References


Characteristics of High-Affinity Folate Binding in Human Leukocytes

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High-affinity binding of [3H]folate in leukocytes from normal subjects was studied in equilibrium dialysis experiments (pH 7.4, 37 °C). Binding displayed positive cooperativity, and the binding affinity increased with decreasing concentration of the binding protein. Both phenomena could be interpreted in terms of ligand binding to a polymerizing system where the affinity of ligand for the oligomer is greater than its affinity for the polymer prevailing at higher concentrations of the binding protein.

High folate binding activity in granulocytes was first demonstrated in some patients with chronic myelogenous leukemia (1) and subsequently in some women who were pregnant or taking oral contraceptives (2). However, we have recently reported that even granulocytes from normal persons of both sexes were in several cases very active in binding folate (3, 4). Lymphocytes and monocytes contained less than 1% of the total folate binding activity (3).

Here, we characterize the mechanism of folate binding in human leukocytes containing large amounts of binding protein. Binding studies were performed by means of a standardized equilibrium dialysis technique we previously used to characterize high-affinity folate binding in milk and serum (5, 6).

Materials and Methods

[3H]Folic acid with specific activity of 4.8 kCi/mol and 26–45 kCi/mol was purchased from Amersham International Ltd., Amersham, U.K. Venous blood (EDTA-stabilized) was sampled from three healthy women (B.V., I.P. and A.M.H. in ref 3), whose hematological values were normal. Blood samples (5 mL) were layered on top of equal volumes of separation medium composed of five volumes of sodium metrizoate (in premixed kit form; Nycomed, Oslo, Norway) and 11 volumes of a 60 g/L solution of dextran (Pharmacia, Hilleroed, Denmark) in isotonic NaCl (3). The upper leukocyte layer, which could be pipetted off after 1 h, was centrifuged and washed several times in isotonic NaCl to remove contaminating plasma. The albumin concentration in the last wash solution was <0.5 g/L as determined by rocket immunoelectrophoresis (7) with monospecific antibody against albumin (Dakopatts, Copenhagen, Denmark). The leukocyte suspensions, which contained from 10 × 10⁶ to 20 × 10⁶ cells per liter, were subjected to freeze–thaw procedures, then the serine protease inhibitor phenylmethyl sulfonyl fluoride (B.D.H., Poole, England) was added to give a concentration of 1 mmol/L. The cells were homogenized and solubilized in 1 g/L Triton X-100 surfactant (Merck, Darmstadt, F.R.G.), then dialyzed against Tris buffer (0.17 mol/L, pH 7.4) for 24 h at 5 °C to remove endogenous folate, and finally centrifuged. The supernatant fluids were pooled and 600-μL aliquots of the pool were dialyzed to equilibrium (20 h) against [3H]folate in 200 mL of the Tris buffer at 37 °C. One gram of Triton X-100 per liter was added to the fluid on both sides of the dialysis membrane (8). Radioactivity was measured as previously described (9).

Results and Discussion

Folate binding in the supernatant fluid from leukocyte lysate was studied in equilibrium dialysis experiments (37 °C, pH 7.4) at three different concentrations of the binding protein (Table 1). The binding data were analyzed in Scatchard and Hill plots. Three conclusions could be drawn from the data shown in Table 1.

- Maximum folate bound is proportional to the concentration of binding protein.
- A decrease in the concentration of folate binding protein resulted in a decrease of S₀.₅, the external folate concentration required for half saturation of binding; that is, there was a parallel increase in the binding affinity (1/S₀.₅). This dependence of ligand affinity on concentration of binding protein seemed to level off in more diluted lysate solutions.
Table 1. High-Affinity Binding of [3H]Folate in Leukocyte Lysate as a Function of Binding-protein Concentration

<table>
<thead>
<tr>
<th>Relative concn of binder</th>
<th>N&lt;sub&gt;0&lt;/sub&gt; nmol/L</th>
<th>S&lt;sub&gt;0.5&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</th>
<th>n (and SD)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19</td>
<td>0.22</td>
<td>2.4 (0.1)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.1</td>
<td>1.8</td>
<td>0.038</td>
<td>1.5 (0.1)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.02</td>
<td>0.32</td>
<td>0.022</td>
<td>1.27 (0.05)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Binding data from equilibrium dialysis experiments (pH 7.4, 37 °C) with undiluted and diluted leukocyte lysate. Lysate containing undetectable amounts of binder was used as diluent to minimize matrix effects. Maximum folate bound (N) is given by the intersection on the abscissa of Scatchard plots. S<sub>0.5</sub> indicates the free folate concentration at half saturation of binding.

<sup>b</sup> Hill coefficients, n, significantly (p < .001, * p < .01) greater than 1.0.

At all three concentrations of binding, protein folate binding displayed positive cooperativity as indicated by Hill coefficients greater than 1.0 and convex upward Scatchard plots (a typical Scatchard plot of data obtained with 10-fold diluted lysate is shown in Figure 1).

In conclusion, high-affinity folate binding in human leukocytes displays two remarkable characteristics: positive cooperativity and increasing ligand affinity with decreasing concentration of binding protein. By analogy with other systems (10) both these phenomena, which are also associated with folate binding in milk and serum (5, 6, 11), can be interpreted in terms of ligand binding to a polymerizing system where the affinity of ligand for the oligomer is greater than its affinity for the polymer prevailing at higher concentrations of the binding protein.

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References


Fig. 1. Scatchard plot: High-affinity binding of [3H]folate in leukocyte lysate
Equilibrium dialysis experiments (single determinations) at 37 °C and pH 7.4