Reactivation of folate-binding protein from cow's milk, purified by affinity chromatography in the presence of lecithin and other surfactants

Steen Ingemann HANSEN, Jan HOLM, and Jørgen LYNGBYE

Department of Clinical Chemistry, Research Division, Central Hospital, DK-3400 Hillerød, Denmark

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Purification by affinity chromatography of the folate-binding protein from cow's milk leads to a drastic lowering of the affinity of the protein for folate. The purified protein was, however, completely reactivated in the presence of a number of surfactants (phospholipids and synthetic detergents) particularly of cationic or non-ionic type. There is a striking analogy between the present results and the well-known lipid/detergent reactivation of certain purified membrane-derived enzymes.

Folate-binding protein isolated from whey of cow's milk after ion-exchange chromatography can be further purified on an affinity matrix of folate- or methotrexate-coupled Sepharose (1-4). However, as recently shown by us, this results in a change of basic binding properties (4). That is, there was a shift from positive to negative cooperativity in diluted solutions of the protein (4). The phenomenon could be reversed upon enrichment of the binder solution with material not adsorbed to the affinity column which indicated that some activator(s) of folate binding had been removed during affinity-chromatography (4). The activator material tolerated boiling and treatment with protease, but not combustion (4). Furthermore, the presence of activator(s) was recently demonstrated in sera and erythrocyte hemolysates (5).

It is now well established that a large number of membrane-derived enzymes which have been purified and thus deprived of their natural lipid environment require the presence of lipids or detergents (surfactants) for optimum activity (6,7). Reasoning by analogy, we considered it of interest to study the effect of some naturally occurring lipids and synthetic detergents on folate binding.

Materials and Methods

The following chemicals were used: egg lecithin (Merck); dipalmitoyl-L-α-phosphatidyl choline, egg L-α-lysophosphatidyl choline, bovine brain L-α-phosphatidylethanolamine, and bovine brain L-α-phosphatidyl-L-serine (Sigma); Triton X-100 (Struers, Denmark); cetyltrimethylammonium bromide, cholic acid, and sodium dodecyl sulfate (SDS) from BDH. [3H]folic acid with a specific activity of 5 Ci/mmol and 40-46 Ci/mmol was supplied by the Radiochemical Centre, Amersham, England.
Highly purified folate-binding protein was produced from cow's whey by a combination of CM-Sepharose® CL-6B cation-exchange chromatography and chromatography on a methotrexate-AH-Sepharose® 4B affinity matrix in accordance with procedures recently developed in our laboratory (4,8). Effluent collected after adsorption of the binding protein to the affinity matrix (4) was boiled for daily periods of 4 h (3 d) and then centrifuged. The supernatant referred to as activator had no folate-binding activity. Binding of [3H]folate was studied in equilibrium dialysis experiments with the binding protein in Tris buffer (pH 7.4) at 37°C as previously described (9).

Results

The binding of folate to solutions of binder purified by affinity chromatography (maximum binding >50 nM folate) displayed positive cooperativity (data not shown). Dilution of the binder solution resulted in a loss of this important binding property (cf. Fig. 1, Scatchard plot in the absence of activator). However, enrichment of the binder solution with activator restored positive cooperativity, as indicated by the downward concavity of the Scatchard plot (Fig. 1).
and a Hill coefficient, $n$, of $1.4 \pm 0.1$ (significantly higher than $1.0$, $P < 0.001$). The folate concentration required for half saturation of binding ($S_{0.5}$) was 0.13 nM in the presence of activator, i.e. almost 50-fold lower than the value in the absence of activator. (The reciprocal of the $S_{0.5}$ value can be taken as an expression of binding affinity.)

As shown in Fig. 2, addition to the binder solution of egg lecithin (0.1 mM) gave rise to an effect which seemed to be indistinguishable from that of the activator ($S_{0.5} = 0.13$ nM and $n = 1.30 \pm 0.07$, $P < 0.001$). The lecithin solution had no folate-binding activity. No effect on folate binding was observed in the presence of 1 µM lecithin, in which case the Scatchard plot (Fig. 2) was indistinguishable from that found in the absence of activator (Fig. 1). Intermediate values were found in the presence of 10 µM lecithin, however, without restoration of positive cooperativity (Scatchard plot not shown).

The binding of folate (1 nM) as a function of the lecithin concentration in the binder solution was studied (Fig. 3). Lecithin present at concentrations ranging from >1 µM to <0.1 mM gave rise to increasing binding of folate, and a maximum value for folate binding similar to that found in parallel experiments with activator-enriched binder solutions was obtained in the presence of 0.1 mM lecithin (Fig. 3). There was no further increase in the amount of folate bound at lecithin concentrations above 0.1 mM.

Other naturally occurring or synthetic surfactants increased folate binding from a 1-nM solution (Table 1). Those were the three cationic phospholipids listed in Table 1, the detergents cetyltrimethylammonium bromide (cationic) and Triton X-100 (non-ionic). Furthermore, 20 mM cholate (anionic) and 1 mM L-α-phosphatidyl-L-serine (anionic) increased folate binding to 70% of the maximum value - however, without restoration of positive cooperativity. No reactivation was seen in the presence of the anionic detergent SDS.

Discussion

The present study has demonstrated that a number of naturally occurring surface-active phospholipids and synthetic detergents reactivate the folate-binding protein from cow's whey, purified by affinity chromatography. Lipids/detergents of cationic or non-ionic
type seemed to act as potent activators, whereas the effect of anionic substances was weak and uncertain. Obvious parallels can be drawn from the present data to the lipid/detergent-reactivation of purified membrane-derived enzymes, e.g. pyruvate oxidase (10,11). Binding of the catalytic ligand, i.e. thiamine pyrophosphate, to this enzyme exhibited positive cooperativity in the presence of phospholipid which acted as an allosteric effector (11).

The biological significance of the lipid-activation phenomenon described herein is obscure. Membrane-derived lipids may play an important role in the overall regulation of high-affinity folate binding. Furthermore, in view of the fact that liposomes can act as vectors for biologically active substances captured in their internal aqueous compartments or associated with their lipid membranes (12,13), there is the interesting possibility that lipid-protein interactions may be involved in trans-membrane transport of folate.

Fig. 2. Scatchard analysis of $[^3H]$folate binding (cf. Fig. 1). Experiments with addition of 1 µM (●) or 0.1 mM (○) lecithin.
Fig. 3. Binding of [3H]folate as a function of the lecithin concentration of the binder solution. Experimental conditions as in Fig. 2. The [3H]-folate concentration was 1 nM.

Table 1. Reactivation of folate-binding protein from cow's milk, purified by affinity chromatography in the presence of various surfactants (lipids and synthetic detergents)

For details see the legends of Figs. 2 and 3.

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Concentration of surfactant required for achievement of maximum folate binding and restoration of positive cooperativity</th>
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<tbody>
<tr>
<td>L-α-phosphatidyl choline</td>
<td>0.1 mM</td>
</tr>
<tr>
<td>L-α-phosphatidyl ethanolamine</td>
<td>0.1 mM</td>
</tr>
<tr>
<td>L-α-lysophosphatidyl choline</td>
<td>0.1 mM</td>
</tr>
<tr>
<td>Cetyltrimethylammonium bromide</td>
<td>0.01 mM</td>
</tr>
<tr>
<td>Triton X-100</td>
<td>1 g/l</td>
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</tbody>
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References