Human Blood Group Glycosyltransferase

II. PURIFICATION OF GALACTOSYLTRANSFERASE*

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A galactosyltransferase, which converts blood group O red blood cells to B-cells, was purified to homogeneity from plasma of blood group B subjects. The stepwise purification procedures include: (a) column chromatography with CM-Sephadex, followed by ammonium sulfate fractionation; (b) Sephadex G-200 gel filtration; (c) column chromatography with DEAE-Sephadex; and (d) column chromatography with hydroxylapatite. The procedures provided about a 400,000-fold increase of specific activity with a 40 to 50% yield. Further purification of the enzyme was performed by small scale preparative acrylamide gel electrophoresis at pH 4.3. The final enzyme preparation showed a single protein band which coincided with enzyme activity, in acrylamide gel electrophoresis, and revealed a single protein band in sodium dodecyl sulfate gel electrophoresis. Judging from the molecular weight, which was estimated by Sephadex gel filtration, and subunit size estimated by sodium dodecyl sulfate-gel electrophoresis, the enzyme is presumably in a dimeric form. The enzyme required Mn++ for its activity and had a pH optimum at 7.0 to 7.5.

EXPERIMENTAL PROCEDURES AND RESULTS

Details of experimental procedures and results are presented in the miniprint supplement which follows.†

DISCUSSION

A galactosyltransferase, which transfers galactose from UDP-galactose to the H-substance of blood group O red cell surfaces, was first purified to homogeneity in the present work. The enzyme was purified 400,000-fold with a 40 to 50% yield by a multistep chromatography and gel filtration procedure. Significant loss of enzyme activity took place in further purification of the enzyme by small scale preparative acrylamide gel electrophoresis at pH 4.3. The enzyme could be clearly separated from contaminating proteins at pH 4.3, but not at pH 8.3 or pH 6.5, on gel electrophoresis. Since the enzyme was labile at pH 4.3, loss of enzyme activity at this last step was inevitable. Various alternative methods, including affinity column chromatography, were attempted without success.

From the approximate purity (25%) and specific activity of the enzyme preparation after the hydroxylapatite chromatography, it can be estimated that the specific activity of the pure enzyme should be about 1.6 × 10^6 units in assay Method 1 and about 1.6 × 10^6 units in assay Method 2.

Judging from the electrophoretic mobility at pH 8.3 and the elution profile from DEAE-Sephadex at pH 7.8, the enzyme is a basic protein, as has been reported with the crude plasma enzyme (6). This enzyme, like the A,-blood group N-acetylgalactosaminyltransferase, described in a previous paper, is in a dimeric form (7).

The success of the purification of galactosyltransferase, which synthesizes blood group B-substance, and N-acetylgalactosaminyltransferase, which synthesizes blood group A-

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† Portions of this paper (including Figs. 1 to 6 and Tables I and II) are presented in the miniprint at the end of this paper. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document 77M-732, cite authors, and include a check or money order for $1.20 per set of photocopies.
substance, permits a study of the structural and immunological homology of these two enzymes and other transferases in human blood. Such studies may lead to an understanding of the underlying genetic mechanism of the expression of blood types.

REFERENCES


Additional references are found below.

TABLE 1

<table>
<thead>
<tr>
<th>Method</th>
<th>Activity after Assay (units/mg)</th>
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<tr>
<td>A</td>
<td>12.3</td>
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<tr>
<td>B</td>
<td>16</td>
</tr>
<tr>
<td>C</td>
<td>14</td>
</tr>
</tbody>
</table>

The partially purified enzyme was obtained from the human liver homogenate by method B.
Human blood group glycosyltransferase. II. Purification of galactosyltransferase.
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