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Multiple Common Properties of Human β_2-Microglobulin and the Common Portion Fragment Derived from HL-A Antigen Molecules

(lymphoid cells/compositional relatedness/histocompatibility)

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ABSTRACT 11,000-Dalton common portion fragments derived from HL-A antigen molecules were isolated and found to have a significantly high homology to β_2-microglobulin in amino-acid composition. Common portion fragments are also very similar to β_2-microglobulin with respect to molecular size, charge, and distribution in tissues. Moreover, both are found in the spent culture media of human cell lines and in human plasma and urine. Thus it appears that β_2-microglobulin may well be the same substance as the common portion fragment of HL-A antigen molecules.

We recently reported the isolation of a small fragment of HL-A antigens which appears to be a characteristic invariant portion of the structure of HL-A antigens. It was isolated from papain-solubilized HL-A antigen molecules of various HL-A allotypes by mild degradation procedures (1, 2). The molecular size is about 11,000 daltons. The small fragment does carry an HL-A common antigenic activity which is a characteristic antigenic marker of HL-A antigens (2, 3) although the fragment is devoid of HL-A alloantigenic activity. This small fragment has been designated HL-A common portion fragment. Cresswell *et al.* (4) recently reported the isolation of a similar 11,000-dalton fragment from papain-solubilized HL-A antigen.

Similar 11,000-dalton fragments are present in human blood plasma (5) and urine and in spent culture media from cultured human cell lines (1). We recently purified the small fragments from spent culture media of cultured human lymphoid cell lines and found them to be identical to the HL-A common portion fragment that was obtained by acid cleavage of papain-solubilized HL-A antigen molecules with respect to molecular size, electrophoretic mobility, and isoelectric point (unpublished). No antigenic differences were observed.

It now appears to us that the above fragment is very closely related or identical to the β_2-microglobulin that was originally described by Berggård and Bearn (6) and that has a structural similarity to the constant parts of human immunoglobulin G (7, 8).

We have determined the amino-acid composition of the HL-A common portion fragment isolated from spent culture media of a human lymphoid cell line and compared it with the composition of the β_2-microglobulin reported by Berggård and Bearn (6). Assessment of compositional relatedness between proteins, by the method described by Metzger *et al.* (9), indicated that β_2-microglobulin has a significantly high homology to HL-A common portion fragment.

The fragment subjected to amino-acid analysis was purified from spent culture media of RPMI 1788 cells by isolation procedures that involved differential ultrafiltration, gel filtration, and column electrophoresis. The HL-A common antigenic activity was followed by radioimmunoassay (3). The culture of RPMI 1788 cells with RPMI 1640 medium supplemented with 10% fetal-bovine serum and with penicillin and streptomycin was harvested at day 3. The spent culture medium was separated by centrifugation and filtered with an HA Millipore filter (Millipore Corp., Bedford, Mass.). Sodium azide was used as a preservative at a final concentration of 0.1%. In each isolation experiment 8 liters of the filtered spent culture medium were passed through a Bio-Fiber 80 Miniplant Ultrafilter with a molecular cut-off of 30,000 (Bio-Rad Laboratories, Richmond, Calif.). About 60% of the HL-A common activity was recovered in the filtrate. The filtrate was concentrated about 200 times by a membrane filter (UM-10) of a molecular cut-off of 10,000 (Amicon Corp., Lexington, Mass.). No significant loss of the HL-A common activity was observed. Reddish-brown concentrate was fractionated by gel filtration on a Sephadex G-75 Superfine column (50 × 500 mm) prewashed with Tris-phosphate buffer (0.04 M Tris, pH 6.6). The HL-A common activity was eluted as a single peak that corresponded to a molecular size of about 11,000 daltons. The fractions carrying the HL-A common activity were concentrated to about 2 ml with a membrane filter (UM-2) with a molecular cut-off of 1,000, and again fractionated on a Sephadex G-75 Superfine column (20 × 550 mm). Upon the second gel filtration, a protein peak was observed which coincided with the HL-A common activity. Most of the colored substance was removed by the gel filtrations and the specific activity (HL-A common activity per A_{280} unit) was increased by about 400 fold. The active fractions were concentrated to about 1 ml with a UM-2 filter and subjected to column electrophoresis in Bio-Gel P-2 (200–400 mesh, 20 × 300 mm) equilibrated with Tris·HCl buffer (pH 8.6 at 4°, $\Gamma/2 = 0.05$). Upon electrophoresis, the HL-A common activity appeared as a single peak within a major protein peak that migrated in the β_2-globulin region. The active fractions were concentrated to about 0.5 ml with a UM-2 filter and finally purified on a Sephadex G-75 Superfine column (14 × 500 mm) prewashed with Tris·HCl buffer (pH 8.6 at 4°, $\Gamma/2 = 0.05$). More than 90% of the protein appeared as a sharp peak and the HL-A common activity was found essentially within the protein peak. The fractions having high specific activity were pooled. The overall recovery of the HL-A common activity was about 18% of the starting material and the overall increase of the specific activity was about 2300 fold. The total number of A_{280} units of the final preparations was 1.5–2.0.

TABLE 1. *Amino-acid composition of the HL-A common portion fragment isolated from spent culture media and of β_2-microglobulin*

Amino acid	Preparation of HL-A common portion fragment (residues/molecule*) I	II	III	β_2-Microglobulin† (residues/molecule)
Aspartic acid	12.0	11.2	11.4	12
Threonine‡	5.1	4.8	4.9	5
Serine§	8.1	7.6	7.9	10
Glutamic acid	11.5	10.9	10.9	11
Proline	6.2	5.2	4.8	5
Glycine	7.7	5.4	4.3	3
Alanine	4.6	3.8	3.7	2
Valine	6.2	6.1	6.3	7
Half-cystine	1.4	1.3	1.7	2
Methionine	1.1	1.3	1.1	1
Isoleucine	5.6	4.8	5.8	5
Leucine	7.0	7.0	7.0	7
Tyrosine	4.7	4.5	4.9	6
Phenylalanine	4.3	4.2	4.4	5
Lysine	8.8	8.9	8.9	8
Histidine	3.7	3.4	3.4	4
Arginine	4.7	4.1	4.3	5
Tryptophan	N.D.	N.D.	N.D.	2
Ammonia	N.D.	N.D.	N.D.	9
Total (excluding ammonia)	102.7	94.5	95.7	100

N.D. = not determined.

* Calculated on the basis of 7.0 residues of leucine per molecule.

† Data from Berggård *et al.* (6).

‡ 3% Correction was made to correct for destruction during acid hydrolysis (15).

§ 10% Correction was made to correct for destruction during acid hydrolysis (15).

Preparations of the fragment isolated from spent culture media in three separate isolation experiments were compared with a preparation of HL-A common portion fragment for molecular size, electrophoretic mobility, and isoelectric point as well as for antigenic properties. The HL-A common portion fragment was isolated by acid dissociation from an HL-A antigen preparation that was obtained by papain digestion of the membrane fraction of RPMI 1788 cells (1, 2). The HL-A antigen preparation was treated with glycine buffer of pH 2.3 for 15 min and fractionated by gel filtration on a Sephadex G-75 Superfine column (14 × 500 mm) at pH 7.8. Fractions containing HL-A common activity and corresponding to a molecular size of about 11,000 daltons were pooled and concentrated.

Upon isoelectrofocusing on acrylamide gel, preparations from both sources and their mixture showed the same major, densely stained sharp band with an isoelectric point of 5.0. All preparations showed in addition a common, faintly stained broad band well displaced from the sharp band. Disc electrophoresis and sodium dodecyl sulfate–acrylamide gel electrophoresis gave single bands for the preparations, thus demonstrating no differences in electrophoretic mobility and molecular size, respectively, between fragments isolated from spent culture media and HL-A common portion fragments. The reaction to antibodies raised in rabbits against human lymphoid cell membrane and against the fragments isolated from spent culture media also did not show any antigenic differences between the common portion fragments from the two sources.

TABLE 2. *Assessment of compositional relatedness of β_2-microglobulin to the HL-A common portion fragment and to the constant parts of immunoglobulin G by comparison of difference indexes**

	Difference index: β_2-Microglobulin	Preparation I	Preparation II	Preparation III
HL-A common portion fragment				
Preparation I	8.5	—	—	—
Preparation II	7.1	3.4	—	—
Preparation III	5.8	4.7	2.5	—
C_L of Eu†	15.5	18.5	17.1	16.6
C_H3 of Eu†	14.8	17.1	15.4	16.0

* 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 multiplying that sum by fifty (9). The compositions of the β_2-microglobulin and the heavy- and light-chain portion used were those reported by Berggård *et al.* (6) and Peterson *et al.* (8).

† These are the constant regions, respectively, of the light chain (residues 109–214) and the heavy chain (residues 342–446) of IgG1 immunoglobulin Eu (8).

The amino-acid compositions of each of the three preparations were determined by a described procedure (10). The amino-acid compositions are given in Table 1, together with that of β_2-microglobulin (6). The amino-acid composition is expressed as the number of amino residues per molecule, based on the assumption that the fragment isolated from spent culture media has the same number of leucine residues as β_2-microglobulin. It is clear that the amino-acid composition of the fragment is very similar to that of β_2-microglobulin. Only few residues, such as glycine and alanine, seem to have significant differences. The significance of this similarity in amino-acid composition was indicated by calculating the difference index, which expresses the differences in the fractional contents of each amino acid (9). Table 2 shows the difference indexes of the three preparations of the HL-A common portion fragment and β_2-microglobulin. The difference indexes of β_2-microglobulin and constant parts of human immunoglobulin G heavy and light chains are also shown because of the reports by Smithies and Poulik (7) and Peterson *et al.* (8) that a structural relationship exists between human β_2-microglobulin and immunoglobolin G. These authors claim, on the basis of amino-acid sequence, there is a high degree of homology of the β_2-microglobulin to the constant regions of G myeloma protein. As a reference, the difference indexes of the preparations of HL-A common portion fragments and the constant parts of immunoglobulin G chains are included.

When the three preparations of the HL-A common portion fragment were compared with β_2-microglobulin, the mean of the difference indexes was 7.1. This value was larger than the value of 3.5 obtained among the preparations of HL-A common portion fragments, but significantly smaller than the values of 14.8 and 15.5 which were obtained when β_2-

microglobulin was compared with the constant part of immunoglobulin G heavy and light chains. These data indicate it is highly probable that β_2-microglobulin is more closely related to HL-A antigens than to immunoglobulins.

The similarities of HL-A common portion fragments and β_2-microglobulin are summarized as follows: (*a*) Their amino-acid compositions are almost the same; (*b*) the molecular weight of the HL-A common portion fragment determined by gel filtration (1, 2) and by sodium dodecyl sulfate–acrylamide-gel electrophoresis is about 11,000 and that of β_2-microglobulin is reported to be 11,600–11,800 (6); (*c*) both migrate in the β_2-globulin region on electrophoresis (6); (*d*) both are present on the cell surface (8, 11, 12); (*e*) neither reacts with any antiimmunoglobulin antisera (6); and (*f*) their production is also very similar. HL-A common portion activity can be found not only in lymphoid cell cultures but also in non-lymphoid cell cultures. Likewise, β_2-microglobulin was recently detected in several human skin fibroblast cultures (13) as well as in lymphoid cell cultures (11, 14).

The fact that β_2-microglobulin activity is produced by human skin fibroblasts, which are not known to produce immunoglobulins, may be taken as an indication that β_2-microglobulin is not derived from immunoglobulin molecules in spite of the apparent homology. Thus it appears that β_2-microglobulin may well be the same substance as HL-A common portion fragments, which were shown to be structural components of HL-A antigens. Final proof will require comparisons of amino-acid sequences and antigenic properties.

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