A high-affinity folate binding protein in normal human leukocytes: Ligand binding characteristics, ionic charge and molecular size

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(Received 26 July 1985)

High-affinity binding of [3H]folate to supernatant from homogenized human leukocytes containing large amounts of binding protein displayed apparent positive cooperativity. The DEAE-Sepharose® CL-6B chromatographic profile of the supernatant at pH 6.3 contained a major peak of folate binding (M_r approx. 25 000) in the front effluent and a smaller more acidic peak (M_r approx. 25 000) that emerged after a rise in NaCl from 30 mmol/l to 1 mol/l. Triton X-100 solubilized cell sediment from the leukocyte homogenate contained some high-affinity folate binding activity (M_r approx 25 000), typically 5-10% of the total binding activity.

A specific folate binder in granulocytes was first demonstrated in patients with chronic myelogenous leukemia (1) and subsequently in women who were pregnant or taking oral contraceptives (2). We have recently reported great interindividual variations, probably genetically controlled, in the folate binding activity of leukocytes from healthy individuals of both sexes (3-5). Thus, values ranging from 2.7 nmol folate bound/10^9 cells to less than 0.01 nmol folate bound/10^9 cells were found (3,4). Lymphocytes and monocytes contained less than 1% of the total folate binding activity (3,4). In the present study the folate binder in normal granulocytes has been characterized as to binding characteristics, ionic charge and molecular size.

Materials and Methods

[3H]Folate with a specific activity of 26-45 kCi/mol was purchased from Amersham International Ltd., Amersham, U.K. Venous blood (EDTA-stabilized) was collected from healthy subjects whose hematological parameters were normal. Preparation of leukocyte homogenate was performed as reported elsewhere (3). After centrifugation of the homogenate the supernatant was harvested while the cell sediment was solubilized in 1 g/l Triton X-100 (Merck, Darmstadt, F.R.G.) and then centrifuged. Supernatant fluids from leukocyte homogenate and Triton X-100 solubilized cell sediment were used for the experiments reported below.
Equilibrium dialysis experiments

Aliquots (600 μl) of the supernatant fluids were dialyzed to equilibrium at 37°C and pH 7.4 against [3H]folate ([3H]pteroylglutamate) as previously described (3).

Ion exchange chromatography

Supernatant fluid from cell homogenate (cf. above) was dialyzed (24 h) at 5°C against 0.05 mol/l imidazole buffer (pH 6.3) prior to DEAE-Sepharose® CL-6B (Pharmacia, Uppsala, Sweden) anion exchange chromatography (6). The column (2.0 cm² × 30 cm) was eluted at 5°C (flow rate 20 ml/h) with 0.05 mol/l imidazole buffer (pH 6.3, 0.03 mol/l NaCl). The NaCl concentration was abruptly raised to 1 mol/l following elution of 200 ml. Maximum folate binding in effluent fractions was determined by equilibrium dialysis against 1.0 nmol/l [3H]folate (cf. above).

Gel chromatography

Front effluent eluted from the DEAE-Sepharose CL-6B column with 0.03 mol/l NaCl was incubated (3 h, 5°C) with [3H]folate (50 nmol/l) in 0.17 mol/l Tris buffer (pH 7.4) containing Triton X-100 (1 g/l) prior to Ultrogel® AcA 44 (LKB) chromatography (6). The column (5.3 cm² × 96 cm) was eluted at 5°C (flow rate 50 ml/h) with Tris buffer containing Triton X-100. Calibration was performed as previously described (6). The above chromatographic procedure was also applied to effluent eluted after the rise in NaCl to 1 mol/l (incubation with 50 nmol/l [3H]folate) as well as supernatant from Triton X-100 solubilized cell sediment (incubation with 100 nmol/l [3H]folate).

Results

High-affinity binding of folate to proteins in supernatant and sediment from leukocyte homogenate

Healthy individuals of both sexes were used as leukocyte donors in experiments where maximum binding of [3H]folate to supernatant from leukocyte homogenate was studied. Maximum binding to supernatant from the Triton X-100 solubilized sediment of the homogenate averaged 5-10% of the total binding activity regardless of interindividual variations in the concentration of leukocyte folate binder.

The remainder of the experiments were performed with leukocytes containing particularly large amounts of folate binding protein. A healthy male individual (N.C.C.) was used as donor. Detailed studies on radioligand binding to aliquots of supernatant from leukocyte homogenate were performed at 37°C and pH 7.4. Fig. 1 shows that folate binding became saturated with a maximum binding of 3.6 nmol/l at a final external concentration of free [3H]folate of 0.1 nmol/l. The final external concentration of free [3H]folate required for half saturation (S₀.₅) was 0.051 nmol/l, which corresponded to an affinity constant (1/S₀.₅) of 2 × 10¹⁰ l/mol (Fig. 1). The binding displayed apparent positive cooperativity as indicated by a convex upward Scatchard plot (not shown) and a Hill coefficient of 2.7 ± 0.5 (h ± SD), significantly (p < 0.05) greater than 1.0.