MACROMOLECULAR BINDING OF [5,6-^{3}H] PROSTAGLANDIN E_1 IN LIVER CYTOSOL *IN VITRO**

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SUMMARY

[^{3}H] Prostaglandin E_1 (PGE_1) is bound extensively to macromolecules in liver cytosol *in vitro*. A principal binding protein accounts for 80% of the binding. This macromolecule is saturated at about 10^{-10} M PGE_1. The partially purified protein has a molecular weight of 50,000 by gel filtration and a pI of about 3.5 by isoelectrofocusing. Binding is primarily noncovalent and the dissociated ligand behaves similarly to the parental [^{3}H] PGE_1 on thin layer chromatography. Possible significance of this interaction is discussed.

A great diversity of physiological actions is attributed to the prostaglandins (1) and they produce biological responses in very low concentrations. Prostaglandin E_1 (PGE_1) is a potent smooth muscle stimulant and shows strong vasodepressor activity (2). Recent work suggests that the mode of action of PGE_1 may involve membrane effects insofar as its activity on the ovary is concerned (3) and many regard the actions of PGE_1 to reside entirely in the cell membrane (4,5).

The distribution of intravenously administered radioactive PGE_1 studied autoradiographically showed that it is concentrated largely in the liver (2) in spite of the fact that much of its metabolism occurs in the lung (6). In studying the accumulation and apparent active transport of prostaglandins by rabbit tissues *in vitro*, Bito showed the ability of liver slices to concentrate PGE_1 substantially above levels in the medium (7). Furthermore the synthesis of PGE_1 in the adrenal from arachidonic acid has been located within the cell in the endoplasmic reticulum involving the cytochrome P-450 system (8). Also,

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PGE_1 has been shown recently to stimulate the activity of solubilized myocardial adenylate cyclase (9). Consequently, assignment of the cell membrane as the sole site of action of PGE_1 remains indefinite.

We have already established the existence of five cytoplasmic binding proteins for corticosteroids (and their anions in some cases) (10-12). One of these functions, containing the glucocorticoid receptor (Binder II), appears to interact with cyclic AMP (13,14). Since PGE_1 increases the intracellular level of cyclic AMP in certain cells (15,16), we decided to investigate the possibility of intracellular binding of PGE_1 in liver to determine if one of the corticosteroid binding macromolecules proved to be the hypothetical PGE_1 binder. We describe here the *in vitro* binding of PGE_1 to a liver cytosol macromolecule which has properties different from the corticosteroid binding series and which binds most of the PGE_1 radioactivity in cytosol.

MATERIALS AND METHODS

$PGE_1[5,6\text{-}^3H]$ (110 Ci/mmole) was obtained from New England Nuclear. It was stored in the freezer under N_2 and checked for purity by thin layer chromatography on pre-coated silica gel plates (10 x 20cm) (Brinkman Instruments). Unlabeled, authentic prostaglandins were obtained through the courtesy of Dr. J. E. Pike of the Upjohn Co.. Dilutions of the isotope were made in ethanol just prior to use.

Column chromatographic methods at 4^o using Sephadex G-25, G-100 and DEAE Sephadex A-50 (Pharmacia) have been described previously (10,12,17). Horse heart cytochrome c and ovalbumin were obtained from Sigma and bovine serum albumin, Fraction V, was obtained from Armour Labs. for use as molecular weight markers in gel filtration chromatograms. Electrofocusing at 4^o was performed as described by Svensson (18) using a pH gradient of 3-10 and the pH of fractions was determined at 4^o.

Relative protein concentration was determined by absorbance of solutions at 280 nm and radioactivity was measured as described previously (19) with an Intertechnique scintillation spectrometer at 12^o.

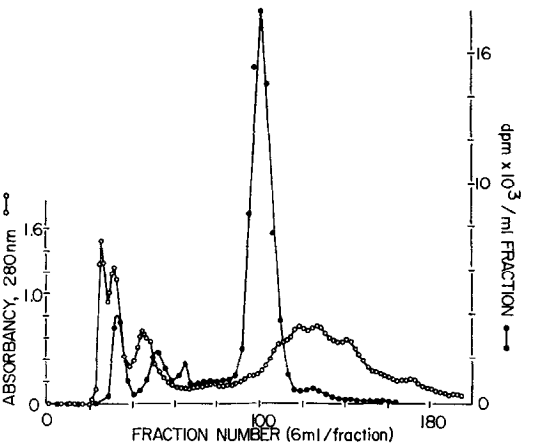

Fig. 1. Chromatogram of bound pool of prostaglandin radioactivity from Sephadex G-25 on DEAE-Sephadex A-50. The resin bed was 3 x 70 cm, the load was 10.5 ml and a linear KCl gradient was used from 0 to 1 M superimposed on 50 mM tris, pH 7.5.

Saline (0.9%) perfused liver cytosols from 50% homogenates were prepared from adrenalectomized male rats (120-180 g) of the Fisher strain (Charles River Breeding Labs.) by previously described techniques (12,20).

RESULTS AND DISCUSSION

In several experiments, 5 to 12.5 ml cytosol were incubated with 10^{-9} to 10^{-8} M [^{3}H] PGE_1 for 90 min at 1 to 4° with gentle stirring. Subsequently, the reaction mixture was chromatographed on a column of Sephadex G-25 (1 mM tris, pH 7.5) to separate bound (excluded) and unbound radioactivity. Approximately 15 to 30 percent, depending on the concentration of prostaglandin, of the recovered radioactivity was in the bound form. The bound pool was lyophilized to about 20% of its original volume and chromatographed on DEAE-Sephadex A-50 using a linear KCl gradient. Results of a typical chromatogram are shown in Fig. 1. Three minor peaks of radioactivity generally elute with proteins in less than 0.04 M KCl whereas the principal binding macromolecule accounting for the majority of bound radioactivity (>80%) is eluted in the range of 0.07 to 0.1 M KCl superimposed upon 50 mM tris, pH 7.5. By column chromatography on Sephadex G-25 the major peak of radioactivity is shown to be in a macromolecular form. Interestingly, this peak elutes just after the glucocorticoid receptor

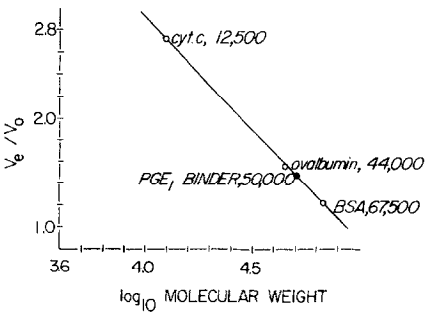

Fig. 2. Determination of molecular weight of PGE_1 binder by chromatography on a 1.8 x 49 cm column of Sephadex G-100 with marker proteins of the indicated molecular weights. Cyt c, cytochrome c, BSA, bovine serum albumin. V_o = 51 ml and V_b = 125 ml. The load volume was 3 ml. 2 ml fractions were collected. The position of the PGE_1 binder was determined by protein concentration and radioactivity.

(12,17) which emerges at about 0.05 M KCl (13,14). Resolution is probably incomplete since unliganded dialyzed glucocorticoid receptor after DEAE chromatography can bind $[^3H]PGE_1$ to a significant extent (ca. 2 pmoles $[^3H]PGE_1$ per mg protein of glucocorticoid receptor fraction). Cyclic AMP, which has been shown to bind to the hormone receptor fraction (13,14) does not compete with the binding of $[^3H]PGE_1$. Moreover, corticosterone or cyclic AMP at 1000 fold higher concentrations than $[^3H]PGE_1$ do not compete with the binding of radioactive prostaglandin in incubations with cytosol. In these cases the extent of binding was determined by gel filtration on Sephadex G-25 columns.

The principal bound form of PGE_1 radioactivity after chromatography on DEAE Sephadex was stable enough for additional studies of physical properties. In Fig. 2 is shown a measurement of its molecular weight by gel filtration on Sephadex G-100. In two experiments the values of molecular weight of the PGE_1 binding protein were 49,600 and 50,000. Considerable stripping of the ligand occurred during this chromatography suggesting that the association of macromolecule and ligand is primarily noncovalent.

The principal macromolecular bound peak of PGE_1 radioactivity from the DEAE chromatogram was isoelectrofocused as shown in Fig. 3. In two such studies the pI values of the radioactive peak were 3.43 and 3.5. The majority of protein moved away from the radioactive peak indicating this to be a useful step in the

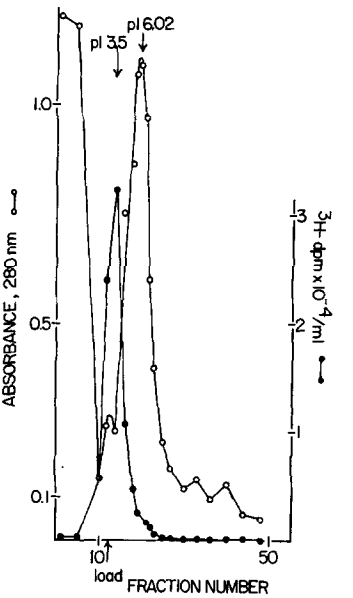

Fig. 3. Electrofocusing of the PGE_1 binder from the DEAE-Sephadex A-50 column. 2.5 ml fractions were collected. The load contained 17.8 $A_{280\ nm}$ units of protein and 591,000 dpm in 1 ml. A pH 3-10 gradient was used. Focusing was started at 100 volts for 12 hrs. then increased to 300 volts, 0.5 ma for the next 48 hrs. at 4^0.

purification of the binder. The pI value of about 3.5 is also different from any of the glucocorticoid binding proteins (12) and different from the major cyclic AMP binding protein associated with the glucocorticoid receptor (13,14), the regulatory subunit of protein kinase (pI 4.5-4.8) (21,22) and the heat stable protein inhibitor of protein kinase (pI 4.2) (23,24).

Although the lung seems to be the major organ for metabolism of PGE_1 (6), we considered the possibility that the principal binding component in liver cytosol could be a metabolizing enzyme. The principal binder was taken from the DEAE-Sephadex chromatogram and chromatographed on Sephadex G-100 as described above. A considerable portion of bound radioactivity stripped from the macromolecule (Fig. 4). The unbound pool was concentrated by lyophilization and applied to a thin layer plate in tandem with the unbound pool from the initial Sephadex G-25 fractionation representing unbound excess ligand in the incubation system as well as the stock isotope. The results are shown in Fig. 5. The

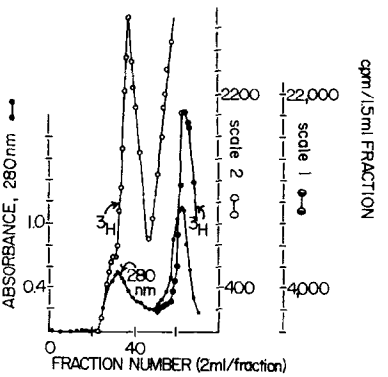

Fig. 4. Stripping of the bound ligand by column chromatography on Sephadex G-100 following chromatography on DEAE-Sephadex A-50. Column dimensions were 1.8 x 49 cm and the load was 3 ml. 590,000 dpm and 18 $A_{280\ nm}$ units of protein were in the load.

profiles are similar in all three cases leading to the conclusion that the ligand in association with the macromolecule is unmetabolized suggesting the protein is not a metabolizing enzyme.

We speculate that the function of this macromolecule may be in transporting PGE_1 either to or from the cell membrane possibly to interact in bound form with an adenylate cyclase or some other membrane associated functional component (25). Further progress depends on experiments, now contemplated, to assess the physiological significance of this binding. These will include *in vivo* experiments and competition studies. In preliminary experiments, there is significant binding to the liver cytosol macromolecular fraction after intraperitoneal injection of 32 ng $[^3H]PGE_1$ per adrenalectomized animal (male, 120 g). In this connection, it is of interest that the binder is saturated *in vitro* in the range of 10^{-10} M PGE_1 with a specific binding of about 40 fmoles $[^3H]PGE_1$/mg protein at the first Sephadex G-25 stage of fractionation before the binder has been physically separated from minor binding components and prior to extensive dissociation of the ligand. With this value for saturation of binding *in vitro* it is clear the macromolecule is saturated in a concentration range consonant with the hypothetical physiological concentrations of the prostaglandin. For example, the effective range of PGE_1 concentration to exert a half-maximal inhibition of epinephrine stimulated cyclic AMP formation in adipocytes is

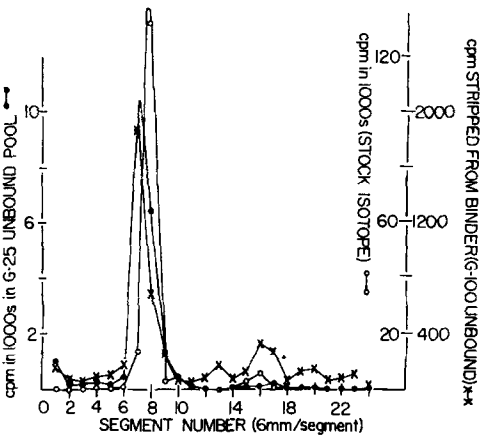

Fig. 5. Thin layer chromatography of stock PGE_1 isotope, unbound pool from Sephadex G-25 step and stripped ligand from binder after DEAE-Sephadex A-50 and Sephadex G-100 column chromatographic procedures. Variations of the positions of the major peak are not greater than duplicate side-by-side runs of the stock isotope.

4×10^{-9} M (25,26), a level substantially higher than that required to saturate the cytosol binder shown here. The most active levels of prostaglandins reported to stimulate cyclic AMP concentrations of a number of tissues range from 10^{-8} to 2×10^{-6} M (25) and levels of prostaglandins to inhibit hormonally induced responses in a number of tissues are in the range of 10^{-7} M (25).

Purification of this macromolecule, now in progress, may lead to a useful protein binding assay for the quantitation of PGE_1.

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