenylmethanesulfonyl fluoride. Nuclei were removed by centrifugation at 2000 × *g*, and 5 μl of normal rabbit serum was added. Extracts were then preabsorbed by gentle shaking for 1 hr with 50 μl of protein A-Sepharose (Pharmacia, Uppsala, Sweden). Insoluble material was removed by centrifugation at 20,000 × *g* for 15 min, and the supernatants were used immediately for immunoprecipitation.

Immunoprecipitations. To labeled spleen cell extracts derived from 10^7 cells either 1 μl of rabbit anti-H-2 antiserum was added, or the extract was divided into equal portions and each one was treated with 5 μl of appropriate mouse alloantiserum. After 30 min on ice, 20 μl of protein A-Sepharose was added, and the reaction mixture was shaken gently for 3 hr. The solid phase was then removed by gentle centrifugation (20 × *g*) and washed three times with 20 mM Tris·HCl, pH 7.4/0.1 M NaCl/0.1% Triton X-100, once with the same buffer containing 0.5 M NaCl, and, finally, once with water. Solid phases were stored at −20°C or prepared immediately for electrophoresis.

Gel Electrophoresis and Fluorography. Proteins were separated by NaDodSO$_4$/polyacrylamide gel electrophoresis in 10–15% gradient gel slabs run under reducing conditions (8). In some experiments, standard 15% acrylamide slab gels were used. Gels were processed for fluorography (9) and exposed for 1–10 days on Kodak X-Omat R film.

Tryptic Peptide Mapping. Concanavalin-A activated spleen cells (3×10^8) from B10.BR and BALB/c mice were labeled with either 200 μCi of [^{14}C]arginine/[^{14}C]lysine mixture (Amersham) or 2 mCi of [^{3}H]arginine/[^{3}H]lysine. β_2m were isolated from detergent lysates by immunoprecipitation with rabbit anti-H-2 antiserum followed by preparative polyacrylamide gel electrophoresis. β_2m were collected from the gel pieces by washing with water containing crystalline bovine albumin (150 μg/ml); the two forms were mixed together and precipitated with cold 20% (wt/vol) trichloroacetic acid. Precipitates were washed twice with acetic acid/water/triethylamine/acetone, 1:1:1:17 (vol/vol) and once with acetone and dried. Tryptic digestion was performed in 0.2 M *N*-ethylmorpholine buffer (pH 8.1) for 3 hr at 37°C at a trypsin/protein ratio of 1:20 (DPC-trypsin, Sigma). Peptides were separated on a C_{18} column (Bondapak) with a 5–60% ethanol gradient containing 5 mM ammonium trifluoracetate (10). Fractions were assayed in a Packard Tri-Carb scintillation counter with double-isotope program and quench correction.

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Abbreviations: β_2m, β_2-microglobulin; $\beta_2$mf and $\beta_2$ms, fast- and slow-migrating types of β_2m, respectively.

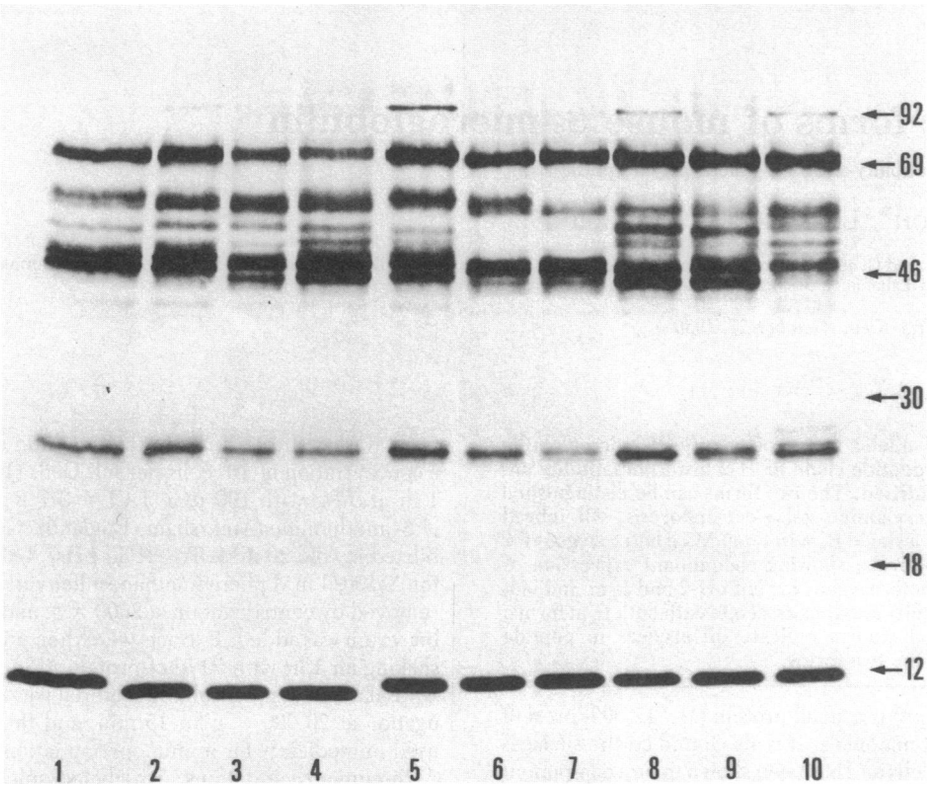

FIG. 1. Strain distribution of β_2m isotypes. Mouse spleen cells were labeled biosynthetically with [^{35}S]methionine and detergent extracts of them were immunoprecipitated with rabbit anti-H-2 antiserum. Immunoprecipitates from the following strains are shown: lane 1, 129; 2, B10; 3, B10.BR; 4, B10.D2; 5, BALB/c; 6, CBA; 7, AKR; 8, A/J; 9, NZB; 10, NZW. M_rs ($\times 10^{-3}$) of marker proteins are shown at the right. Fluorograms were exposed for 24 hr.

RESULTS

β_2m Typing of Mice. We have observed that β_2m from different strains of mice differ in their apparent M_r when analyzed by polyacrylamide gel electrophoresis. Two forms could be distinguished by virtue of their different mobilities and, for convenience, the faster and slower migrating forms were provisionally named "$\beta_2$mf" and "$\beta_2$ms," respectively. To determine the strain distribution of β_2m alleles, 10 laboratory inbred mouse strains were examined. It was found that all strains of mice tested carried β_2m of either the $\beta_2$ms or the $\beta_2$mf type, but not both. $\beta_2$mf is therefore unlikely to be a proteolytic breakdown product of $\beta_2$ms.

Mouse spleen cells were labeled biosynthetically with [^{35}S]methionine and β_2m was isolated by using a rabbit antiserum against mouse whole H-2 antigens. This antiserum contained antibodies against mouse β_2m (7). Most strains tested synthesized $\beta_2$ms. Three congeneic strains of the C57BL/10 (B10) background carrying different *H-2* haplotypes were found to synthesize $\beta_2$mf (Fig. 1, lanes 2, 3, and 4). These were B10 (*H-2*^{b}), B10.BR (*H-2*^{k}), and B10.D2 (*H-2*^{d}). These mice were produced by an initial cross between B10 and a strain carrying the required *H-2* type, followed by repeated backcrossing of B10 mice with progeny carrying the desired *H-2* haplotype. Six further H-2-different B10 congeneic strains have subsequently been typed for β_2m (not shown). Because all nine strains carry $\beta_2$mf, it can be concluded that β_2m genes are not linked to the major histocompatibility complex.

In another experiment, the inbred strains C3H and DBA/2 were found to synthesize $\beta_2$ms whereas C57BL/6 produced $\beta_2$mf (not shown).

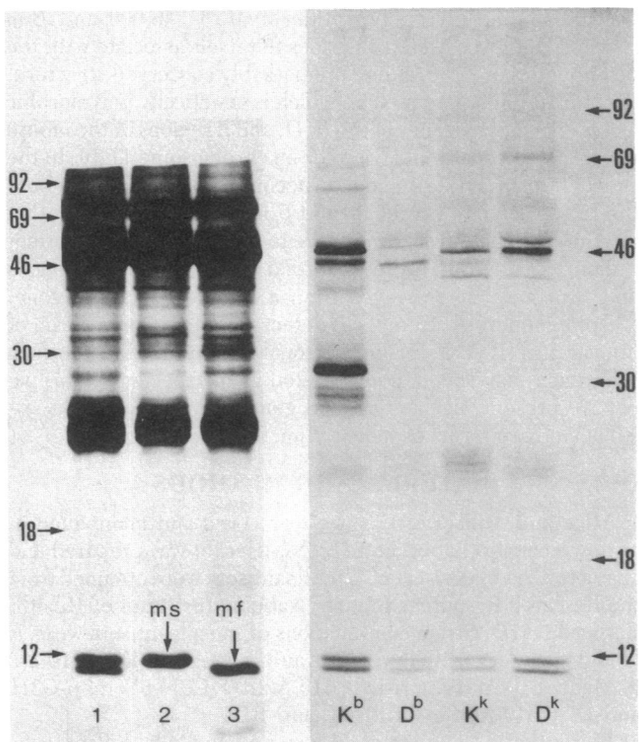

FIG. 2. β_2m from heterozygous mice. (*Left*) Cell extracts were immunoprecipitated with rabbit anti-H-2 antiserum. Lanes: 1, C57BL/10 × A/J; 2, DBA/2 ($\beta_2$ms control); 3, C57BL/10 ($\beta_2$mf control). Gel is 15% acrylamide. (*Right*) Immunoprecipitates with anti-H-2 alloantisera separated on a 10–15% acrylamide gradient gel. M_rs ($\times 10^{-3}$) are indicated at the sides. Fluorograms were exposed for 4 days.

β_2m Phenotypes of Heterozygotes. To determine whether mice heterozygous for β_2m express both forms of the protein, β_2m were isolated from labeled extracts of C57BL/10 ($\beta_2$mf) × A/J ($\beta_2$ms) spleen cells. Equal numbers of counts corresponding to $\beta_2$ms and $\beta_2$mf were precipitated by the rabbit antiserum (Fig. 2 *Left*, lane 1). A similar result was obtained in the strain combination C57BL/6 × CBA (not shown). These results show that both β_2m alleles are codominantly expressed in heterozygotes.

Association of β_2m with Individual H-2 Molecules. To determine whether H-2 molecules preferentially associate with a particular β_2m type, individual H-2 antigens were immunoprecipitated from labeled extracts of CBA × C57BL/6 spleen cells by using alloantisera specific for determinants on the H-2 heavy chains. These antisera were produced in strain combinations that excluded the possibility of anti-β_2m antibodies being present. Thus, β_2m was precipitated by virtue of its association with H-2 heavy chains. In one experiment, four molecules were precipitated: H-2K^k, H-2K^b, H-2D^k, and H-2D^b (Fig. 2 *Right*). All four H-2 precipitates were found to contain β_2m of both types. Therefore, H-2 molecules do not preferentially associate with one β_2m type but appear to bind equal amounts of each type.

Tryptic Peptide Mapping of β_2m Isotypes. To determine the degree of structural homology between the two β_2m types, tryptic cleavage products of both proteins were compared by high-pressure liquid chromatography. Approximately 15 peptides were detected by using this technique, of which 12 were identical in both $\beta_2$mf and $\beta_2$ms (Fig. 3). Three peptides were different, with $\beta_2$mf having one peptide less than $\beta_2$ms. The results indicate a high degree of structural homology between the two forms. The observed peptide differences could be explained by a single amino acid interchange involving a tryptic cleavage site.

DISCUSSION

These experiments demonstrate that mouse β_2m exists in at least two allelic forms which are similar in structure and in their association with mouse H-2 histocompatibility antigens. The results confirm and extend the findings of Michaelson *et al.* (11) who recently showed an electrophoretic difference between β_2m from mouse strains B10.A and A. Genetic polymorphism of β_2m has not yet been demonstrated in other species. Reports of molecular heterogeneity of guinea pig (12) and human (13) β_2m have not established whether such differences are determined genetically or are due to protein modifications.

These results indicate further structural polymorphism of mouse H-2 molecules. One important consequence of these results is that mouse strains sharing a common major histocompatibility gene region but differing at the β_2m locus do not express identical H-2 molecules on their cell surface. One example is B10.D2, which carries the major histocompatibility region from DBA/2 but β_2m of the $\beta_2$mf type. It is necessary to determine the biological significance, if any, of such structural differences. Experiments with mice heterozygous for β_2m suggest that both forms are produced in approximately equal amounts and can bind equally well to individual mouse H-2 antigen heavy chains. Thus, structural differences between the two proteins do not drastically affect their association with H-

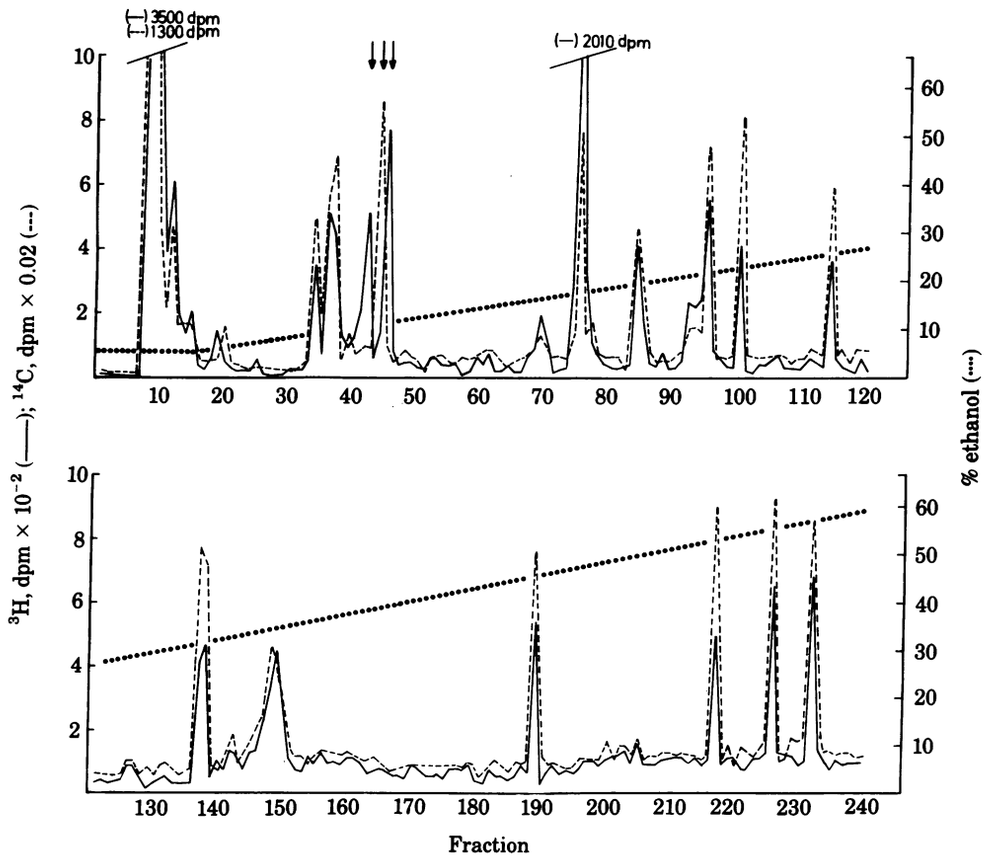

FIG. 3. High-pressure liquid chromatography of tryptic fragments of $\beta_2$mf (– – –) and $\beta_2$ms (———). Differences are indicated by vertical arrows. $\beta_2$mf was prepared from B10.BR spleen cells, labeled with [^{14}C]arginine/[^{14}C]lysine mixture. $\beta_2$ms was isolated from [^{3}H]arginine/[^{3}H]lysine-labeled BALB/c cells.

2 antigens. It is possible, however, that some anti H-2 alloantisera may distinguish molecules with the same heavy chain but different β_2m type.

The observed allelism of β_2m enables the chromosomal location of β_2m genes to be established. Preliminary results obtained by typing a series of DBA/2 × C57BL/6 recombinant inbred mouse strains show that the distribution of β_2m isotypes follows closely that of the Ly4 antigen (14) which is encoded by genes on mouse chromosome 2.

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1. Tanigaki, N. & Pressman, D. (1974) *Transplant. Rev.* **21**, 15–34.
2. Östberg, L., Lindblom, B., Fernstedt, Y. & Peterson, P. (1974) *Transplant. Rev.* **21**, 85–105.
3. Zinkernagel, R. M. (1978) *Immunol. Rev.* **42**, 224–270.
4. Dobberstein, B., Garoff, H., Warren, G. & Robinson, P. J. (1979) *Cell* **17**, 759–769.
5. Klein, J. (1979) *Science* **203**, 516–521.
6. Trägardh, L., Rask, L., Wiman, K., Fohlmann, J. & Peterson, P. A. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5839–5842.
7. Kvist, S., Klareskog, L. & Peterson P. A. (1978) *Scand. J. Immunol.* **7**, 447–452.
8. Blobel, G. & Dobberstein, B. (1975) *J. Cell Biol.* **67**, 835–851.
9. Laskey, R. A. & Mills, A. D. (1975) *Eur. J. Biochem.* **56**, 335–341.
10. Altevogt, P., Fohlmann, J., Kurnick, J. T., Peterson, P. A. & Wigzell, H. (1981) *Eur. J. Immunol,* in press.
11. Michaelson, J., Rothenberg, E. & Boyse, E. A. (1980) *Immunogenetics* **11**, 93–95.
12. Cigén, R., Ziffer, J. A., Berggard, B., Cunningham, B. A. & Berggard, I. (1978) *Biochemistry* **17**, 947–956.
13. Hall, P. W., Ricinati, E. S. & Vacca, C. V. (1977) *Clin. Chim. Acta* **77**, 37–42.
14. Snell, G. D., Cherry, M., McKenzie, I. F. C. & Bailey, D. W. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 1108–1111.