Individual Difference in Non–Hemoglobin Proteins of Red Cell Lysate

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SAGISAKA, K., YAMASHITA, H. and IWASA, M. Individual Difference in Non-Hemoglobin Proteins of Red Cell Lysate. Tohoku J. exp. Med., 1980, 132 (2), 239-240 — Non-hemoglobin proteins (NHP) were effectively isolated from red cell lysate by CM-Sephadex chromatography using 0.01 M phosphate buffer pH 6.3. NHP from 180 subjects was analyzed with polyacrylamide gel disc electrophoresis. The individual difference was observed at the intermediately migrating zone after protein staining. Group I had two of the three bands migrating fastly, group II all the three bands, and group III two of the three bands migrating slowly. Incidences of groups I, II and III were 67, 20 and 13 percent, respectively. 

Recent development in protein analysis, electrophoresis, isoelectric focusing or microcolumn chromatography, has been capable of increasing information about polymorphisms in constituents of body fluids. It is well known that red cell lysate is composed of numerous enzymes some of which is revealed to be controlled genetically. These polymorphisms of red cell lysate were determined as a rule by enzyme staining using specific substrate after electrophoresis. In this experiment, NHP was effectively isolated from red cell lysate and analyzed with polyacrylamide gel disc electrophoresis.

MATERIALS AND METHODS

NHP. Adult healthy bloods were kindly provided by the regional Red Cross Blood Bank. NHP was isolated from red cell lysate by CM-Sephadex chromatography as described in a preceding paper (Sagisaka et al. 1980). After dialyzing against 0.01 M phosphate buffer pH 6.3, hemolysate was applied on a column of CM-Sephadex which was equilibrated with the same buffer. Elution with the equilibrating buffer produced three peaks and the initial two ones were pooled and concentrated with an ultrafiltration (Toyokagaku Co., cut off mol. wt. 10,000).

Electrophoresis. Electrophoretic analysis of NHP was performed by the method of Davis (1964); 7% polyacrylamide running gel of pH 8.9 and spacer gel of pH 6.7. After running, the gels were stained with Amidoblack 10 B.

RESULTS AND DISCUSSION

Polyacrylamide gel electrophoretic patterns are shown in Fig. 1. Usually 14 to 15 bands were observed, and intense bands migrating slowly were regarded as carbonic anhydrase isozymes. At the moderately migrating zone consisting of mainly three bands, individual difference was observed; group I had two of the three bands migrating fastly, group II all the three bands and group III two of the three bands migrating slowly. It

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Fig. 1. Examples of polyacrylamide gel disc electrophoretic patterns of NHP.
Nos. 1, 5, 6, 7, 8, 10 and 12 were grouped as Group I, Nos. 4, 9, and 11 as Group II, and Nos. 2 and 3 as Group III. The zone showing individual difference was indicated by the bars. In this figure, some part of anodal zone was cut off.

TABLE 1. Incidence of individual difference of NHP

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of incidence</th>
<th>Percent</th>
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<tbody>
<tr>
<td>I</td>
<td>119</td>
<td>67</td>
</tr>
<tr>
<td>II</td>
<td>36</td>
<td>20</td>
</tr>
<tr>
<td>III</td>
<td>24</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>179</td>
<td>100</td>
</tr>
</tbody>
</table>

One case possessing no band was omitted.

was revealed in the preceding paper (Sagisaka et al. 1980) that the CM-Sephadex chromatography recovered the majority of NHP except for carbonic anhydrase C from the lysate. Since the individual difference on the disc electrophoresis might be caused by the selection of a certain fraction of the chromatography, electrophoretic analyses on the serial eluates from each of the groups were performed, in which all the fractions from each group showed the identical and reproducible pattern. Incidence of each of the three groups among 180 subjects is shown in Table 1. One subject possessing no band was found among the subjects.

The nature of the electrophoretic bands and its genetic study are under investigation.

References