

Preferential binding of growth inhibitory prostaglandins by the target protein of a carcinogen

(liver fatty acid binding protein/hepatocytes/2-acetylaminofluorene)

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ABSTRACT Liver fatty acid binding protein (L-FABP) is the principal target protein of the hepatic carcinogen *N*-(2-fluorenyl)acetamide (2-acetylaminofluorene) in rat liver. In addition, the cyclopentenone prostaglandins (PG), PGA, PGJ₂, and Δ^{12} -PGJ₂, inhibit the growth of many cell types *in vitro*. This report describes the preferential binding of the growth inhibitory prostaglandins by L-FABP and the reversible inhibition of thymidine incorporation into DNA by PGA₂ and Δ^{12} -PGJ₂ in primary cultures of purified rat hepatocytes. As a model ligand, [³H]PGA₁ bound to L-FABP specifically, reversibly, rapidly, and with high affinity. Its dissociation constants were 134 nM (high affinity) and 3.6 μ M (low affinity). The high-affinity binding of [³H]PGA₁ was 9- and \approx 13-fold more avid than the binding of the conventional fatty acid ligands, oleic acid and arachidonic acid, respectively. The abilities of different prostaglandins to compete with the high-affinity binding of [³H]PGA₁ correlated with their growth inhibitory activities reported previously and here. The growth inhibitory cyclopentenone prostaglandins (PGA₁, PGA₂, Δ^{12} -PGJ₂, and PGJ₂) were the best competitive ligands, intermediate competitors were the weak growth inhibitors PGE₁ and PGD₂, and the poorest competitors were PGE₂ and PGF_{2 α} , which stimulate rather than inhibit DNA synthesis in rat hepatocytes in primary culture. The *in vitro* actions of L-FABP are compatible with those of a specific and dissociable carrier of growth inhibitory prostaglandins in rat hepatocytes and suggest that the carcinogen may usurp the cellular machinery of the growth inhibitory prostaglandins.

Early events during chemical carcinogenesis usually involve a growth inhibition that in effect localizes the proliferation of chemically transformed cells to regions of focal hyperplasia surrounded by growth-suppressed nontransformed cells (1). The liver carcinogen *N*-(2-fluorenyl)acetamide (2-acetylaminofluorene, FAA) is especially effective in bringing about such growth inhibition and is used for that purpose in the resistant cell model of Solt and Farber for the rapid induction of focal hyperplasia in rat liver (1). Liver fatty acid binding protein (L-FABP), generally considered to be an intracellular carrier of fatty acids (2-4), is the principal early target protein of the reactive metabolites of FAA in rat liver (5, 6). L-FABP is also a minor target of the liver carcinogenic aminoazo dyes (7). The marked specificity of L-FABP as the target protein of the FAA metabolites *in vivo* and the exceptional ability of FAA as a growth inhibitor raised the possibility of a direct association between L-FABP and the growth inhibition of hepatocytes. This connection is further supported by the recent finding that L-FABP is also a principal target protein of selenium in mouse liver (8). Selenium compounds reversibly block cell multiplication in cultures, inhibit carcinogenesis in animals, and correlate with lower cancer mortality

rates in humans (reviewed in refs. 9 and 10). In addition, L-FABP belongs to a protein family (11) in which five members are linked to the depression of cell multiplication (see *Discussion*).

Prostaglandins are local tissue hormones (12, 13). In liver, they are produced mainly by Kupffer and endothelial cells and appear to act on adjacent hepatocytes (14, 15). The predominant prostanoid synthesized by these nonparenchymal cells is prostaglandin D₂ (PGD₂) (14), which is converted spontaneously and catalytically to PGJ₂ and then to Δ^{12} -PGJ₂ (16, 17). PGEs undergo similar reactions in the formation of PGAs (16). These products (PGJ₂, Δ^{12} -PGJ₂, PGA₁, and PGA₂) inhibit cell growth and do so apparently by virtue of their α,β -unsaturated double bonds adjacent to keto groups in their cyclopentenone ring (ref. 18, reviewed in ref. 19). Such double bonds react with protein thiols and glutathione (20, 21). Δ^{12} -PGJ₂ contains two such double bonds, whereas PGJ₂ and the PGAs each have one. Several groups have reported that these cyclopentenone prostaglandins suppress the growth of many types of human and animal tumor cell lines *in vitro* and *in vivo* and normal mouse fibroblasts and smooth muscle cells in culture (reviewed in ref. 19). To our knowledge, effects of these prostaglandins on primary cultured hepatocytes have not been described. These prostaglandins have been found to be actively taken up by various cells, in which they migrate in part to nuclei apparently by a ligand-selective and carrier-mediated process that correlates with growth inhibitory activity and its reversibility (22-24). In comparison, PGD₂ and PGEs exhibit lower growth inhibitory activities, in part attributed to their gradual conversion to the cyclopentenone prostaglandins (18, 25, 26). In contrast, PGF_{2 α} and PGE₂ itself have been reported to stimulate DNA synthesis in primary cultures of adult rat hepatocytes (27).

We demonstrate here that the growth inhibitory cyclopentenone prostaglandins preferentially bind to L-FABP *in vitro* and reversibly suppress incorporation of thymidine into DNA in normal rat hepatocytes in primary culture. The rank order of the binding of the different prostaglandins to L-FABP is in accord with their relative growth inhibitory activities. The evidence suggests that L-FABP may be a preferential and dissociable carrier of the growth inhibitory cyclopentenone prostaglandins in normal rat hepatocytes.

MATERIALS AND METHODS

Materials. [5,6(n)-³H]PGA₁ (40.0 Ci/mmol; 1 Ci = 37 GBq; 95-98% radiochemically pure; NEN) was stored as multiple samples in ethanol at -75°C under argon gas. [1-¹⁴C]Oleic acid (58.0 mCi/mmol) of 99% radiochemical purity (NEN), nonradioactive oleic acid (Sigma), and unlabeled prostaglandins (Biomol Research Laboratories, Plymouth Meeting, PA)

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Abbreviations: L-FABP, liver fatty acid binding protein; PG, prostaglandin; FAA, *N*-(2-fluorenyl)acetamide (2-acetylaminofluorene). *To whom reprint requests should be addressed.

were likewise divided and maintained. Lipidex-1000 (Packard Instrument) was kept at 4°C in methanol until used.

Ligand Binding. L-FABP of high purity according to SDS/polyacrylamide gel electrophoresis was previously prepared, characterized in part, and stored in 10 mM potassium phosphate buffer (pH 7.5) at -60°C (28). Binding to L-FABP was measured essentially as reported (28) (see Fig. 1 and Tables 2 and 3).

Hepatocyte Cultures. Hepatocytes of 1-4 normal male rats (249-333 g) of the in-house-bred F344-NIH strain were isolated by differential centrifugation after perfusion with collagenase (type IV, Sigma) at 37°C (29). Before plating, the hepatocytes were 96% pure and usually about 55% viable by the criterion of trypan blue exclusion. The cells (5×10^5 per 1.6 ml) were suspended in Williams' E medium containing 10% fetal bovine serum, 2 nM insulin, penicillin, streptomycin, and kanamycin and plated on 35-mm culture dishes coated with rat tail collagen type I (Collaborative Research). Test substances were in 1% ethanol (final) as solvent. After incubation with or without test compounds in the absence of serum, DNA synthesis was measured in terms of incorporation of [³H]thymidine and normalized to DNA content as assayed in triplicate employing the fluorescent dye 4',6-diamidino-2-phenylindole (30) (see Table 1).

RESULTS

Reversible Inhibition of Hepatocyte DNA Synthesis. PGA₂ inhibited DNA synthesis in primary cultures of purified rat hepatocytes. Exposure to concentrations of 5-30 μg of PGA₂ per ml for 6 hr resulted in graded suppression of [³H]thymidine incorporation (Table 1). The inhibition did not affect the viability of the hepatocytes as measured by exclusion of trypan blue. The IC₅₀ was ≈15 μg of PGA₂ per ml (≈45 μM). The inhibition by 7 μg of PGA₂ per ml was reversed by removal of the PGA₂ (Table 1). The reduction of [³H]thymidine incorporation by PGA₂ in the cultured hepatocytes was confirmed by autoradiography using PGA₂ at the IC₅₀ (15 μg/ml) and 1 μCi or 5 μCi of [³H]thymidine per dish (as in Table 1). Virtually all cells appeared morphologically to be hepatocytes. More than 2000 cells were counted in each arm of the experiment. Nuclei were labeled in 8.3% (1 μCi) and 5.1% (5 μCi) of the control cells, compared to 0.8% and 0.6% in the PGA₂-treated cells, respectively.

Neither a lower rate of entry of thymidine into the hepatocytes nor an increase in the intracellular pool size of thymidine nucleotide appeared to be responsible for the observed PGA₂-induced inhibition of [³H]thymidine incorporation into hepatocyte DNA. In the presence of 0.238 nmol or 1.6 μCi of [³H]thymidine per culture, 100- and 1000-fold excesses of nonradioactive thymidine equally reduced, on a relative basis, the incorporation of labeled thymidine into the DNA of hepatocytes treated with 15 μg of PGA₂ per ml (IC₅₀), compared to those of control cells (64% vs. 63% and 84% vs. 84%, respectively), normalized to DNA content.

Δ¹²-PGJ₂ also inhibited DNA synthesis in rat hepatocytes. The IC₅₀ was between 2.5 and 5.0 μg/ml (7.5-15 μM) (Table 1). Higher concentrations were cytotoxic. The inhibitions brought about in the narrow range of 0.5-1.0 μg/ml were in part reversible (Table 1). However, exposure to Δ¹²-PGJ₂, combined with serum-free conditions for two 6-hr periods, brought about effects bordering on significant loss of viability. PGJ₂ (0.63-40 μg/ml; i.e., ≈2-120 μM) and PGD₂ (1.25-40 μg/ml; i.e., 3.5-114 μM) also inhibited thymidine incorporation in initial experiments (data not shown).

Rapid, Saturable, and Dissociable Binding of PGA₁ by L-FABP. Binding of PGA₁ to L-FABP was complete at 15 sec using the standard concentrations of 0.4 nmol of [³H]PGA₁ and 1.0 nmol of L-FABP (data not shown). In tests for saturable binding, association of [³H]PGA₁ with L-FABP was

Table 1. Inhibition of DNA synthesis by PGA₂ and Δ¹²-PGJ₂ in normal rat hepatocytes

PGA ₂			Δ ¹² -PGJ ₂		
Conc., μg/ml	Inhibition, %	Viability, %	Conc., μg/ml	Inhibition, %	Viability, %
Inhibition studies					
0		96, 99	0		97, 97
5	28, 24	95	0.25	7	96
10	42, 46	92	0.5	16	95
20	59, 55	99	1.0	38, 45	93, 93
30	78, 70	93	2.5	40, 67	88, 82
			5	53	70
			10	67	38
			20	71	25
Reversible inhibition studies					
0		97	0 → 0		95
7	36	92			
0 → 0		94			
7 → 0	0	86	0.5 → 0	NS	91
			1.0 → 0	5	82

For the inhibition studies, culture medium containing serum was renewed at 4 hr after plating. At 45 hr, cultures were washed twice and administered PGA₂ at the above concentrations for 6 hr (hr 45-51) without serum. In the last 3 hr (hr 48-51), cultures were exposed to 1.6 μCi of [³H]thymidine (6.7 Ci/mmol). In experiments with Δ¹²-PGJ₂, cells were simultaneously presented prostaglandin and [³H]thymidine for 3 hr (hr 48-51). The decreases in [³H]thymidine incorporation into trichloroacetic acid-insoluble material relative to those of control dishes (no prostaglandins) were normalized to 1 μg of DNA. At the end of the culture and treatment periods, 500-1000 cells were counted for viability by dye exclusion. Paired values denote duplicate results. For the reversible inhibition studies, cells were inhibited as above, except that exposure to 7 μg of PGA₂ per ml and [³H]thymidine was simultaneous for 6 hr (hr 45-51). In reversal experiments (7 → 0), parallel cultures were treated as above (no thymidine), fed serum-containing medium without PGA₂ for 16 hr (hr 51-67), washed twice, and exposed to [³H]thymidine for 6 hr (hr 67-73). Virtually identical experiments were carried out with Δ¹²-PGJ₂. Inhibitions were compared to control cultures (0 → 0). NS, not significant; Conc., concentration.

near maximal at 8 nmol of ligand (Fig. 1). That value corresponded to 3.4 nmol of PGA₁ bound per nmol of L-FABP, in approximate agreement with Scatchard analysis (see below). In examination of the reversibility of the binding to L-FABP, [³H]PGA₁ could be displaced in part by excess nonradioactive ligand. After incubation for 30 min with 0.4, 0.5, or 0.6 nmol of [³H]PGA₁, another 30-min incubation with a 10-fold excess of unlabeled PGA₁ displaced 39%, 44%, or 42%, respectively, of the bound radioactive ligand. Likewise, following exposure to the higher concentration of 4.0 or 5.0 nmol of [³H]PGA₁, a 5-fold excess of the nonradioactive PGA₁ displaced 27% and 31%, respectively, of the bound [³H]PGA₁. Furthermore, only 8% of the [³H]PGA₁ bound with high affinity under the standard conditions was fixed to L-FABP after direct isolation from SDS/polyacrylamide gels (data not shown).

High-Affinity Binding of PGA₁. Two classes of PGA₁ binding sites were present in L-FABP (Fig. 2), as revealed by Scatchard analysis (31). Below 1.0 nmol of total PGA₁, high-affinity sites with a dissociation constant (K_d) of 134 nM bound up to 0.65 nmol of PGA₁ per nmol of L-FABP. At the higher concentrations, low-affinity sites with a K_d of 3.6 μM bound up to 2.8 nmol of ligand per nmol of protein (extrapolated). The high-affinity binding of PGA₁ was 9-fold more avid than with oleic acid ($K_d = 1.2 μM$) (28) and almost 13-fold more avid than with arachidonic acid ($K_d = 1.7 μM$) (28). In fact, it was the low-affinity K_d of PGA₁ that was comparable to the reported K_d values of these and other fatty acids (2-4).

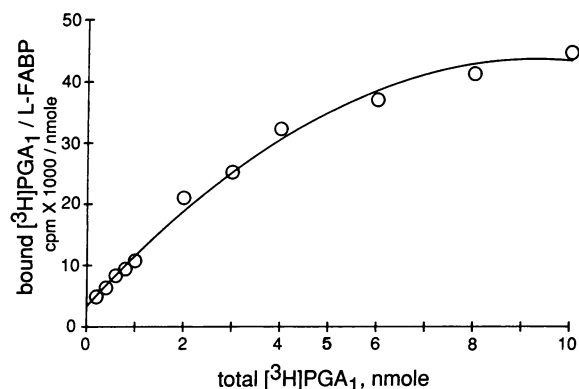


FIG. 1. Saturable binding of [^3H]PGA $_1$ to L-FABP. One nanomole of L-FABP was incubated with different amounts of [^3H]PGA $_1$ in 0.5 ml of 10 mM potassium phosphate buffer (pH 7.5) and 1.0% ethanol (final concentration) as solvent of the ligand. After incubation for 30 min with mild shaking in a water bath at 25°C, solutions were cooled to 0°C (5 min) before addition of 0.125 ml of an ice-cold suspension of Lipidex-1000 in potassium phosphate buffer (pH 7.5) (1:1, vol/vol). After periodic shaking at 0°C for 15 min, the Lipidex granules containing approximately two-thirds of the non-protein-bound labeled ligand were removed by centrifugation at 1–4°C. Approximately one-half of the remaining free ligand in the supernatant fluid was then removed by a second cycle of incubation with an additional 0.125 ml of the Lipidex suspension for 15 min at 0°C and centrifugation thereafter. The radioactivity in the second supernatant fluid was counted as L-FABP-bound ligand. Analyses were performed in duplicate.

Preferential Binding of Growth Inhibitory Prostaglandins.

Several unlabeled prostaglandins and oleic acid, each at a 50-fold greater concentration (20 nmol), were examined for their abilities to compete simultaneously with the high-affinity binding of [^3H]PGA $_1$ to L-FABP (Table 2). The greatest reductions (84–79%) in binding were caused by the potent growth inhibitory prostaglandins, PGA $_1$, PGA $_2$, Δ^{12} -PGJ $_2$, and PGJ $_2$. Intermediate depressions in binding were caused by oleic acid and PGE $_1$ (68% and 61%), and the least inhibition of binding was caused by PGD $_2$, PGF $_{2\alpha}$, and PGE $_2$ (40% to 25%). The data indicate that the growth inhibitory prostaglandins were preferentially bound to L-FABP and suggest that the ability to bind the ligands was associated with their capacity to inhibit growth.

Protein Specificity of High-Affinity PGA $_1$ Binding. Five proteins (1.0 nmol of each) were compared as to their abilities both to bind and also to release [^3H]PGA $_1$; all were tested under the standard conditions of high-affinity binding by L-FABP (Table 3). L-FABP bound the most [^3H]PGA $_1$ (i.e., 0.22 nmol per nmol of protein, or 55% of available labeled

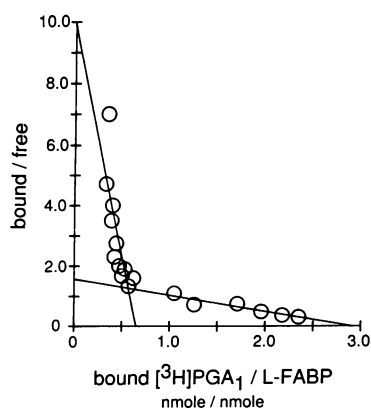


FIG. 2. Scatchard analysis of the binding of [^3H]PGA $_1$ to L-FABP. Assays were performed as described in the legend of Fig. 1.

Table 2. Preferential binding of growth inhibitory prostaglandins by L-FABP in competition binding assays of [^3H]PGA $_1$

Unlabeled ligand	Inhibition of binding, %
PGA $_1$	84
PGA $_2$	85
Δ^{12} -PGJ $_2$	82
PGJ $_2$	79
Oleic acid	68
PGE $_1$	61
PGD $_2$	40
PGF $_{2\alpha}$	38
PGE $_2$	25

Assay mixtures contained 0.4 nmol of [^3H]PGA $_1$ and 20 nmol (50-fold excess) of the listed unlabeled ligands in 0.5 ml of 10 mM potassium phosphate buffer (pH 7.5) and 1% ethanol (final concentration). Binding was started by addition of 1.0 nmol of L-FABP and continued for 30 min in a shaking water bath at 25°C. After cooling the mixture to 0°C, [^3H]PGA $_1$ bound to L-FABP was assayed by the two-stage Lipidex assay (Fig. 1). Values were corrected for Lipidex assays without L-FABP. Inhibition of binding is expressed relative to the binding of [^3H]PGA $_1$ without unlabeled ligand.

ligand). Bovine serum albumin had significantly less activity (0.14 nmol; 35%). The other proteins, β -lactoglobulin, ribonuclease, and ovalbumin, exhibited low or insignificant binding. In addition, after binding to each protein, the mixtures were exposed to a 10-fold excess of unlabeled PGA $_1$ in displacement assays. One-half of the [^3H]PGA $_1$ bound to L-FABP was displaced by the 10-fold PGA $_1$, compared to 21% with bovine serum albumin and essentially none with the others. L-FABP thus bound the most ligand, and its binding was the most dissociable and presumably noncovalent.

DISCUSSION

Growth of hepatocytes may be subject to paracrine influences. Fewer than 1 in 1500 hepatocytes divide at any time in adult rat liver (32). Kupffer and endothelial cells, located adjacent to hepatocytes, produce relatively large amounts of PGD $_2$ and PGE $_2$ (14, 15), which spontaneously and catalytically convert to the growth inhibitors PGJ $_2$, Δ^{12} -PGJ $_2$, and PGA $_2$ (16–18, 22, 23). In apparent accord, PGA $_2$ and Δ^{12} -PGJ $_2$ suppressed thymidine incorporation into DNA of purified normal rat hepatocytes in culture.

That micromolar concentrations of PGA $_2$ and Δ^{12} -PGJ $_2$ inhibited DNA synthesis is striking evidence of their sequestration in hepatocytes. Free cyclopentenone prostaglandins

Table 3. Protein specificity of binding of [^3H]PGA $_1$

Protein	Unlabeled PGA $_1$ added, nmol	Protein-bound [^3H]PGA $_1$		Displacement, %
		nmol	%	
L-FABP	0	0.22	55	
	4.0	0.11	28	50
Bovine serum albumin	0	0.14	35	
	4.0	0.11	28	21
β -Lactoglobulin	0	0.09	22	
	4.0	0.09	22	0
Ribonuclease	0	0.07	18	
	4.0	NS	NS	NS
Ovalbumin	0	0.06	15	
	4.0	NS	NS	NS

Assays involved incubation of 0.4 nmol of [^3H]PGA $_1$ with 1.0 nmol of the listed proteins. In parallel assays, ligand binding was followed by displacement with 4.0 nmol of unlabeled PGA $_1$ (see Fig. 1 and Table 2). Values are corrected for assays without L-FABP. NS, not significant.

can react with glutathione without and with the catalytic aid of glutathione *S*-transferases (20, 21, 33, 34). Since normal rat liver contains a high concentration (≈ 10 mM) of glutathione (35) and the transferases may constitute as much as 10% of all cytosolic proteins of normal rat liver (33), any free cyclopentenone prostaglandin would be consumed. Instead, Δ^{12} -PGJ₂ is incorporated into cells, where they migrate to nuclei and fix irreversibly to nucleoproteins (22–24). PGA₂ localizes more in cytoplasm, where the prostaglandin can be eliminated by removal from the culture medium (22–24). Importantly, the cyclopentenone prostaglandins inhibit the growth of several cell lines through a block at the G₁ phase near the G₁/S interface in the cell cycle (36–38). In agreement, inhibition of DNA synthesis by PGA₁ and PGA₂ is not due to a depression of thymidine uptake in cultured melanoma cells (39), and in cultured hepatocytes it is not the result of either a lowered rate of cell entry of thymidine or an increase in intracellular pool size of thymidine nucleotide (this report). Considering these findings, as well as the specific and reversible high affinity binding of PGA₁ and the preferential binding of the inhibitory prostaglandins by L-FABP (below), the evidence suggests that L-FABP may act as a carrier of growth inhibitory prostaglandins in rat hepatocytes.

The cyclopentenone prostaglandins were preferentially bound by L-FABP. The potent growth inhibitory cyclopentenone prostaglandins, PGA₁, PGA₂, Δ^{12} -PGJ₂ and PGJ₂, competed maximally with the high-affinity binding of [³H]PGA₁. Next in decreasing order were oleic acid and PGE₁, and last were PGD₂, PGF_{2 α} and PGE₂. Dutta-Roy *et al.* reported previously that PGE₁ binds to L-FABP in a specific manner but with an affinity that was observed in the presence of 3 mM MgCl₂ (40). A correlation thus exists between the relative affinities of the different prostaglandins for L-FABP and their growth inhibitory activities. The cyclopentenone prostaglandins, which potently inhibit DNA synthesis and cell multiplication (refs. 18, 19, 22–24; this report), bind preferentially. Next, PGE₁ and PGD₂ are intermediate competitors. In agreement, Δ^{12} -PGJ₂ is 7- to 17-fold and PGA and PGJ₂ are 3- to 7-fold more growth inhibitory than are PGE₁ and PGD₂ (19), both of which are converted gradually to the growth inhibitory prostaglandins (18, 19, 22–24). Finally, the two poorest competitors were PGE₂ and PGF_{2 α} , which are actually reported to stimulate DNA synthesis in primary cultures of adult rat hepatocytes (27).

The ligand-binding site(s) of L-FABP apparently binds the growth inhibitory cyclopentenone prostaglandins both non-covalently and covalently. The competitive binding between PGA₁ and oleic acid, and that previously reported between 15-hydroperoxyeicosatetraenoic acid (15-HPETE) and oleic acid (28), indicates that these ligands bind at or near the same site in L-FABP. However, the binding of PGA₁ by L-FABP differed from those of fatty acids (2–4, 28), 15-HPETE (28), and 15-hydroxyeicosatetraenoic acid (15-HETE) (28). Whereas the molar ratios of the latter group of ligands relative to L-FABP were 1 or 2 (2–4, 28), the molar ratio of PGA₁ reached ≈ 3 . PGAs, PGJ₂, and Δ^{12} -PGJ₂ can interact with protein thiols (20), and L-FABP contains only one cysteine residue, which is situated midway in the molecule (41). Nevertheless, the following data suggest that the high-affinity binding of the first PGA₁ molecule is noncovalent in nature. First, the high-affinity *K*_d value of PGA₁ at 134 nM is similar in magnitude to those of other nonreactive, high-affinity eicosanoid ligands of L-FABP, namely 15-HPETE (76 nM) and 5-HETE (175 nM) (28). Second, it was the low-affinity binding at elevated concentrations of PGA₁ that extrapolated to a molar ratio of ≈ 3 in the Scatchard plot (Fig. 2). Third, like the reversibly bound 15-HPETE (28), the high-affinity bound [³H]PGA₁ was displaced to the extent of 50% by excess unlabeled PGA₁ (Table 3). Fourth, only 8% of the [³H]PGA₁

bound to L-FABP under the standard high-affinity conditions actually migrated with L-FABP in SDS/polyacrylamide gels. It is reasoned accordingly that a covalent interaction at the thiol residue may account for the third PGA₁ bound to L-FABP and that that occurs at relatively high concentrations of the ligand.

In 1981 Jonsson and Powers (42) concluded that PGA is not present *in vivo* in significant amounts. Since then it was found that PGD₂ at about 144 ng/g is the main prostaglandin in rat liver homogenate, while PGE₂ is in next lower amounts (134 ng/g) (15) (each at ≈ 0.5 μ M). Thus, there appears to be ample concentrations of the precursors of the cyclopentenone prostaglandins in rat liver. It is also noteworthy that Δ^{12} -PGJ₂ is present in human urine, indicative that that prostaglandin is a natural metabolite (43). Furthermore, the reported existence of cellular machinery for their specific uptake and carrier-mediated nuclear localizations, both of which correlate with growth inhibitory activities (22–24), support the existence and roles of these cyclopentenone prostaglandins in cells. Finally, the current demonstration that PGA₁ binds reversibly to L-FABP raises the possibility that in previous analyses of tissues there may have occurred a release of sequestered PGAs, which then may have covalently interacted with glutathione and thiol proteins, resulting in failure to detect these prostaglandins.

The high-affinity binding of PGA₁ by L-FABP was protein specific in the physicochemical sense. Not only did L-FABP bind PGA₁ specifically, reversibly, and with high affinity, but, of all the tested proteins, L-FABP bound the most PGA₁, and its ligand was the most dissociable. Only serum albumin, a nonspecific carrier of hydrophobic ligands in blood, approached in magnitude those properties of L-FABP. The high-affinity binding characteristics of L-FABP *in vitro* thus appear to be compatible with those of a specific dissociable carrier of the growth inhibitory prostaglandins in rat hepatocytes. Nonhepatic organs have other fatty acid binding proteins (2–4), which conceivably may act similarly.

L-FABP belongs to a family of proteins whose genes share homology in their first exons (11). In the family are five proteins that appear to be connected with depression of cell multiplication in six biological systems. First, L-FABP itself is the target protein of the reactive metabolites of the hepatocarcinogens FAA (5, 6) and aminoazo dyes (7) *in vivo*. Early effects of these and other carcinogens involve growth inhibition of target cells (Introduction). Second, L-FABP is a principal target protein of selenium in mouse liver (8). Selenium compounds reversibly block cell multiplication in cultured cells (9, 10). Third and fourth, cellular retinol binding protein I and cellular retinoic acid binding protein, which appear to be intracellular carriers of retinol and retinoic acid, respectively, are associated with the cessation of cell multiplication that accompanies cellular differentiation (reviewed in refs. 44 and 45). Fifth, the potent mammary-derived growth inhibitory protein from bovine lactating (non-growing) mammary glands reversibly suppresses cell multiplication and thymidine incorporation in a variety of epithelial cells (46). Sixth, the fibroblast growth regulator-soluble, a protein that is secreted by growth-inhibited NIH 3T3 cells at confluence, reversibly blocks DNA synthesis and cell replication at 0.1 nM in a variety of fibroblast lines (47, 48). Collectively, these different systems support the present finding of a link between L-FABP and inhibition of cell multiplication.

The preferential binding of the growth inhibitory cyclopentenone prostaglandins by L-FABP *in vitro* may be related to the specific covalent binding of the reactive metabolites of FAA carcinogen to that same protein during hepatocarcinogenesis in rats (5, 6). The actions of L-FABP as the common and specific target of both types of ligands suggest the possibility that the reactive metabolites of the carcinogen

may usurp the cellular machinery of the growth inhibitory prostaglandins in rat hepatocytes in a process important in carcinogenesis.

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