Genetic Analysis of Human Lymphocyte Proteins by Two-Dimensional Gel Electrophoresis: 4. Genetic Polymorphism of Cytosol 100k Polypeptide

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Summary. We describe a genetic polymorphism of a human cellular polypeptide with mol. wt. 100,000, detected in peripheral blood lymphocytes by high resolution two-dimensional electrophoresis. Three different electrophoretic types (1-1, 2-1, and 2-2) of the polypeptide have been identified. Family and population studies indicate that the three phenotypes of the polypeptide are determined by two common alleles at a single autosomal locus. The polypeptide occurs in the cytosol and is one of the abundant polypeptides of B-lymphoblastoid cells, T-lymphoblastoid cells, fibroblasts, and HeLa cells. The data indicate that the cytosol polypeptide with mol. wt. 100,000 shows a genetic polymorphism determined by a new autosomal locus. It is proposed that the polypeptide and its locus be temporarily designated cytosol 100k polypeptide (C100k polypeptide) and C100P, respectively. In a Japanese population, the gene frequencies of C100P¹ and C100P² were 0.907 and 0.093, respectively.

Introduction

The use of high-resolution two-dimensional electrophoresis in combination with family and population studies is obviously the most effective procedure to reveal which cellular abundant polypeptides show genetic polymorphism. The presence of a genetic polymorphism in lymphocyte cytosol 64k polypeptide has been shown by the use of this procedure (Hamaguchi et al. 1981, 1982a). Furthermore, the procedure revealed that common genetic variants are present in some of the other lymphocyte-abundant polypeptides (Hamaguchi et al. 1982b). Among them is a polypeptide with a mol. wt. of 100,000 and pl value of 6.85. In the known human polymorphic proteins, hexokinase III and glucose dehydrogenase have subunit sizes with a mol. wt. of about 100,000 (Povey et al. 1975; Hopkinson et al. 1976; King and Cook 1981). These enzymes show characteristic cell distribution or intracellular localization (Metzger et al. 1965; Povey et al. 1975). The present work was carried out to determine whether the polypeptide with mol. wt. 100,000 shows a genetic polymorphism defined by a new locus.

Materials and Methods

Sample Preparation

Two-dimensional electrophoresis samples of phytohemagglutinin (PHA)-stimulated peripheral blood lymphocyte proteins were prepared by the method described elsewhere (Hamaguchi et al. 1982b). Two-dimensional electrophoresis samples of [¹⁴C]-labeled proteins from HeLa cells and a human skin fibroblast line (CH2) were prepared according to the procedure described previously (Hamaguchi et al. 1982a). HPB-ALL cells, which belong to the human T-lymphoblastoid cell line (Morikawa et al. 1978), were suspended at a density of 2 x 10⁵ cells per ml in 20 ml of RPMI-1640 medium containing 10% fetal calf serum (Gibco, N.Y.) and grown in a 75 cm² Falcon plastic flask at 37°C in a humidified atmosphere containing 5% CO₂ for 2 days. A two-dimensional electrophoresis sample of HPB-ALL cell proteins was prepared according to the method described by Hamaguchi et al. (1982b). HPB-ALL cells were kindly provided by Prof. Morikawa, Shimane Medical College. Protein concentrations were determined by the method of Bradford (1976). Radioactivity incorporated into proteins was determined on the basis of trichloroacetic-acid-precipitable activity.

Two-Dimensional Electrophoresis

Two-dimensional electrophoresis was carried out according to the method of O'Farrell (1975). Polycrylamide slab gel with 1-mm thickness and 10% concentration were used in the second dimension. Two-dimensional electrophoresis patterns of un-labeled polypeptides from PHA-stimulated peripheral blood lymphocytes and HPB-ALL cells were visualized in gels with Coomassie Blue staining. Autoradiograms of electrophoresis patterns of [¹⁴C]-labeled polypeptides from HeLa cells and fibroblasts were made by exposing X-ray film (Fuji Photo Co., Tokyo) to a dried gel at −80°C for 240–360 h. For proteins from HeLa cells and fibroblasts, the map position corresponding to the spot of the polypeptide with mol. wt. 100,000 and pl 6.85 in the gel was determined by a separate experiment, in which peripheral blood lymphocyte proteins from an individual with type 2-1 of the polypeptide were mixed, co-electrophoresed, and subsequently detected by Coomassie Blue staining before making an autoradiogram.

Results

Electrophoresis Patterns of Cytosol 100k Polypeptide

Figure 1 shows three observed electrophoretic phenotypes of the polypeptide with mol. wt. 100,000 from peripheral blood lymphocytes. The electrophoretic phenotypes of the polypeptide are characteristic for individual lymphocytes. Since the polypeptide is present in the cytosol (Hamaguchi et al. 1982b) and since it is one of the abundant polypeptides at least in several cell types described below, we propose that the polypeptide be
Table 1. Cytosol 100k polypeptide types in families

<table>
<thead>
<tr>
<th>Parental types</th>
<th>No. of families</th>
<th>Offspring types</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1-1</td>
</tr>
<tr>
<td>1-1 × 1-1</td>
<td>15</td>
<td>34 (34)</td>
</tr>
<tr>
<td>1-1 × 2-1</td>
<td>9</td>
<td>8 (10)</td>
</tr>
<tr>
<td>1-1 × 2-2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>42 (44)</td>
</tr>
</tbody>
</table>

* Expected numbers are given in parentheses.

Table 2. Cytosol 100k polypeptide types among unrelated individuals

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>1-1</th>
<th>2-1</th>
<th>2-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed</td>
<td>94</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>Expected</td>
<td>93.0</td>
<td>19.1</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* Expected values calculated from gene frequencies: C100P1 = 0.907 and C100P2 = 0.093

Table 1 presents C100k polypeptide phenotypes in families. The pattern of inheritance is consistent with codominant inheritance of two alleles: types 1-1 and 2-2 are homozygous for one of two alleles and type 2-1 is heterozygous for the two alleles. The locus is autosomal since some males are heterozygotes and fathers transmit the alleles to children of both sexes. We propose that the locus be temporarily designated C100P1 and the alleles C100P1 and C100P2. This designation means that phenotype 1-1 is determined by C100P1 and phenotype 2-2 by C100P2.

Table 2 shows the distribution of phenotypes in a random population of 113 unrelated healthy Japanese. It can be seen that the observed numbers are close to those calculated on the assumption that the Hardy-Weinberg equilibrium is observed.

Family and Population Studies

Table 1 presents C100k polypeptide phenotypes in families. The pattern of inheritance is consistent with codominant inheritance of two alleles: types 1-1 and 2-2 are homozygous for one of two alleles and type 2-1 is heterozygous for the two alleles. The locus is autosomal since some males are heterozygotes and fathers transmit the alleles to children of both sexes. We propose that the locus be temporarily designated C100P1 and the alleles C100P1 and C100P2. This designation means that phenotype 1-1 is determined by C100P1 and phenotype 2-2 by C100P2.

Fig. 1A–C. Three observed phenotypes of C100k polypeptide in the two-dimensional electrophoresis pattern of PHA-stimulated peripheral blood lymphocyte proteins visualized in gels with Coomassie Blue staining. About 150 μg of proteins was subjected to electrophoresis. C100k and variant C100k polypeptides are indicated by bold arrows. A Type 1-1; B type 2-1; C type 2-2. The polypeptide marked a is actin. Variant 49k and 40k polypeptides are also shown by arrowhead marks. The explanation of other polypeptides indicated by thin arrows is in the text. Isoelectric focusing was from left to right and molecular weight separation from top to bottom. The basic end of the isoelectric focusing gel is on the left.

Fig. 2. Demonstration of C100k polypeptide in a T-lymphoblastoid cell line (HPB-ALL) by two-dimensional electrophoresis. Proteins from HPB-ALL cells (120 μg) were subjected to electrophoresis; Coomassie Blue staining. C100k polypeptide is shown by a bold arrow. The polypeptide marked a is actin. The explanation of the other polypeptides indicated by thin arrows is in the text. Gel orientation is the same as in Fig. 1.

Cell Distribution

The C100k polypeptide is one of the abundant polypeptides in T-lymphoblastoid cells, as indicated by a bold arrow in Fig. 2. The polypeptide also exists as one of the 100 or so most intensely radioactive proteins in both fibroblasts and HeLa cells (Fig. 3). The polypeptides shown by thin arrows in Figs. 2 and 3 are some...