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Characterization of the folate-binding proteins associated with the plasma membrane of rat liver

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Unsaturated folate-binding proteins (i.e., apo forms) have been identified with the plasma membranes of rat liver by the binding of [^{3}H]pteroylglutamic acid. Normal rat liver contains very little of the folate-binding apoproteins, but the folate-binding capacity increases substantially when the rats are made folate-deficient. This increase appears to be due to unsaturation of the folate-binding holoproteins rather than to synthesis of additional protein, because the binding capacity of the plasma membranes from normal rat liver following dissociation of the bound folate is equivalent to the binding capacity of the preparation from folate-deficient liver. Two molecular forms of folate-binding protein were identified by gel filtration of the solubilized plasma membrane fraction, a high-molecular-weight form ($M_r < 100\,000$), representing 25% of the binding capacity, and a smaller protein ($M_r \approx 55\,000$), representing 75% of the binding capacity. Whereas the larger species can be solubilized only with a detergent, the smaller form appears to be hydrophilic and dissociates spontaneously from the membrane preparation. The binding of [^{3}H]pteroylglutamic acid by the membrane preparation was specific, saturable, and pH- and temperature-dependent. Scatchard analysis of the binding could be fitted to a curvo-linear plot, indicating at least two orders of binding sites which probably correspond to the two molecular forms identified by gel filtration. Competitive inhibition by folate analogues demonstrated that the apoproteins have higher affinity for oxidized folate than for N^5-methyltetrahydrofolate and virtually no affinity for N^5-formyltetrahydrofolate or methotrexate.

Introduction

Membrane-associated and soluble forms of folate-binding proteins have now been found in many different mammalian cells, tissues and biological fluids [1]. Many of these folate-binding proteins have been identified by the binding of [^{3}H]pteroylglutamic acid (PteGlu) because they are present as apoproteins. The liver, however, which contains the largest store of folates, has very little unsaturated folate-binding protein, though the intracellular folates are bound to several macromolecules [2,3,4]. These folate-binding macromolecules were identified by administering [^{3}H]PteGlu to rats and then analyzing the protein-bound forms of labeled folate in the subcellular fractions of the liver. The folate-binding proteins which became saturated with labeled folates in vivo were present in the cytosol and

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Abbreviations: PteGlu, pteroylglutamic acid; HBSS, Hanks' balanced salts solution; 5-methylH$_4$PteGlu, N^5-methyltetrahydrofolate; 5-formylH$_4$PteGlu, N^5-formyltetrahydrofolate.

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mitochondria, and have now been purified and characterized [5–9]. Although these fractions bound very little [^{3}H]PteGlu in vitro, one folate-binding apoprotein which did bind was associated with the plasma membrane. The binding of PteGlu to this protein was saturable and pH- and temperature-dependent [4]. However, the structural properties of this membrane-associated folate-binding protein, which was present in very low concentration, were not studied any further.

Because the livers of folate-deficient rats contained several-fold less bound folates than normal rat liver [10], and because folate deficiency appeared to induce the folate-binding apoprotein in cultured KB cells, a human epidermoid cell line [11], we made rats folate-deficient to similarly augment the folate-binding apoprotein in the liver. This report describes this experiment and the properties of the folate-binding apoprotein(s) obtained from this organ.

Materials and Methods

[^{3}H]PteGlu (spec. act. 37–40 Ci/mmol) was obtained from Amersham. Its purity, determined by $ZnSO_4$ precipitation [12], was greater than 95%. PteGlu, dihydrofolic acid, N^5-methyltetrahydrofolate (5-methylH_4PteGlu), phenylmethylsulfonyl fluoride and Norit A charcoal were purchased from Sigma. N^5-Formyltetrahydrofolate (5-formylH_4PteGlu) and methotrexate were obtained from Lederle Laboratories. The methotrexate was purified before use by the method of Gallelii and Yokoyama [13]. The purity and concentration of PteGlu and its analogues were determined spectrophotometrically [14]. Hanks' balanced salts solution (HBSS) was purchased from Gibco Laboratories. Trasylol was obtained from the Mobay Chemical Corp. Millipore filters (type HA, 0.45 μm) were purchased from Millipore. Folate-deficient rat chow was purchased from ICN Laboratories. Holtzman rats were purchased from Charles River Laboratories. Complete scintillation cocktail (3a70) was purchased from Research Products International, 2,5-diphenyloxazole (PPO), 1,4-bis-(2(4-methyl-5-phenyloxazolyl)) benzene (POPOP) and NCS solubilizer were purchased from Amersham. All other chemicals used were of reagent grade.

Preparation of liver homogenates

Rats were made folate-deficient by feeding them the folate-deficient diet and water containing neomycin (1 mg/l) for 4–6 weeks. Control rats were fed normal laboratory chow. To obtain the livers, rats were fasted for 24 to 48 h, killed by decapitation, and, after draining the blood by gravity, the livers were removed as rapidly as possible and placed in ice-cold 0.15 M NaCl. Each liver was then quickly blotted to remove excess fluid, weighed, finely minced, and suspended in 5 vols. of cold 0.02 M Tris buffer (pH 7.4)/0.32 M sucrose/0.02 M KCl/0.003 M $MgCl_2$/0.5 mM phenylmethylsulfonyl fluoride/1000 U/l Trasylol. These and subsequent steps were carried out at 4 °C. The suspension was gently homogenized with a motor-driven Potter-Elvehjem homogenizer using a Teflon pestle. The homogenate was centrifuged at $1000 \times g$ for 10 min, and the pellet containing the tissue debris, intact cells and nuclei was discarded. The supernate was then subjected to differential centrifugation, first at $5000 \times g$ and then at $11\,000 \times g$ for 20 min each, followed by $100\,000 \times g$ for 1 h. The final supernatant fraction (cytosol), was stored at −40 °C. Each of the above pellets was washed once in HBSS and repelleted by centrifugation at the g force used to pellet it initially, and then it was suspended in 5 vols, HBSS containing the proteinase inhibitor, and stored at −40 °C.

Measurement of folate

Ascorbic acid was added to an aliquot of each subcellular fraction (final concentration 5 mg/ml) which was suspended in the HBSS. One aliquot of the suspension was then boiled for 10 min and the deproteinated extract was assayed for total folate using a sequential radioligand-binding assay [15]. Another aliquot of each suspension was dissolved in 1 M NaOH and the protein concentration was determined [16].

Measurement of folate-binding apoprotein

An aliquot of the thawed particulate suspension was incubated with 2.3 pmol of [^{3}H]PteGlu and increasing concentrations of unlabeled PteGlu in a total volume of 0.5 ml of HBSS for 30 min, and this was then passed through a Millipore filter (Type HA, 0.45 μ). The filter which retained the

bound [^{3}H]PteGlu was washed with 10 ml of HBSS, dried, solubilized in 1 ml of NCS solubilizer containing 0.1 ml of 30% H_2O_2 and counted in a toluene scintillation cocktail containing PPO (7 mg/l) and POPOP (150 mg/l). Nonspecific binding was determined in a parallel reaction in which the sample was preincubated with 2.3 nmol unlabeled PteGlu.

The total binding capacity of the corresponding fractions prepared from normal rat liver was determined by an exchange of the bound endogenous folate with [^{3}H]PteGlu at an acidic pH. The pH of the incubation mixture containing an aliquot of the membrane preparation and a 10-fold excess of [^{3}H]PteGlu over the endogenous folate concentration was lowered to 3.0 with 1 M citric acid. After 30 min, the reaction was neutralized with 1 M sodium phosphate buffer (pH 7.4), and the incubation was continued for an additional 30 min. The reaction mixture was then passed through the Millipore filter as described above.

The relative affinity of folate analogues (vis-à-vis PteGlu) for the folate-binding protein was determined from the competitive inhibition by the folate analogue of the binding of [^{3}H]PteGlu. Accordingly, the test sample was added to a mixture of 2.3 pmol [^{3}H]PteGlu and 2.3–23.0 pmol of the folate analogues. The reactions containing the reduced folate analogue also contained 1 mM 2-mercaptoethanol. Bound [^{3}H]PteGlu was determined as described above for assaying the apoprotein in the particulate fraction.

Radioactivity was measured using a TM Analytical scintillation spectrometer with automatic external standardization. Sufficient counts were accumulated for a counting error of approx 3% or less.

Sucrose-density-gradient centrifugation

The 11 000 and 100 000 × g pellets were preincubated with [^{3}H]PteGlu, washed to remove unbound [^{3}H]PteGlu, suspended in 1 ml of 5% sucrose and applied to a discontinuous sucrose gradient of 5, 15, 30, 45 and 60% sucrose in a 1.5 × 15 cm tube and centrifugated at 100 000 × g for 18 h. Fractions (0.5 ml) were removed from the top of the gradient tube and each fraction was counted for bound radioactivity and assayed for marker enzymes.

Marker enzyme assays

5′-Nucleotidase and glucose-6-phosphatase, markers for plasma membranes and endoplasmic reticulum, respectively, were measured by the method of Aronson and Touster [17]. Acid phosphatase, a lysosomal marker, was measured by the method of Trouet [18], succinate dehydrogenase, a mitochondrial marker, was measured by the method of Lester and Smith [19].

Isolation of plasma membranes

The plasma membranes were isolated from a folate-deficient rat liver by homogenizing the liver in 0.25 M sucrose followed by separation of the subcellular fractions by zonal centrifugation through sucrose, following the method of Aronson and Touster [17]. The plasma membranes which segregated with the nuclear and microsomal fractions were isolated from both of these fractions and designated P_1 and P_2, respectively. The folate-binding capacity was measured in the two fractions containing the isolated plasma membranes, in the fraction which contained the mitochondria and lysosomes, and in the microsomal fraction using [^{3}H]PteGlu. Each fraction was subjected also to analysis for the enzymatic activity listed above.

Solubilization and gel filtration

An aliquot of the 100 000 × g pellet from the folate-deficient liver preparation and an aliquot of the P_2 plasma membranes were suspended in 1 ml of HBSS and 1 ml of 5 mM Tris buffer (pH 7.4), respectively, and sufficient [^{3}H]PteGlu was added to exceed its binding capacity. The suspension was then incubated at 37 °C for 30 min and solubilized in Triton X-100 (1%, v/v) for 18 h at 4 °C. The insoluble debris was pelleted by centrifugation at 100 000 × g for 60 min and the supernatant fraction was subjected to gel-filtration chromatography.

A second aliquot of the 100 000 × g pellet from the folate-deficient liver preparation was incubated with [^{3}H]PteGlu in 1 ml of HBSS for 30 min at 37 °C and the suspension was centrifuged at 100 000 × g for 1 h before solubilization. The supernatant fraction was separated and the pellet was then solubilized in 1 ml of HBBS containing 1% Triton X-100 (v/v). Any insoluble material

was removed by centrifugation at 100000 × *g* for 1 h. Both supernatant fractions were subjected to gel-filtration chromatography.

A Sephadex G-100 column (1.5 × 80 cm) was equilibrated in 0.01 M phosphate buffer (pH 7.4)/0.15 M sodium chloride/1% Triton X-100 (v/v) at a flow rate of 30 ml/h at 4°C. The column was calibrated with blue dextran, hemoglobin, ovalbumin, cytochrome *C* and free [^{3}H]PteGlu. The sample (1 ml) was applied to the column and 1-ml fractions were collected. The radioactivity in an aliquot of each fraction was then determined.

Results

Following 4 weeks on the folate-deficient diet, the folate content of the 5000 × *g*, 11000 × *g* and 100000 × *g* pellet prepared from the liver homogenates ranged from 12% to 35% of the value obtained from the corresponding fractions from normal rat liver (data not shown). All the rats used for this study were on the folate-deficient diet for no less than 4 weeks.

The binding of [^{3}H]PteGlu by the subcellular fractions of three normal and three folate-deficient rat livers is shown in Table I. Each particulate subcellular fraction from the folate-deficient liver bound substantially more [^{3}H]PteGlu than did the corresponding fraction from normal liver. However, because the number of animals studied was small, the statistical significance of this difference was not computed. There was no difference in the binding capacity of the fractions from normal and folate-deficient livers when measured after removal of the endogenous folate (data not shown). In addition, saturable and specific binding of [^{3}H]PteGlu could not be detected in the cytosolic fraction from either the folate-deficient or normal livers.

Because of the higher unsaturated binding capacity of the particulate fractions from folate-deficient livers, only those preparations were used to characterize the properties of the binding protein(s).

The results of the sucrose-density-gradient fractionation of the 11000 × *g* and 100000 × *g* pellets of the liver preparation are shown in Fig. 1. The peaks of bound [^{3}H]PteGlu and 5′-nucleotidase activity corresponded exactly in the gradient fractions and were clearly separated from the acid phosphatase, succinate dehydrogenase and glucose-6-phosphatase activities, the marker enzymes for lysosomes, mitochondria and microsomes, respectively. Thus, the folate-binding apoprotein in the preparation appeared to be associated with the plasma membranes.

The process of isolation of the plasma membranes from the liver of a folate-deficient rat by zonal centrifugation of the homogenate prepared in 0.25 M sucrose yielded a number of fractions which were assayed for the binding of [H^3]PteGlu and marker enzymes (Table II). In addition, electron microscopy of the fractions containing the plasma membranes demonstrated little contamination with other subcellular organelles (not shown). The fractions which contained the isolated plasma membranes (P_1 and P_2) contained, respectively, 8% and 11% of the total [^{3}H]PteGlu binding capacity of the liver homogenate and 12.4% and

TABLE I

BINDING OF [^{3}H]PteGlu BY THE SUBCELLULAR FRACTIONS OF LIVERS FROM NORMAL AND FOLATE-DEFICIENT RATS

The binding capacity for [^{3}H]PteGlu was measured in an aliquot of each fraction and then computed for the total fraction in the liver homogenate.

Fraction	[^{3}H]PteGlu binding capacity (ng/total fraction)							
	Normal rats			Mean ± S.D.	Folate deficient rats			Mean ± S.D.
	1	2	3		1	2	3	
5000 × *g* pellet	0.24	0.10	0.80	0.38 ± 0.37	–	6.9	4.1	5.5 ± 1.97
11000 × *g* pellet	0.56	0.12	1.10	0.59 ± 0.49	12.8	21.0	15.3	16.4 ± 4.2
100000 × *g* pellet	0.21	0.10	1.10	0.47 ± 0.49	5.8	8.4	1.5	5.2 ± 3.3

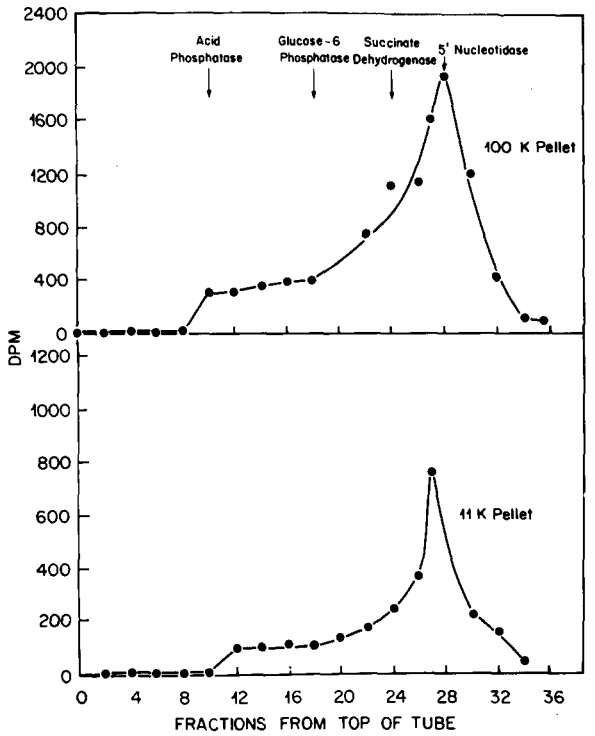

Fig. 1. Binding of [^{3}H]PteGlu by the subcellular fractions of rat liver subjected to centrifugation through a discontinuous sucrose-density gradient. Each fraction (0.5 ml) was removed from the top of the centrifuge tube and counted for bound [^{3}H]PteGlu and analyzed for the activity of marker enzymes.

24.2% of the total 5′-nucleotidase, the plasma membrane enzyme marker. These fractions contained less than 1% of the total glucose-6-phosphatase and succinate dehydrogenase activity, the enzyme markers for microsomes and mitochondria, respectively. The mitochondrial and microsomal fractions, which contained 9% and 4% of the total bound [^{3}H]PteGlu, also contained 13.5% and 18.7% of the 5′-nucleotidase activity. There was, therefore, considerable contamination of these fractions with plasma membranes, and this would account for the observed binding of [^{3}H]PteGlu. If the folate-binding protein was associated with the mitochondria or the microsomes, the binding of [^{3}H]PteGlu by these fractions would have been much greater, since they contained more than 70% of these subcellular organelles. The [^{3}H]PteGlu binding capacity of the folate-binding apoprotein did not parallel the 5′-nucleotidase activity of the plasma membranes, because a fraction of the folate-binding apoprotein is unstable in the absence of its binding ligand (vide infra) and is lost during the preparation. However, the specific activity of bound [^{3}H]PteGlu (pg per mg protein) was highest in the fractions containing the isolated plasma membranes.

Because the folate-binding apoprotein pelleted with the plasma membranes at $11\,000 \times g$ and $100\,000 \times g$, in subsequent studies, either the $11\,000 \times g$ or $100\,000 \times g$ pellet was used to characterize the properties of the folate-binding protein.

The binding of [^{3}H]PteGlu was pH- and temperature-dependent, as shown by the data in Fig.

TABLE II

DISTRIBUTION OF [^{3}H]PteGlu BINDING AND ENZYME ACTIVITY IN SUBCELLULAR FRACTIONS OF RAT LIVER

Values were calculated from the total protein, total binding capacity for [^{3}H]PteGlu or the total enzyme activity in the whole liver homogenate.

Fraction	Percentage of total activity					
	protein	[^{3}H]PteGlu binding	5′nucleotidase	glucose-6-phosphatase	succinate dehydrogenase	spect. act. pg [^{3}H]PteGlu bound per mg protein
Plasma membranes from nuclear fraction (P_1)	1.68	8	12.4	0.55	0.83	26
Plasma membranes from microsomal fraction (P_2)	1.82	11	24.2	0.93	0.42	48
Mitochondrial and lysomal	22.7	9	13.5	20.7	72	4.2
Microsomal	29.2	4	18.7	78.2	4.8	10

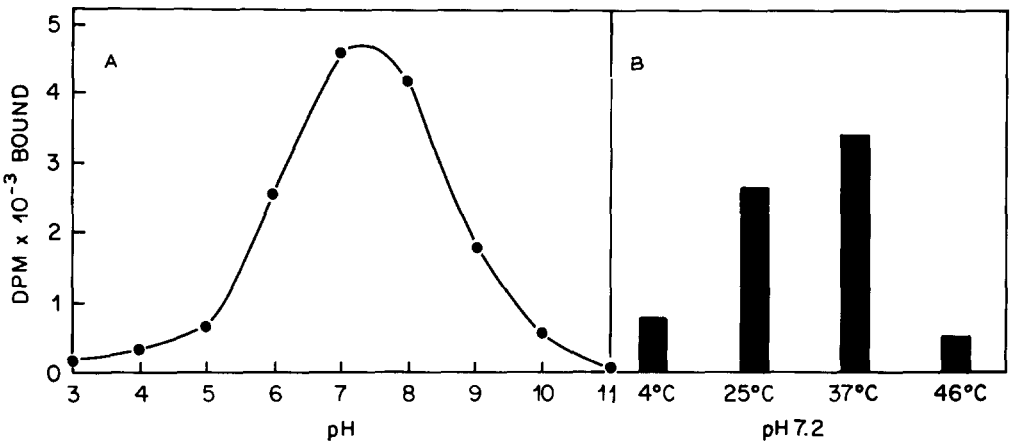

Fig. 2. Effect of pH and temperature on the binding of [^{3}H]PteGlu by the 11000 × g pellet prepared from the homogenate of the liver of a folate-deficient rat.

2. The binding was maximal at pH 7.0 and decreased substantially below and above this pH. The binding of [^{3}H]PteGlu was also maximum at 37 ° C, with considerably less binding at 4 or 46 ° C.

The kinetics of the binding of [^{3}H]PteGlu by the folate-binding protein associated with the 100000 × g pellet was determined over a range of ligand concentrations, and the results were subjected to Scatchard analysis [20] (Fig. 3). The curvolinear plot indicates the presence of at least two orders of binding sites. Tangents were drawn to the extremities of the curves, as described by Berson and Yalow [21], and the slopes of these lines were used to obtain the dissociation constants (K_d). Although K_d values of 1 and 7.4 nM were computed, the true values are likely to be

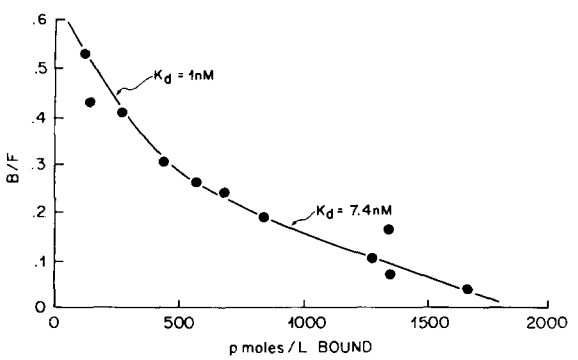

Fig. 3. Scatchard plot of the binding of [^{3}H]PteGlu by the 100000 × g pellet prepared from the homogenate of the liver of a folate-deficient rat.

lower, since nonspecifically bound endogenous folate present in the sample may have eluted from the pellet and decreased the specific activity of the radiolabeled folate.

The relative affinity of folate analogs, vis-à-vis PteGlu, was determined for the folate-binding apoproteins associated with the 100000 × g pellet. The concentration of each analog required to inhibit by 50% the binding of [^{3}H]PteGlu is listed in Table III. The tri-, penta- and heptaglutamates of PteGlu inhibited the binding of [^{3}H]PteGlu at a concentration equal to PteGlu, while a 17-fold and 40-fold greater concentration of dihydrofolic acid and 5-methylH_4PteGlu, respectively, were required to produce a similar inhibition. Even an 80-fold greater concentration of 5-formylH_4PteGlu and methotrexate did not inhibit the binding of [^{3}H]PteGlu.

The results of the gel filtration studies are shown in Fig. 4. When the 100000 × g pellet suspension, following incubation with [^{3}H]PteGlu, was solubilized with Triton X-100 and filtered through the Sephadex G-100 column, the bound [^{3}H]PteGlu eluted as two peaks (Fig. 4A). The larger molecular weight species eluted just after the void volume, and thus had an apparent M_r of less than 100000. This peak represented 25% of the bound radioactivity, while the smaller molecular weight species, which had an apparent M_r of approx. 55000, represented 75% of the bound [^{3}H]PteGlu. When the 100000 × g pellet suspen-

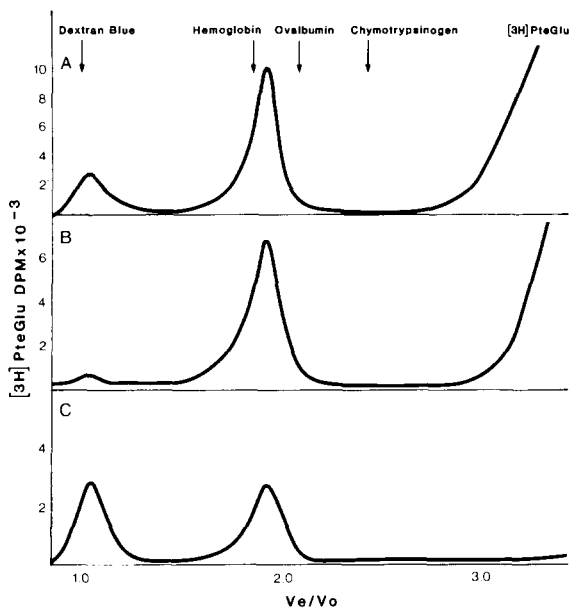

Fig. 4. Gel-filtration chromatography of [^{3}H]PteGlu bound to preparations of the 100000 × *g* pellet and isolated plasma membranes of a homogenate of the liver from a folate-deficient rat. (A) 1 ml of the 100000 × *g* pellet was suspended in HBSS and incubated for 30 min at 37 °C with 23 pmol [^{3}H]PteGlu and then solubilized in 1% Triton X-100 for 24 h. The 100000 × *g* supernate of the solubilized material was applied to the column. (B) 1 ml of the 100000 × *g* pellet suspension was incubated for 30 min at 37 °C with 23 pmol [^{3}H]PteGlu and then centrifuged for 2 h at 100000 × *g*. The supernate was applied to the column without Triton X-100. (C) The pellet obtained from the above incubation (B) was solubilized in Triton X-100 for 24 h, and the solubilized material was applied to the column.

TABLE III

RELATIVE AFFINITY OF THE MEMBRANE-ASSOCIATED FOLATE-BINDING APO-PROTEIN IN THE 100000 × *g* PELLET FOR FOLATE ANALOGUES

Folate analogue	Concentration required to inhibit by 50% the binding of [^{3}H]PteGlu by the analogue (nM)
PteGlu, $PteGlu_3$, $PteGlu_5$, $PteGlu_7$	1.0
Dihydrofolic acid	17.0
5-MethylH_4PteGlu	40.0
5-FormylH_4PteGlu	– [a]
Methotrexate	– [a]

[a] There was no inhibition of the binding of [^{3}H]PteGlu at the maximum concentration (80 nM) of these analogues tested.

sion, following incubation at 37 °C with [^{3}H]PteGlu, was first centrifuged at 100000 × *g* for 1 h, and the supernate (without Triton X-100) and pellet (solubilized in Triton X-100) were chromatographed separately, the larger molecular species was virtually absent in the supernate, and virtually all of the bound [^{3}H]PteGlu eluted as the low-molecular-weight species (Fig. 4B). The solubilized pellet from this experiment still contained the two forms of bound folate (Fig. 4C), but the proportion of the higher-molecular-weight species was now greater because of the dissociation of the smaller species from the membrane component of this subcellular fraction.

Discussion

In this study, we describe the properties of a folate-binding apoprotein which is associated with the plasma membrane of rat liver. Zamierowski and Wagner [4] first identified this apoprotein when they observed that, 30 min after injecting rats in vivo with [^{3}H]PteGlu, most of the bound radioactivity in the liver was present in the subcellular fractions which contained the plasma membranes. The same subcellular fraction also bound a small amount of [^{3}H]PteGlu in vitro. This membrane folate-binding apoprotein, which is present in very small quantities in normal rat liver, had not been characterized any further, though the folate-binding proteins in the cytosol and mitochondria of rat liver have been purified and their properties studied [5–9]. By making rats folate-deficient, the folate binding protein(s) associated with the plasma membrane fraction became unsaturated, permitting the characterization of the protein in the native state.

The folate-binding apoprotein, which was present in the particulate fraction of the liver homogenate, fractionated with the plasma membranes through a sucrose gradient. Furthermore, when the plasma membranes were purified by zonal centrifugation, they contained the highest specific activity for the binding of [^{3}H]PteGlu, confirming that this folate-binding apoprotein is indeed associated with this component of the liver cell. We were not able to identify any specific unsaturated folate-binding protein in the cytosol of these livers.

The folate-binding protein which we found with

the plasma membranes has properties that are distinctly different from those previously purified from the cytosol and mitochondria of rat liver. The cytosolic folate binding proteins were reported to have M_r values of approx. 350000, 150000 and 25000 by gel filtration [4]. The 350 kDa protein apparently binds the pentaglutamate of tetrahydrofolate with the highest affinity, and has very little affinity for PteGlu. The 150 kDa folate-binding protein binds the pentaglutamate of 5-methylH_4PteGlu with the highest affinity, and has been shown to be the enzyme glycine *N*-methyltransferase [9]. The folate-binding protein which was associated with rat liver mitochondria had an M_r of approx. 90000, bound the fully reduced polyglutamates of folate with the highest affinity, and has now been shown to consist of two closely related enzymes, dimethylglycine dehydrogenase and sarcosine dehydrogenase [6,7].

The properties of the plasma membrane folate-binding apoproteins of the rat liver, on the other hand, are more like those of the membrane-associated folate-binding proteins identified and isolated from other tissues, such as rat intestinal epithelial cells [22], rat kidney epithelial cells [23], human placenta [24], liver [25], leukemia cells [26,27], and the cultured KB cell line [28], all of which have a higher affinity for PteGlu than for the reduced folates.

Another similarity between this folate-binding protein in the plasma membrane of the rat liver and the membrane-associated folate-binding proteins from other tissues is that, following solubilization, they separate chromatographically into a larger hydrophobic protein and a smaller protein with apparently hydrophilic properties. This has been demonstrated for the membrane-associated folate-binding protein in human leukemia cells [26,27] and KB cells [28]. Correcher et al. [25] also observed that the folate-binding proteins in isolated plasma membranes from human liver can be separated into a large hydrophobic molecular weight protein and a smaller peripheral protein which dissociated from the membrane in 0.5 M NaCl solution.

The hydrophilic folate-binding protein in the plasma membrane of the rat liver dissociates spontaneously from the membrane. This dissociation was not observed initially, because this species of the folate-binding protein is unstable as a soluble apoprotein and it was only identified when excess PteGlu was added to the suspension of the particulate fraction. This instability has hampered efforts to study the properties of this form of folate-binding protein.

The location of the folate-binding protein in the plasma membrane of cells has prompted the notion that this protein functions in the cellular uptake of folate. Indeed, Antony et al. [29] have demonstrated that an antiserum to the purified membrane-associated folate-binding protein from KB cells inhibited the uptake of 5-methylH_4PteGlu by intact KB cells. However, there are other data which indicate that the membrane-associated folate binder may have other functions. For example, McHugh and Cheng observed that the membrane-associated folate-binding protein of KB cells contained polyglutamate forms of folate [11] and this would suggest that this protein may play same role in the storage of folate or in the biosynthesis of folyl polyglutamates.

In this study, we observed that the folate-binding protein associated with the plasma membrane of rat liver has such low affinity for methotrexate and 5-formylH_4PteGlu as to be virtually undetectable by the method of competitive inhibition, yet this protein has substantial affinity for 5-methylH_4PteGlu. Studies of folate transport in perfused rat livers, however, show that the uptake of methotrexate is substantially higher than the uptake of 5-methylH_4PteGlu and PteGlu [30], and in isolated rat hepatocytes, 5-methylH_4PteGlu uptake is completely inhibited by methotrexate [31]. Thus, the hypothesis that the prime function of the membrane-associated folate-binding protein is that of folate transport is not supported by our finding that this protein in rat liver has little or no affinity for either methotrexate or 5-formylH_4PteGlu.

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