PHOSPHORYLCHOLINE: A SPECIFIC PROMOTER OF HEPARIN AND SERUM β-LIPOPROTEIN INTERACTION[1]

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Received June 8, 1973

SUMMARY

The mechanism of formation of heparin-β-lipoprotein complex was studied. Precipitation of fatty acid labelled β-lipoproteins and optical density changes of the reaction were used as measures for the formation of insoluble complex. Equimolar amounts of Ca-phosphorylcholine were found to be more effective than $CaCl_2$ to form the insoluble complex. In presence of 10 mM $CaCl_2$, minute amounts (2-16 μM) of Ca or Na-phosphorylcholine increased the interaction between heparin and serum β-lipoprotein. Equimolar amounts of choline, ethanolamine, acetylcholine, phosphoserine, phosphothreonine, α-glycerophosphate, α-glycerophosphate plus choline, NaH_2PO_4, galactose, glucosamine and N-acetylglucosamine had no effect on the reaction. The results suggested that phosphorylated quaternary nitrogen groups play a role in the interaction of β-lipoprotein with heparin and divalent cation.

The interaction of macromolecular polyanions, in particular of polysaccharide sulfates, with serum lipoproteins has been widely studied and this reaction in presence of Ca^{2+} has been utilised as a basis for the quantitative measurement of β-lipoproteins in serum (1,2). The hypothesis that the mucopolysaccharides of the arterial connective tissue may in some way render serum lipoproteins insoluble and thereby set the stage for the development of atherosclerotic lesions, underlines the significance of these mucopolysaccharide and lipoprotein interactions.

β-Lipoproteins, comprised of very low- (VLDL) and low density (LDL) lipoproteins, carry large loads of apolar lipids in its core compared to α-lipoproteins. Therefore the β-lipoprotein fraction is more dependent on its surface-oriented phospholipids for

[1] Supported by research grants from the Ontario Heart Foundation and the Medical Research Council of Canada. The expert technical assistance of Mrs. K.K.M. Chen is acknowledged.

solubility (3). It is generally agreed that a complex is formed through Ca^{2+} acting as a bridge between N-sulfate groups of heparin and phosphate groups of phospholipids of β-lipoproteins (4). Activation of heparin-dependent lipoprotein lipase by phospholipids may also reflect a common mechanism of interaction between phospholipids, heparin and the enzyme (5).

Isolated LDL is rapidly precipitated by phospholipase C (6). In contrast, phospholipase D which released about 80% of choline did not precipitate lipoprotein. Also incubation of serum (7) or LDL with phospholipase A caused no turbidity of serum which suggested that the phosphorylcholine molecule may have some special significance for the stability of β-lipoprotein and thereby influence its interaction with mucopolysaccharides and Ca^{2+}.

Our interest in understanding the role of choline and phosphorylcholine in lipoprotein metabolism (8,9) and also recent studies on a specific haptenic function of phosphorylcholine molecule in the interaction between C-polysaccharide and myeloma and other proteins (10-12) prompted us to investigate a possible role of phosphorylcholine in the interaction between polysaccharide heparin and β-lipoprotein.

METHODS

The method of Jordan *et al*. (1) was followed to study the reaction between heparin and serum β-lipoproteins. The complete incubation mixture contained rat serum 0.15 ml; heparin (Connaught Medical Research Laboratories) 50 units, 0.05 ml; $CaCl_2$, 0.05 M, 0.5 ml or as specified and distilled water to make a volume of 2.5 ml. The tubes were incubated at 40°C for 1 hr and the change of optical density (O.D.) was measured at 600 mμ against a blank incubation tube without $CaCl_2$. In preliminary experiments with rat serum it was found that the O.D. of the reaction increased in

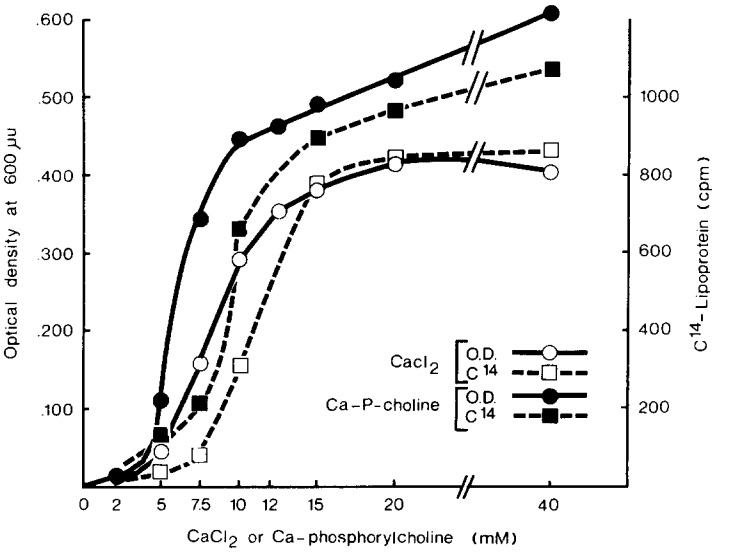

Fig. 1 Effects of $CaCl_2$ and Ca-phosphorylcholine on β-lipoprotein precipitation. Incubation conditions were as in the text. $CaCl_2$ or Ca-phosphorylcholine concentrations were varied. A total of 1,500 counts were recovered from the total lipid extract of 0.15 ml unincubated serum. There was no significant precipitation in absence of heparin.

presence of 5 to 15 mM $CaCl_2$. When Ca-phosphorylcholine was used to replace $CaCl_2$, a sharp increase of O.D. occurred in the range of 5 to 10 mM and at all equimolar concentrations, Ca-phosphorylcholine produced greater turbidity than $CaCl_2$. Furthermore, when μmolar amounts of Ca^{2+} or Na^{+} phosphorylcholine was added the turbidity developed by 10 mM $CaCl_2$ was greatly amplified (3- to 5-fold). In order to make these observations more quantitative and less dependent on O.D. measurements alone, the following methods were used.

Rats (250 to 300 g body wt) fed Purina chow and water were injected through jugular vein 0.6 to 1 ml of palmitic acid-1-^{14}C-albumin complex (13) containing 7.74 x 10^{-6} cpm/ml to label the serum lipoproteins *in vivo*. Animals were bled by aortic exsanguination 30 min after the injection and serum was prepared. Aliquots of fresh serum, containing fatty acid labelled lipoproteins, were

used for incubation with heparin, $CaCl_2$ and other agents as described above. The turbidity formed by incubation was read at 600 mμ and then the tubes were centrifuged (7000 rpm/30 min in Sorvall RC-2B) to sediment the lipoproteins as pellets. The pellets were rinsed with cold distilled water and dissolved in 0.5 ml of 0.3 M NaCl. The lipoproteins were extracted with 20 volumes of chloroform:methanol (2:1), washed several times with water and a chloroform:methanol:0.5 M KCl mixture (3:47:48 v/v). The lipids recovered in the chloroform phase were dried and counted for radioactivity. In trial experiments the lipids recovered from the pellets were separated by thin layer chromatography (13) and over 90% radioactivity of the total lipid extract was recovered in triglycerides indicating that the labelled palmitic acid was incorporated into heparin precipitable β-lipoproteins which contained most of the triglycerides.

RESULTS AND DISCUSSION

Fig. 1 shows the O.D. measurements and ^{14}C-radioactivity recovered in lipoproteins as a function of $CaCl_2$ and Ca-phosphorylcholine concentrations. Ca-phosphorylcholine reacted with lipoprotein at a concentration lower than $CaCl_2$ and had an overall effect greater than $CaCl_2$. The point of sharp increase in turbidity or lipoprotein radioactivity due to $CaCl_2$ or Ca-phosphorylcholine sometimes varied, but this was probably dependent on the amount of lipoproteins present in serum. For example, the turbidity and radioactivity changes appeared at concentrations of Ca salts higher than shown in Fig. 1 in case of serum obtained from fasted rats and, in contrast, for lipemic serum lower concentrations were effective.

Fig. 2 presents evidence of a dramatic effect of small amounts of phosphorylcholine (in the range of 10^{-6} M) in promoting Ca-

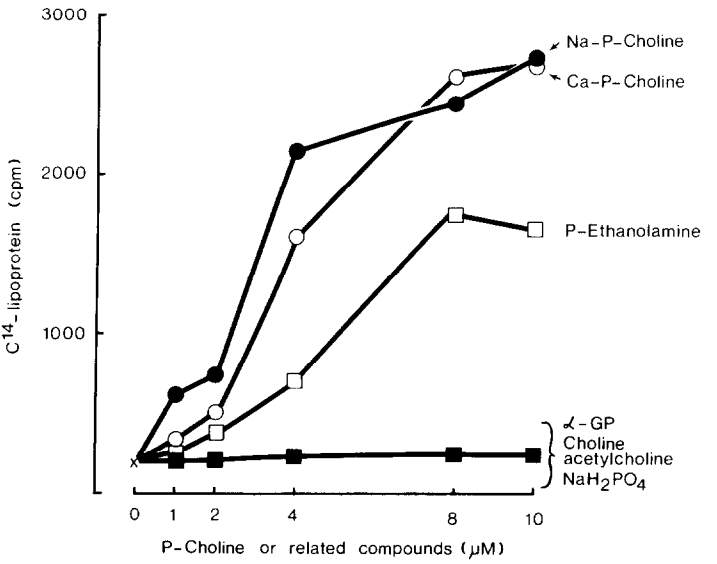

Fig. 2 Specific effect of the phosphorylated quaternary nitrogen compounds on the heparin-β-lipoprotein interaction. Incubation conditions were as in the text. Symbols: incubation with 10 mM $CaCl_2$ —x—; 10 mM $CaCl_2$ + Ca-phosphorylcholine —o—; 10 mM $CaCl_2$ + Na-phosphorylcholine —●—; 10 mM $CaCl_2$ + phosphorylethanolamine —□—; 10 mM $CaCl_2$ + α-glycerophosphate or (choline, acetylcholine, NaH_2PO_4) —■—. A total of 3,600 counts were recovered from the total lipid extract of 0.15 ml unincubated serum.

heparin interaction with lipoproteins when 10 mM $CaCl_2$ was used for the reaction. Only a small amount of ^{14}C-lipoprotein was sedimented in presence of 10 mM $CaCl_2$ alone but this reaction was readily increased by either Ca- or Na-phosphorylcholine and the dependence on the doses of phosphorylated quaternary compounds showed a sigmoidal kinetics. With 8 μM phosphorylcholine, the effect became about equivalent to what was obtained by 20 to 40 mM Ca-phosphorylcholine or $CaCl_2$ (Fig. 1). The effect of Ca^{2+} or Na^+ salts of phosphorylcholine was about equal. Phosphorylethanolamine in equimolar concentrations showed a similar but small effect. In presence of 10 mM $CaCl_2$, α-glycerophosphate, NaH_2PO_4, choline, acetylcholine in concentrations up to 16 μM showed no effect on the reaction. In separate experiments (results not shown) equimolar

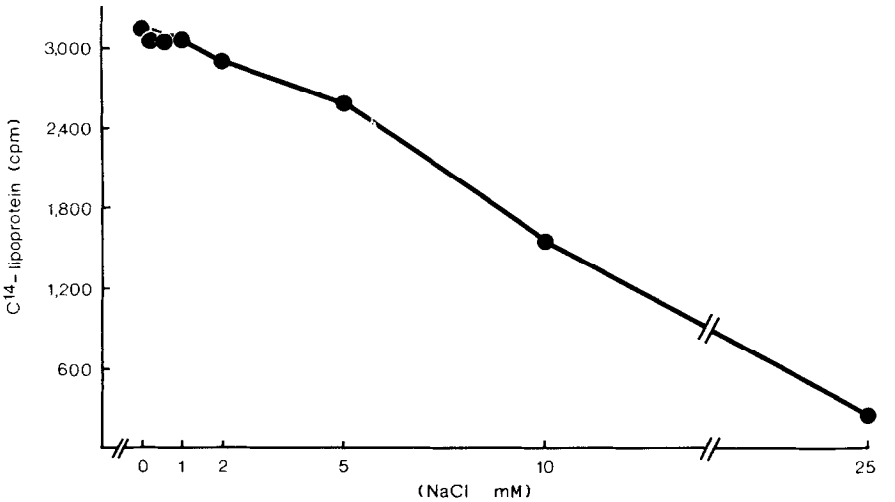

Fig. 3 Dissociation of heparin-β-lipoprotein interaction by NaCl. Incubation conditions were as in the text except that 10 mM $CaCl_2$ plus 4 μM Na-phosphorylcholine were present in the incubations. A control incubation without Na-phosphorylcholine yielded 580 cpm in the sedimented lipoprotein. Variations of NaCl concentrations were as shown.

concentrations of ethanolamine, choline plus α-glycerophosphate, phosphoserine, phosphothreonine, glucosamine, N-acetylglucosamine and galactose were without any effect on the reaction. NaCl in concentrations higher than 1 mM started to inhibit and at a concentration of 25 mM completely inhibited the phosphorylcholine effect indicating the electrostatic nature of the reaction (Fig.3).

The results suggested certain degrees of specificity for the phosphorylated quaternary compounds in promoting the reaction. It also appears that the intact phosphorylcholine molecule is needed for the reaction since other related compounds were unable to mimic the phosphorylcholine effect. In support of a role of phosphorylcholine in surface recognition phenomenon compelling evidence has been obtained (10,11,12) that murine IgA myeloma proteins react with and recognize the phosphorylcholine moiety present in the C polysaccharide of Pneumococcus. For the mechanism

of reaction, it has been suggested (10) that an initial binding of the quaternary nitrogen of phosphorylcholine might alter the conformation of the IgA to bring about binding of the phosphate group. These myeloma proteins also precipitated serum β-lipoprotein and agglutinated erythrocytes coated with human β-lipoprotein, which again indicated that myeloma proteins recognize phosphorylcholine on the surface of β-lipoproteins. Work is in progress to test the possibility of binding of phosphorylcholine with lipoproteins or with heparin in our incubation system.

The results presented in this paper clearly show that phosphorylcholine in a minute amount plays a role to accelerate the β-lipoprotein and heparin-Ca^{2+} interaction. Heparin and divalent cations participate in many intra and extra cellular reactions in relation to phospholipids of the membrane. It will be of interest to examine the role of phosphorylcholine and other related quaternary compounds in these physiological systems. In particular, the relatively high concentration of sphingomyelin in human and primate aortic tissues is known to increase with age and degree of atherosclerosis (14,15). There is also evidence of an active hydrolytic enzyme in human and other arterial tissues which splits sphingomyelin into ceramide and phosphorylcholine (16). A release of minute amounts of phosphorylcholine by such hydrolytic reactions may be a prime factor in modifying the β-lipoprotein and mucopolysaccharide interaction at these sites.

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