Vol. 141, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 26, 1986 Pages 151-157

INHIBITION OF THE BINDING OF LOW DENSITY LIPOPROTEINS TO LIVER MEMBRANE RECEPTORS BY RAT SERUM PHOSPHORYLCHOLINE BINDING PROTEIN

Uday Saxena, Arun Nagpurkar and Sailen Mookerjea

Department of Biochemistry,
Memorial University of Newfoundland,
St. John's, Newfoundland Canada A1B 3X9

Received September 23, 1986

Rat serum phosphorylcholine binding protein (PCBP) is characterized by its Ca^{2+} dependent property to bind phosphorylcholine ligand. PCBP immobilized on sepharose has been shown to selectively bind human plasma apo B and E containing lipoproteins. The present report describes an inhibitory effect of PCBP on the binding of human ^{125}I-LDL to LDL receptors on estradiol treated rat liver membranes. Pre-incubation of liver membranes with PCBP did not affect the binding of ^{125}I-LDL to the membranes. Gel filtration analysis of the incubation products from the LDL-receptor assay showed a concentration dependent binding of ^{125}I-PCBP to LDL. The inhibitory effect of PCBP is likely due to the formation of LDL-PCBP complex and not due to the binding of PCBP to the LDL receptor site.

Rat serum PCBP, a member of the pentraxin family of proteins (1), has been shown to inhibit the precipitation of rat, rabbit and human serum lipoproteins, by heparin (2,3,4). PCBP is present in normal rat serum in high concentrations (0.6-0.7 mg/ml) (2). The demonstration of binding of PCBP to phosphatidylcholine containing liposomes (5) led to more recent findings that PCBP immobilized on sepharose binds preferentially to human apo B and E containing lipoproteins (6,7). The present investigation examines the effect of such PCBP-lipoprotein interactions on the binding of human LDL to LDL receptors on liver membranes from estradiol treated rats. Since the specific objective of this study was to determine the effect of PCBP on lipoprotein-LDL receptors, human LDL containing exclusively apo B, has been utilized, thereby, minimizing the participation of apo E and apo A-I specific receptors which are known to be present in rat liver (8,9).

0006-291X/86 $1.50

EXPERIMENTAL PROCEDURES

Isolation and radioiodination of PCBP: PCBP isolated from normal rat serum was radioiodinated enzymatically as described before (5). The specific activity was 144 cpm/nmol of PCBP (based on mr = 125,000). Radiolabelled PCBP was diluted with unlabelled PCBP as indicated in the legends.

Isolation and radioiodination of LDL: LDL (density 1.030 to 1.063 gm/ml) was isolated from human plasma by ultracentrifugation (5) and its purity checked by polyacrylamide gel electrophoresis (6). Purified LDL was radioiodinated by modification of Mcfarlanes method (10) as described by Wong and Rubinstein (11). The ^{125}I-LDL protein concentration ranged from 0.25 mg to 0.32 mg/ml while the specific activity was 121-248 CPM/ng protein. Labelled LDL was stored in sterile plastic tubes in 0.01M Tris-HCl buffer (pH 7.4) containing 0.15M NaCl and used within 2 weeks of preparation.

Preparation of rat liver membranes: Liver membranes were prepared from 17α-ethinyl estradiol treated rats according to the method of Kovanen et al. (12). The 100,000 x g pellet prepared from whole liver homogenate was assayed either immediately or stored at -80°C and used within 3 weeks.

Binding of ^{125}I-LDL to rat liver membranes: The binding assays were carried out essentially as described by Kovanen et al. (12) except that the incubation buffer contained 20 mM NaCl. Liver membranes (100-120μg protein), ^{125}I-LDL (as indicated in individual assays) were incubated in 20 mM Tris-HCl (pH 7.5) containing 20 mM NaCl, 20 mg/ml BSA and Ca^{2+} (5mM) ('Buffer A') either in the presence or absence of EDTA (20 mM) in a total volume of 150μl. Incubations were carried out in Beckman microfuge tubes (1.5 ml capacity) at 0°C for 90 min. After the incubation 75 μl of the assay mixture was layered onto 200 μl of fetal calf serum in Beckman Type 25 rotor tubes and centrifuged at 92,000 x g for 30 min at 4°C. The supernatant was aspirated and replaced with 150 μl of fetal calf serum and the centrifugation repeated for 10 min. The supernatant was then removed, and the pellet counted for radioactivity in a Beckman 5500 gamma counter. Results presented are average of duplicate assays.

Preincubation of liver membranes with PCBP: Preincubation of liver membranes (120 μg protein) with increasing concentrations of PCBP in 'buffer A' in a total volume of 150μl was carried out for 90 min. at 0°C. The tubes were centrifuged at 12,800 x g for 30 min. at 4°C, which resulted in quantitative sedimentation of the membranes. The supernatant was aspirated and replaced with ^{125}I-LDL (47.3 μg protein/ml) and 'Buffer A'. The membranes were gently resuspended and incubated for an additional 90 min at 0°C, and then centrifuged as described for the liver membrane binding assays. The amount of ^{125}I-LDL bound to the liver membrane was determined from the radioactivity associated with the membrane sediments. The results presented are average of duplicate assays. In a separate experiment, liver membranes (120μg) were incubated in the presence of 1 μg of ^{125}I-PBCP plus unlabelled PCBP as indicated in 'Buffer A' for 90 min at 0°C, in a final volume of 150μl. The incubation mixture was centrifuged at 12,800xg for 30 min and the supernatants discarded. The membrane sediments were washed (x2) with 'Buffer A' and counted for radioactivity to determine the amount of ^{125}I-PCBP bound.

Gel-filtration experiments: LDL (0.6 mg protein) and ^{125}I-PCBP (0.5μg ^{125}I-PCBP diluted with 36.5μg unlabelled PCBP) were incubated in 0.01M Tris-HCl buffer (pH 7.4) containing 100 mM NaCl either in the absence or presence of Ca^{2+} (5mM) in a total volume of 0.2 ml at 0°C for 90 min and the mixture applied to a Sephacryl S400 column (1.5 x 45 cm). The column

was eluted with 0.01M Tris-HCl buffer (pH 7.4) containing 100 mM NaCl, and Ca^{2+} (5 mM) at a flow rate of 24 ml/h. The fractions (2 ml) were counted for radioactivity in a Beckman 5550 gamma counter. The elution volumes for LDL and ^{125}I-PCBP were determined in separate experiments.

In another set of experiments, the supernatants (100μl) from the LDL receptor binding assays carried out in Buffer A (containing 2 mg/ml BSA instead of 20 mg/ml) were chromatographed on Sephacryl S400 column as described above. The supernatants were obtained by centrifugation of the assay mixture at 12,800xg for 30 min at 4^{o}C.

RESULTS

In the absence of EDTA, the progression of binding of ^{125}I-LDL to liver membranes was non-linear suggesting the presence of saturable binding sites on the membranes (Fig. 1A). In the presence of EDTA the overall binding of ^{125}I-LDL was reduced as expected (12) and the difference between binding of ^{125}I-LDL in the absence and presence of EDTA gave a measure of the specific high affinity binding (Fig. 1A). The effect of PCBP on the ^{125}I-LDL binding to liver membranes showed that the binding of ^{125}I-LDL to liver membranes decreased in the presence of increasing concentrations of PCBP (Fig. 1B). Two different concentrations of PCBP (87 and 600 μg/ml) were required to achieve about 40% inhibition of binding

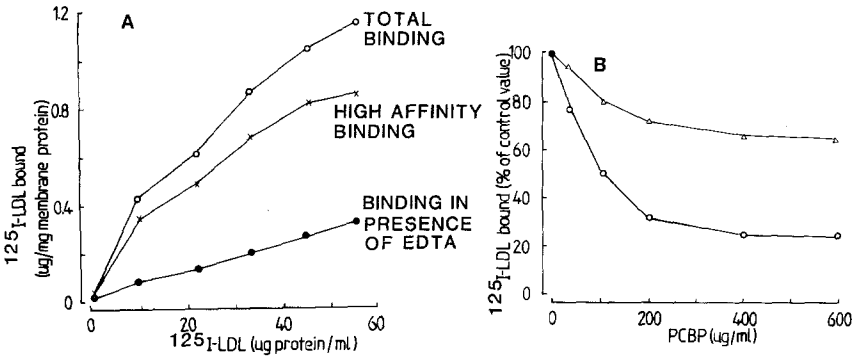

Fig. 1.Binding of ^{125}I-LDL to liver membranes (A) and effect of PCBP on the binding (B):
A) The binding assays containing 100 g membrane protein and indicated concentration of ^{125}I-LDL (124 cpm/ng protein) were incubated at 0^{o}C for 90 min either in absence (○) or presence (●) of 20 mM EDTA. High affinity binding was calculated by subtracting the ^{125}I-bound in the presence of EDTA from that bound in the absence of EDTA.
B) Liver membranes (100 μg protein) were incubated with, either 34 μg/ml (-○-) or 152.6 μg/ml of ^{125}I-LDL protein (-△-) (Specific activity = 124 cpm/ng protein) and increasing concentrations of PCBP. The 100% value for ^{125}I-LDL bound in the absence of PCBP was 0.85 μg/mg with 34 μg/ml and 2.25 μg/mg with 152.6 μg/ml of ^{125}I-LDL protein.

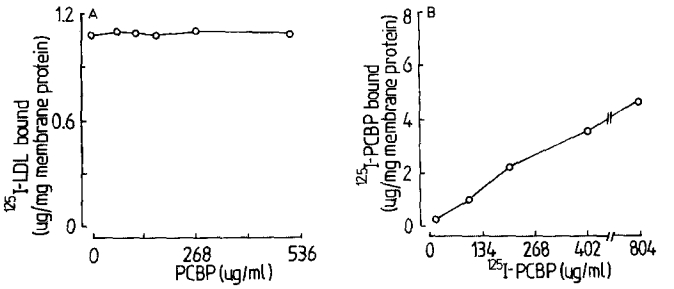

Fig. 2. Effect of preincubation with PCBP on the binding of ^{125}I-LDl to liver membranes (A) and the binding of ^{125}I-PCBP to liver membranes (B).
A) Liver membranes (120μg protein) were preincubated at 0°C for 90 min with increasing concentrations of PCBP as indicated, followed by the addition of ^{125}I-LDL (48μg protein/ml, 172 cpm/ng protein) and further incubated for 90 min at 0°C. The amount of ^{125}I-LDL bound to the membrane sediments was determined.
B) Incubation mixture in Buffer A contained liver membranes (120μg protein), ^{125}I-PCBP (1μg) diluted with unlabelled PCBP to give concentrations as indicated. After incubation at 0°C for 90 min. the tubes were centrifuged and ^{125}I-PCBP bound to membranes was determined.

when 34 μg and 152.6 μg protein/ml of ^{125}I-LDL respectively were present in the assays.

Preincubation of liver membranes with PCBP did not affect the binding of ^{125}I-LDL (Fig. 2A) even though some PCBP (<5%) was bound to the membranes as determined from parallel incubations (Fig. 2B).

Chromatography of a mixture of LDL and ^{125}I-PCBP on Sephacryl S-400 column in presence of Ca^{2+} (Fig. 3B) resulted in coelution of ^{125}I-PCBP

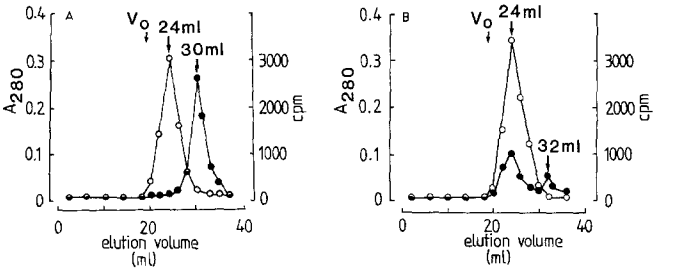

Fig. 3. Gel-filtration of LDL and ^{125}I-PCBP on Sephacryl S400 column after incubation either in absence (A) or presence of Ca^{2+} (B).
A) LDL (0.6 mg protein), ^{125}I-PCBP (0.5μg) diluted with 36.5μg unlabelled PCBP were incubated at 0°C for 90 min in 0.01M Tris-HCl buffer (pH 7.4) containing 100 mM NaCl in a total volume of 0.2 ml. The mixture was applied to a Sephacryl S400 column (1.5x45 cm) and eluted with 0.01M Tris-HCl buffer (pH 7.4) containing 100 mM NaCl, at a flow rate of 24 ml/h.
B) LDL and ^{125}I-PCBP were incubated and chromatographed exactly as described in A except that the incubation and elution buffers contained 5 mM Ca^{2+}. The recovery of LDL was 90% and that of ^{125}I-PCBP was 80%. Void volume (Vo) was determined using Blue Dextran 2000. (○) denotes elution of LDL and (●) of ^{125}I-PCBP.

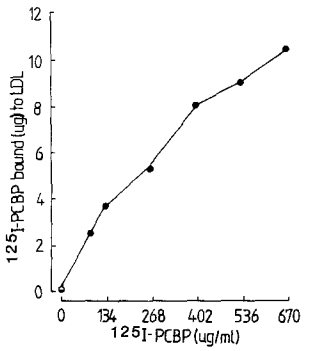

Fig. 4. Detection of LDL-PCBP complex in supernatant from the binding assays by gel-filtration analysis.
Binding assays containing liver membranes (100μg protein), LDL (33.9 μg protein/ml), ^{125}I-PCBP (1μg) diluted with unlabelled PCBP to give final concentrations as indicated were incubated in Buffer A (containing 2.5 mg/ml BSA instead of 20 mg/ml BSA) for 90 min at 0°C. The assay mixtures were centrifuged at 12,800xg for 30 min and the supernatants (100μl) applied to a Sephacryl S400 column equilibrated with 0.01M Tris-HCl buffer (pH 7.4) containing 100 mM NaCl and 5 mM Ca^{2+}. ^{125}I-PCBP complexd with LDL in the supernatants was determined from the radioactivity co-eluting with LDL. Column size and flow rate were as described in Fig. 3A.

with LDL, whereas, in absence of Ca^{2+} (Fig. 3A) the two peaks elute separately. The formation of an LDL-PCBP complex was examined by analyzing aliquots of the supernatants from the binding assays by gel-filteration on Sephacryl S400. When increasing concentrations of ^{125}I-PCBP were incubated, increasing amounts of ^{125}I-PCBP complexed in the supernatant with LDL (Fig. 4).

DISCUSSION

Studies showing a selective binding of apo B and E containing lipoproteins to PCBP immobilized on sepharose (6,7) have prompted the current investigation on the interaction between PCBP, LDL and LDL receptors. Results show that PCBP can inhibit the binding of LDL to the liver membrane LDL receptors (Fig. 1B). A stoichiometric relationship between LDL and PCBP is apparent, since increased concentration of PBCP is required to achieve 40% inhibition, when higher concentration of LDL is used in the binding assays (Fig. 1B). Preincubation of the liver membranes with PCBP did not affect the binding of LDL to the membranes (Fig. 2A) suggesting that the LDL-receptor sites on membranes were not bound by PCBP, although some PCBP (<5%) was bound to the membranes (Fig. 2B). Investigations to

test the binding of PCBP to LDL in the fluid phase of the assay system demonstrated that ^{125}I-PCBP co-eluted with LDL in presence of Ca^{2+} (Fig. 3B). Furthermore, in the LDL-receptor assay, incubation of increasing concentrations of ^{125}I-PCBP with LDL showed increased LDL-PCBP complex formation in the supernatants (Fig. 4). We, therefore, conclude that the inhibition of binding of LDL to the receptors on liver membranes by PCBP resulted from a fluid phase interaction between LDL and PCBP and is not due to PCBP binding to receptor sites.

It has previously been shown by Brown et al., (13) that certain positively charged proteins are capable of inhibiting the LDL-receptor interaction by binding either to the receptor or to an adjacent site. However, results presented in this paper offer another mechanism involving a fluid phase interaction between LDL and PCBP.

Rats have low levels of plasma LDL and it has been suggested (14) that apo E-rich HDL, acting as a substitute for LDL, may be a major vehicle for transport of cholesterol to cells containing active LDL receptors. It seems reasonable to speculate that in vivo, PCBP may interact with apo E-rich HDL and alter the accessibility of these lipoproteins to LDL receptors, in light of our previous observation that apo E-rich rat HDL also binds to PCBP (7).

ACKNOWLEDGMENTS: U.S. is a recipient of a Canadian Heart Foundation Traineeship. We acknowledge financial support by the Canadian Heart Foundation and the technical assistance of Ms. Joan Collins with some of the experiments.

REFERENCES

1. Coe, J.E. (1983) in contemporary topics in molecular immunology: vol. 9 (Inman, F.P. and Kindl, T.J. Eds.) pp. 211-238, Plenum Publishing Corporation.
2. Saxena, U., Nagpurkar, A. and Mookerjea, S. (1985) Can. J. Biochem. Cell Biol. 63, 1014-1021.
3. Nagpurkar, A. and Mookerjea, S. (1981) J. Biol. Chem., 256, 7440-7448.
4. Mookerjea, S. (1978) Can. J. Biochem., 56, 740-752.
5. Nagpurkar, A., Saxena, U. and Mookerjea, S. (1983) J. Biol. Chem. 258, 10518-10523.
6. Saxena, U., Nagpurkar, A., Dolphin, P.J. and Mookerjea, S. (1986) J. Biol. Chem. (submitted).

7. Nagpurkar, A., Saxena, U., Dolphin, P. and Mookerjea, S. (1985) Proc. 7th International Symposium on Atherosclerosis, Melbourne. Australia, Abstract #377, pg. 178.
8. Weisgraber, K.H., Innerarity, T.L., Rall, S.J. Jr. and Mahely, R.W. (1985) Can. J. Biochem. Cell Biol. 63, 898-905.
9. Nakai, T., Otto, P.S., Kennedy, D.L. and Whayne, T.F. (1976) J. Biol. Chem. 251, 4914-4921.
10. McFarlane, A.S. (1958) Nature (Lond.) 182, 53.
11. Wong, L. and Rubinstein, D. (1979). Atherosclerosis, 34, 249-258.
12. Kovanen, P.T., Brown, M.S. and Goldstein, J.L. (1979) J. Biol. Chem. 254, 11367-11373.
13. Brown, M.S., Demel, T.F., Basu, S.K. and Goldstein, J.L. (1978) J. Supramolecular Structure, 8, 223-234.
14. Van't Hoof, F. and Havel, R.J. (1982) J. Biol. Chem. 257, 10996-11001.