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Review

Purification of lipases, phospholipases and kinases by heparin-Sepharose chromatography

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Abstract

Heparin interacts with lipases, phospholipases and kinases. Immobilized heparin can be used for the purification of diacylglycerol and triacylglycerol lipases, phospholipases A_2 and C and protein and lipid kinases. The use of heparin-Sepharose is an important development in analytical and preparative techniques for the separation and isolation of lipases, phospholipases and kinases.

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1. Introduction

In recent years heparin-Sepharose chromatography has revolutionized the purification of enzymes and macromolecules. This method utilizes the specific and electrostatic interactions of heparin with enzymes, growth factors, receptors and blood coagulation factors [1–3]. The general

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scheme of heparin-Sepharose chromatography involves the covalent attachment of heparin to Sepharose. A crude tissue preparation is then applied to the column; only those enzymes and macromolecules that specifically interact with heparin are retained on the column while the other proteins having no affinity for heparin are washed out. The retained enzymes and macromolecules can be eluted from the column either by adding an excess of heparin to the equilibrating buffer or by using substances such as salts or denaturants [2]. Theoretically, the success of heparin-Sepharose chromatography depends largely upon how closely the conditions used in the experiment mimic the native or biological interactions.

Heparin-Sepharose chromatography has been useful for the purification of lipases, phospholipases and kinases [4–13]. These enzymes, along with glycosaminoglycans, play an important role in cell division, cell differentiation and signal transduction [14,15]. The purpose of this review is to discuss the use of heparin-Sepharose for the purification of lipases, phospholipases and kinases. It is hoped that this discussion will promote further studies on the uses of heparin-Sepharose in analytical and preparative biochemistry and will result in the development of procedures for better understanding of heparin–enzyme interactions and evaluation of heparin–enzyme binding constants.

Heparin is a linear, naturally occurring, highly sulfated glycosaminoglycan with anticoagulant and antilipemic properties [16]. It is characterized by the presence of 2-acetamido-2-deoxy-α-D-glucopyranosyl and α-D-glucopyranosyl-uronic acid residues containing various proportions of O-sulfate, N-sulfate and acetyl groups. In the disaccharide repeating units of heparin, glucuronic acid and D-glucosamine are linked by α-1,4-glycosidic linkages [2]. Heparin exists in a wide range of molecular masses (5000–40 000). It is highly charged and appears to occur entirely in mast cells. Heparin has many structural features in common with heparan sulfate except it is more highly sulfated and contains a large proportion of iduronic acid. Because of its unique structure and surface charge distribution, heparin is able to interact strongly with many enzymes and macromolecules in two ways: a positive, cooperative binding and a specific binding [16,17]. In positive, cooperative binding, heparin binds to a protein at different sites, and the affinity shown at each individual interacting site contributes to the overall affinity of the heparin for its protein ligand. An example of a positive, cooperative interaction is that between heparin and a lipoprotein particle with several apolipoprotein molecules [18,19]. In specific binding of heparin to protein, other glycosaminoglycans cannot substitute for heparin in its binding nor in exerting a biological activity. An example of a specific heparin protein interaction is the binding between heparin and antithrombin III [2].

When a protein is bound to heparin, its biological activity is either enhanced or decreased; when the heparin is then removed, the original activity of the protein is restored. This restorative property plays an important role in the purification of enzymes and growth factors by heparin-Sepharose chromatography, and also in the evaluation of heparin–enzyme equilibrium constants and other quantitative features of protein–heparin reactions. Effects of heparin on lipases, phospholipases and kinases are shown in Table 1.

2. Interactions of heparin with lipases

Two triacylglycerol lipases are released into the bloodstream after intravenous injections of heparin [20]. Lipoprotein lipase, released from extrahepatic tissues, is required for the removal of both chylomicron and very low-density lipoprotein triacylglycerol from plasma. This enzyme is inhibited by sodium chloride and requires a specific apolipoprotein for optimal activity. A second enzyme that is released from the liver is hepatic triacylglycerol lipase. This enzyme differs from lipoprotein lipase in that there is no requirement for an apoprotein cofactor, and 1 *M* NaCl stimulates rather than inhibits the enzymic activity.

Rat lipoprotein lipase is markedly stimulated

Table 1
Effects of heparin on lipases, phospholipases and lipid and protein kinases

Enzyme	Effect
Diacylglycerol kinase	Inhibition [12]
Phosphatidyl inositol 4-kinase	Inhibition [35]
Protein kinase C	Inhibition [32]
Casein kinase II	Inhibition [30]
Nuclear kinase	Inhibition [67]
β-Adrenergic receptor kinase	Inhibition [34]
Protein kinase A	Inhibition [32]
Myosin II heavy chain kinase A	Activation [31]
Tyrosine protein kinase	Inhibition [32]
Lipoprotein lipase	Inhibition [22]
Triacylglycerol lipase (Plasma)	Activation [43]
Triacylglycerol lipase (Brain)	Inhibition [23]
Diacylglycerol lipase	Inhibition [4]
Lysophospholipase	Inhibition [4]
Monoacylglycerol lipase	No effect [4]
Phospholipase A_2 (pancreas)	Inhibition [24]
Phospholipase A_2 (synovial fluid)	No effect [25]
Phospholipase A_2 (brain, M_r 110 000)	No effect [26]
Phospholipase A_2 (brain, M_r 39 000)	Inhibition [26]

by heparin, perhaps by binding of the enzyme to chylomicrons. Heparin has no effect on the stability of the enzyme nor on its activity once the enzyme–chylomicron complex is formed. It has been proposed that heparin functions as a specific ligand that acts as an allosteric modifier of lipoprotein lipase and alters the kinetics of substrate–enzyme interactions [21]. In contrast, milk lipoprotein lipase is strongly inhibited by heparin [22].

Hepatic triacylglycerol lipase participates in the clearance of chylomicron remnants and in conversion of high-density lipoprotein subfractions (HDL2 into HDL3). Like lipoprotein lipase, this enzyme is also stimulated by heparin [11]. Mammalian brain triacylglycerol lipase is strongly inhibited by heparin. At 5 μg/ml, heparin produces 50% inhibition of enzymic activity [23]. Heparin also inhibits bovine brain diacylglycerol lipase in a concentration-dependent manner. It has no effect on monoacylglycerol lipase, whereas lysophospholipase is partially inhibited (Fig. 1). The inhibition of diacylglycerol lipase by heparin is quite specific

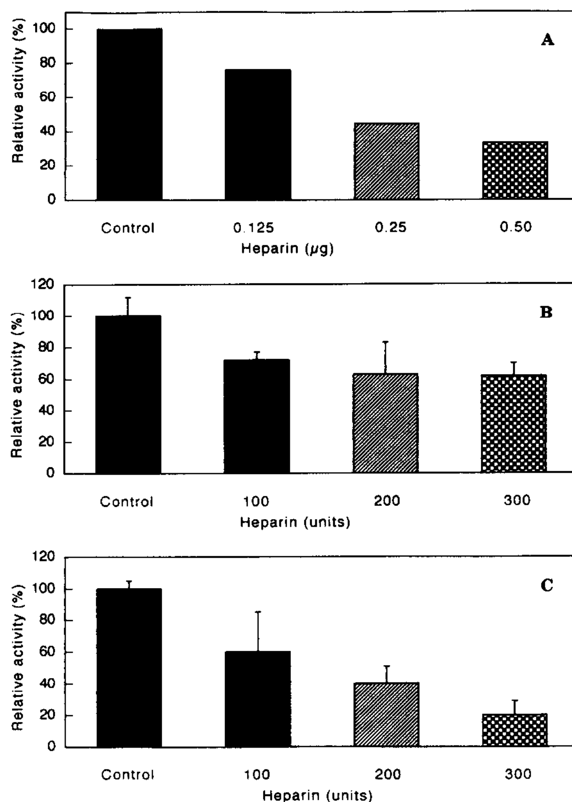

Fig. 1. Effect of heparin on triacylglycerol lipase, lysophospholipase and diacylglycerol lipase. (A) Milk triacylglycerol lipase [22], (B) bovine brain lysophospholipase [4] and (C) bovine brain diacylglycerol lipase [4].

because other glycosaminoglycans do not affect the enzymic activity [4].

3. Interactions of heparin with phospholipases

Heparin markedly inhibits the activity of porcine pancreatic phospholipase A_2 [24]. It has been suggested that interactions of porcine pancreatic phospholipase A_2 are electrostatic, and Ca^{2+}, which is required for optimal activity of phospholipases A_2, is not required for heparin binding to pancreatic phospholipase A_2. Based on circular dichroism and fluorescence probe studies, the conformation of the amino-terminus region of pancreatic phospholipase A_2 is important in heparin binding [24]. This region of

pancreatic phospholipase A_2 contains Arg, His and Lys. These amino acids may play an important role in the binding of pancreatic phospholipase A_2 to heparin. Synovial fluid phospholipase A_2, which resembles pancreatic enzyme in its kinetic properties, also binds to heparin, but its catalytic activity is not affected [25].

Glycosaminoglycans affect the activities of bovine brain cytosolic phospholipases A_2 [26]. Heparin and other glycosaminoglycans markedly inhibit phospholipase A_2 of M_r 39 000, whereas phospholipase A_2 of M_r 110 000 is not affected by heparin or other glycosaminoglycans (Fig. 2). Inhibition of phospholipase A_2 of M_r 39 000 by heparin and other glycosaminoglycans was reversed with protamine sulfate and histone. This suggests that at least some interactions of heparin with this phospholipase A_2 are electrostatic. Rat liver lysosomal phospholipases A_1 and A_2 are also inhibited by heparin [27]. The mechanism of inhibition of lipases and phospholipases is not known. However, three possibilities should be carefully considered. Heparin may bind directly to the amino-terminus of lipases and phospholipases (which is rich in basic amino acids), blocking the interfacial recognition site by steric hindrance. Another possibility is that the conformation of the interfacial recognition site may be altered by the binding of heparin to another site on the enzymes. Finally, heparin is known to bind phospholipids [28] and may interfere with the interaction between enzyme and substrate.

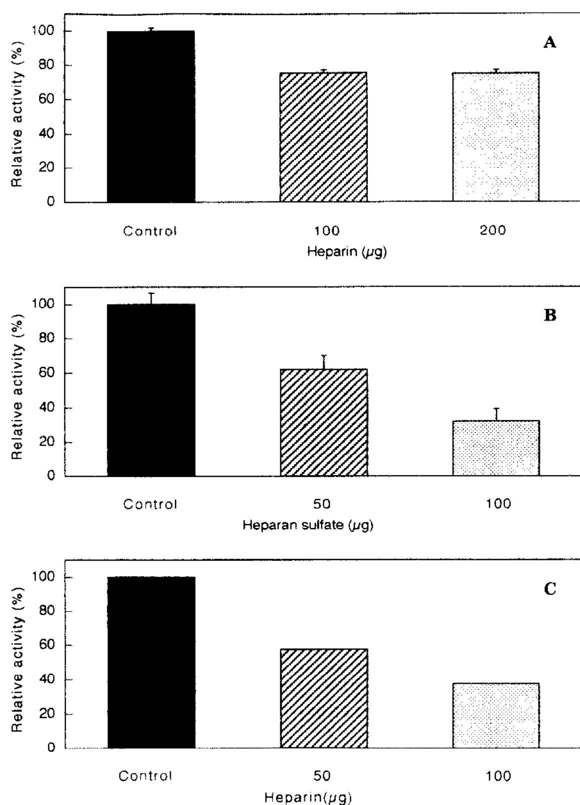

Fig. 2. Effect of heparin and heparan sulfate on phospholipases A_2. (A) Bovine brain phospholipase A_2, M_r 39 000 [26], (B) bovine brain phospholipase A_2, M_r 39 000 [26] and (C) pancreatic phospholipase A_2 [24].

4. Interactions of heparin with protein and lipid kinases

Protein kinases are classified into several groups. Cyclic nucleotide-dependent kinases are active only with ATP as the phosphate donor and preferentially phosphorylate basic proteins such as histone *in vitro*. Cyclic nucleotide-independent protein kinases utilize GTP as well as ATP as the phosphate donor and prefer acidic proteins such as casein and phosvitin. Heparin is a potent inhibitor of phosvitin and casein kinases but has no effect on histone kinase [29–31]. For casein kinase the nature of inhibition is competitive (inhibitor constant, $K_i = 1.4$ nM). The inhibition is not reversed by ATP. However, in rabbit smooth muscle cells, protein kinases are markedly inhibited by heparin [32]. Dictyostelium myosin II heavy-chain kinase A is stimulated by heparin [31], whereas protein kinase C is strongly inhibited in a dose-dependent manner [32]. The nature of inhibition is non-competitive with respect to ATP ($K_i = 0.2$ μM). The molecular size of heparin is a critical determinant for inhibition of protein kinase C and other protein kinases [32]. Very-low-molecular-mass fractions of heparin (M_r 600–20 000) have no effect on the activities of protein kinases, but high-molecular-mass heparin (*ca.* 40 000) produces maximal inhibition. Heparin also inhibits formation of

c-fos mRNA in control vascular smooth muscle cells but does not inhibit serum stimulation of c-fos mRNA in protein kinase down-regulated vascular smooth muscle cells. Thus, heparin may selectively inhibit protein kinase C-dependent but not protein kinase C-independent stimulation of gene expression [33].

β-Adrenergic receptor kinase is also inhibited by heparin in a concentration-dependent manner [34]. In this system, heparin is a competitive inhibitor with respect to rhodopsin. Fibroblast growth factor receptor kinase has an essential heparin-binding domain that markedly affects its kinase activity [3]. One of the early cellular events induced by the binding of growth factors to their respective receptors is the stimulation of phospholipases A_2 and C. These enzymes hydrolyze membrane phospholipids and generate second messengers, arachidonate, eicosanoids, diacylglycerol and inositol 1,4,5-trisphosphate [14,15]. The messengers are required for normal cell function.

Lipid kinases (phosphatidylinositol 4-phosphate kinase and diacylglycerol kinases) are also inhibited by heparin in a dose-dependent manner [12,35,36]. The effects of heparin on these enzymes may be involved in the mechanism of oncogene-induced cell transformation.

5. Attachment of heparin to solid supports

The ideal matrix for the attachment of heparin should be hydrophilic, inert, rigid, spherical and stable to chemical and microbial degradation. Sepharose is the trade name (Pharmacia, Uppsala, Sweden) of the beaded agarose (a linear polysaccharide consisting of alternating residues of D-galactose and 3,6-anhydro-L-galactose). This matrix has been used extensively for the coupling of heparin [2]. The major requirements for the immobilization of heparin to matrix are the synthesis of a stable linkage between the matrix and heparin and, perhaps more importantly, retention of specific heparin-binding characteristics of the immobilized heparin. Heparin has been coupled to Sepharose, Affi-Gel, polyacrylamide and Toyopearl gel by several procedures described elsewhere in detail [2,37–39]. Several factors affect the coupling of heparin to the matrix. They are the pH of the coupling buffer, temperature, reaction time and amount of reacted heparin.

Success of the purification of lipases, phospholipases and kinases depends upon the following requirements: (1) the protein to be isolated should bind in a reversible way to heparin with an affinity such that it can be retained on the heparin-Sepharose while the other proteins are washed away; (2) the enzymes should interact with heparin-Sepharose with an affinity allowing their elution by the free ligand or by other non-denaturing agents; and (3) accompanying proteins should not bind to the matrix, but, if retained, should not be eluted from it under the conditions used for the desired protein.

6. Assay of heparin in heparin-Sepharose samples

The heparin content of heparin-Sepharose can be estimated by determining the hexosamine, uronic acid, and sulfate contents of the gel. With this method the above heparin constituents in the reaction mixture are determined prior to coupling and are compared to the free heparin that is left in the supernatant and washing after coupling. The difference is taken as the amount of heparin bound to Sepharose [2]. The amount of heparin in heparin-Sepharose can also be determined potentiometrically by the titration of heparin carboxyl groups [40]. Some investigators have used the dye-binding interactions of *o*-toluidine blue to colorimetrically or fluorimetrically quantitate unbound heparin before and after coupling [41]. The amount of heparin covalently bound to Sepharose can also be determined by assessing the interference by heparin in the Bradford dye-binding assay for proteins [42]. This method is extremely sensitive, rapid, and inexpensive, and it is capable of distinguishing between heparin and other polysaccharides.

7. Purification of lipases, phospholipases, lysophospholipases and kinases by heparin-Sepharose chromatography

Lipases, phospholipases and kinases have been successfully purified by heparin-Sepharose column chromatography (Table 2). Heparin-Sepharose is packed into a column and washed with 4 to 6 bed volumes of the desired buffer. Crude preparations of lipolytic enzymes or kinases are dialyzed against the equilibrating buffer and applied to the heparin-Sepharose column at a flow-rate of 0.2 ml/min. Enzymes that interact with immobilized heparin are retained and all other proteins are washed out. The retained enzymes can be eluted from the column with a gradient of NaCl or heparin.

7.1. Purification of lipases

Lipoprotein lipase and hepatic triacylglycerol lipase from human plasma bind tightly to heparin-Sepharose, but can be eluted with a linear gradient of sodium chloride. This procedure resulted in a 7400-fold purification of lipoprotein lipase with 95% recovery of enzymic activity. Hepatic triacylglycerol lipase was purified 6500-fold with 48% recovery under similar conditions by heparin-Sepharose chromatography [5]. The elution profile and fold purification of canine hepatic triacylglycerol lipase [43] was similar to that of the human plasma triacylglycerol lipase. Bovine brain diacylglycerol lipases have also been purified by heparin-Sepharose chromatography. About 60 to 65% of the protein was washed out while diacylglycerol lipase was completely retained. The enzyme appeared as a sharp peak when the column was washed with 0.5 *M* NaCl [4]. Heparin-Sepharose chromatography yielded a 4-fold purification with 75% recovery over the previous step [44].

Table 2
Lipases, phospholipases and kinases purified by heparin-Sepharose chromatography

Enzyme	Fold purification
Diacylglycerol lipase [4]	10
Triacylglycerol lipase [5]	6250
Lipoprotein lipase [5]	7430
Phospholipase A_2 [7]	17
Phospholipase A_2 [48]	4
Phospholipase A_2 [47]	61
Phospholipase A_2 [46]	3
Phospholipase C [13]	7
Phospholipase C [11]	17
Phospholipase C [11]	9
Diacylglycerol kinase [12]	3
Diacylglycerol kinase [12]	7

7.2. Purification of phospholipases A_2

Heparin-Sepharose chromatography has been used extensively for the purification of rabbit and bovine platelet phospholipases A_2 [8,45,46]. Some phospholipases A_2 were retained on the heparin-Sepharose column; others were washed out [8,45,46]. In rabbit platelets the heparin binding form of phospholipase A_2 had a molecular mass of 14 000 and reacted with antihuman phospholipase A_2 monoclonal antibody of M_r 14 000. The phospholipase A_2 activity found in the heparin unbound fraction did not appreciably react with the above antibody [8,45,46]. Heparin-Sepharose chromatography gave a 3-fold purification of phospholipase A_2 with 70% recovery of enzymic activity. Similarly, pancreatic phospholipase A_2 can also be separated into heparin-bound and heparin-unbound fractions [32]. A phosphatidylserine-hydrolyzing phospholipase A_2 has been purified by heparin-Sepharose chromatography from cultured mast cells. In this case, heparin-Sepharose chromatography yielded a 61-fold purification with 83% recovery of enzymic activity [47]. Heparin-Sepharose chromatography of human monocytic cell cytosolic phospholipase A_2 resulted in a 4-fold purification of this enzyme [48].

7.3. Purification of phospholipase C

Heparin-Sepharose chromatography has been useful for the purification of phospholipase C from several sources. Heparin-Sepharose chromatography gave a 5-fold purification of human platelet phospholipase C with 73% recovery of enzymic activity [49]. Similarly, bovine liver [10],

spleen [50], and iris sphincter muscle [11] phospholipases C have been purified by this procedure. In all cases heparin-Sepharose chromatography resulted in good purification and yield of enzymic activity (Fig. 3).

7.4. *Purification of protein kinase C and casein kinase II*

Protein kinase C and casein kinase II can be purified by heparin-Sepharose chromatography [51,52]. Heparin-Sepharose chromatography yielded a 17-fold purification of bovine kidney protein kinase C with a recovery of 42% [51]. Similarly, casein kinase II was completely retained on a heparin-Sepharose column and was eluted with 0.5 *M* NaCl. This step resulted in an increase of specific activity with 55% yield of enzymic activity.

7.5. *Purification of diacylglycerol kinase and phosphatidylinositol 4-kinase*

Diacylglycerol kinases and phosphatidylinositol 4-kinases were retained on heparin-Sepharose and heparin-acrylamide columns [12,35,36]. They can be eluted from heparin-Sepharose with a linear gradient of NaCl. Heparin-Sepharose chromatography of diacylglycerol kinase preparations resulted in two peaks designated as diacylglycerol kinase I and diacylglycerol kinase II [12]. Phosphatidylinositol 4-kinase also bound to a heparin–acrylamide column and could be eluted by a linear NaCl gradient. This procedure resulted in a 15-fold purification with 81% recovery of enzymic activity [36].

An important feature of heparin-Sepharose chromatography is the resolution of multiple forms (isozymes) of lipases, phospholipases and kinases. It is not possible to separate the multiple forms of the above enzymes by ion-exchange and hydrophobic chromatographies. Multiple forms of phospholipases A_2 [7], phospholipase C [13,53–55], diacylglycerol kinase [12] and protein kinase C [51] can be separated easily from crude extracts using heparin-Sepharose chromatography. This suggests that multiple forms of these enzymes have different affinities for heparin. Heparin-Sepharose chromatography can also be used as an alternative to equilibrium dialysis for the direct determination of enzyme–heparin affinity constants [56].

Heparin-Sepharose columns can be regenerated for reuse by washing the gel alternatively with a 10 × bed volume of high pH (8.5) and low pH (5.0) buffers containing 0.5 *M* NaCl. The

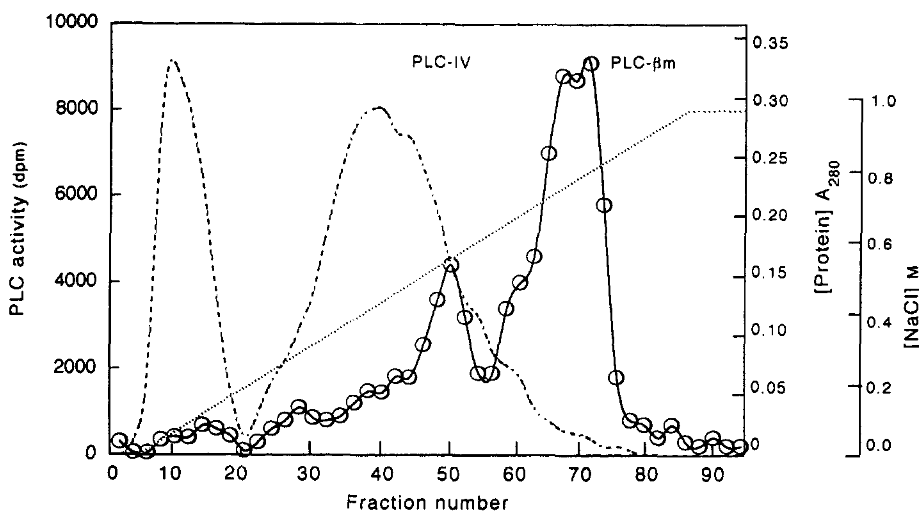

Fig. 3. Separation of multiple forms of rabbit brain phospholipase C by heparin-Sepharose chromatography. ○ = Phospholipase C (PLC) activity; broken line = A_{280}; dotted line = NaCl. Data taken from ref. 13.

addition of 6 *M* urea or guanidine hydrochloride to the washing buffer removes all proteins from the heparin-Sepharose columns.

8. Advantages and disadvantages of heparin-Sepharose chromatography

Heparin has a stable structure, and its functional groups can be easily coupled to Sepharose, Affi-Gel or polyacrylamide. The immobilized heparin can interact reversibly with many enzymes and proteins [2]. The swollen heparin-Sepharose is stable for at least two years at 4°C in the presence of 0.02% merthiolate [57]. Heparin-Sepharose can be sterilized by autoclaving at pH 7.0 at 120°C. It has excellent flow properties and packs well into columns. Because of its polymeric and polyanionic properties, the immobilized heparin has a very high binding capacity, and this allows the use of a small bed volume. As a result, enzymes are eluted from the column in small volumes with very high activities. The elution of lipases, phospholipases or kinases from heparin-Sepharose columns by heparin or other polyanionic polysaccharides gives sharper peaks than elution by a salt gradient.

Heparin is a general ligand that inhibits a variety of biological reactions. Some of these interactions are specific; others are electrostatic. The presence of negatively charged groups on heparin results in retention of many positively charged proteins on the heparin-Sepharose column. Thus, electrostatic interactions can modify the efficiency of the protein separation process. These interactions can be eliminated by packing the column in high-ionic-strength buffer and eluting it with either heparin or other glycosaminoglycans. Trehalose phosphate synthetase and hyaluronidase have been successfully eluted from a heparin-Sepharose column by heparin and hyaluronic acid, respectively [58,59]. Under high ionic strength the elution of specifically bound proteins can also be achieved by using low concentrations of denaturants. In some cases this procedure has resulted in the elution of proteins that interact with heparin by specific interactions.

Another major disadvantage of heparin-Sepharose chromatography is its lack of specificity. It is not possible to obtain homogeneous preparations of lipases, phospholipases or kinases in a single step. Furthermore, the chemical modification of heparin or the matrix during coupling can introduce additional binding sites. Blocking of these sites, as well as prior knowledge of the isoelectric points of the enzymes to be purified and their behavior on ion-exchange columns, are required in order to select the appropriate spacer and buffer for optimal elution of enzymic activities.

9. Conclusions

The unique structure and surface charge distribution of heparin allow it to interact with lipases, phospholipases, lysophospholipases and kinases in a specific manner (Fig. 4). These interactions inhibit or stimulate activities of the

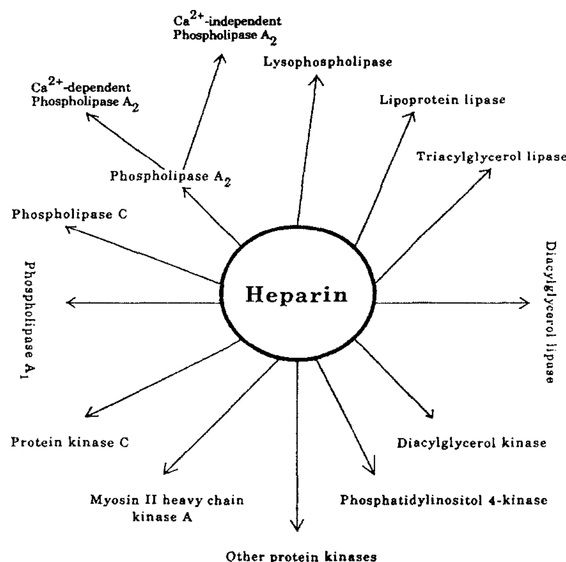

Fig. 4. Interactions of lipases, phospholipases and kinases with heparin.

above enzymes. The crucial question, whether these interactions occur *in vivo* and play some role in the regulation of lipases, phospholipases, lysophospholipases and kinases, cannot be answered at this time because our knowledge of heparin–enzyme interactions is incomplete. However, it is possible that in the plasma membrane, heparan sulfate, a glycosaminoglycan similar to heparin in structure, and other glycosaminoglycans may serve to anchor lipases, phospholipases and kinases from cytosol to the plasma membrane and regulate signal transduction, cell proliferation, cell differentiation and cell division [14,15]. Tyrosine kinase and phospholipases A_2 and C are linked to epidermal and fibroblast growth factors (FGFs) [15,60]. FGFs bind heparin, which potentiates or inhibits specific actions on different cell types [61,62]. Heparin also protects the FGF from proteolytic degradation [62,63]. Based on these studies, it has been suggested that heparin or heparan sulfate, along with protein kinases, may be involved in translocation and regulation of lipases and phospholipases [26]. Heparin has also been shown to possess anti-cancer activity. It may cause tumor regression and inhibit metastasis [64]. Recent studies have also indicated that heparin suppresses local immune response leading to tissue destruction. In fact, heparin reduces glomerulosclerosis, whereas the administration of protamine accelerates the development of this pathology [65,66]. There is a growing body of evidence to indicate that heparin–enzyme interactions are important in connective tissues and in the circulatory system [16]. This suggests that heparin and other glycosaminoglycans play important roles in cellular metabolism *in vivo*. *In vitro*, heparin–protein interactions can be used successfully for the isolation and characterization of lipases, phospholipases and kinases.

10. Acknowledgement

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11. References

[1] A.A. Farooqui, *J. Chromatogr.*, 184 (1980) 335.
[2] A.A. Farooqui and L.A. Horrocks, *Adv. Chromatogr.*, 23 (1984) 127.
[3] M. Kan, F. Wang, J. Xu, J.W. Crabb, J. Hou and W.L. McKeehan, *Science*, 259 (1993) 1918.
[4] A.A. Farooqui, W.A. Taylor and L.A. Horrocks, *Biochem. Biophys. Res. Commun.*, 122 (1984) 1241.
[5] C.-F. Cheng, A. Bensadoun, T. Bersot, J.S.T. Hsu and K.H. Melford, *J. Biol. Chem.*, 260 (1985) 10720.
[6] J. Peinado-Onsurbe, C. Soler, M. Soley, M. Llobera and I. Ramirez, *Biochim. Biophys. Acta*, 1125 (1992) 82.
[7] M. Hayakawa, I. Kudo, M. Tomita and K. Inoue, *J. Biochem.*, 103 (1988) 263.
[8] D.K. Kim, I. Kudo, Y. Fujimori, H. Mizushima, M. Masuda, R. Kikuchi, K. Ikizawa and K. Inoue, *J. Biochem.*, 108 (1990) 903.
[9] S. Higashi, T. Kobayashi, I. Kudo and K. Inoue, *J. Biochem.*, 103 (1988) 442.
[10] K. Shaw and J.H. Exton, *Biochemistry*, 31 (1992) 6347.
[11] C.-J. Zhou, R.A. Akhtar and A.A. Abdel-Latif, *Biochem. J.*, 289 (1993) 401.
[12] Q. Chen, N. Klemm and I. Jeng, *J. Neurochem.*, 60 (1993) 1212.
[13] H.R. Carter, M.A. Wallace and J.N. Fain, *Biochim. Biophys. Acta*, 1054 (1990) 119.
[14] Y. Nishizuka, *Science*, 258 (1992) 607.
[15] A.A. Farooqui, Y. Hirashima and L.A. Horrocks, in N.G. Bazan, G. Toffano and M. Murphy (Editors), *Neurobiology of Essential Fatty Acids*, Plenum Press, New York, 1992, pp. 11–25.
[16] M.-C. Bourin and U. Lindahl, *Biochem. J.*, 289 (1993) 313.
[17] F. Zhou, T. Hook, J.A. Thompson and M. Hook, *Adv. Exp. Med. Biol.*, 313 (1992) 141.
[18] M. Gigli, G. Ghiselli, G. Torri, A. Naggi and V. Rizzo, *Biochim. Biophys. Acta*, 1167 (1993) 211.
[19] D. Gómez-Coronado, G.T. Sáez, M.A. Lasunción and E. Herrera, *Biochim. Biophys. Acta*, 1167 (1993) 70.
[20] P.E. Fielding, V.G. Shore and C.J. Fielding, *Biochemistry*, 13 (1974) 4318.
[21] R.L. Patten and C.H. Hollenberg, *J. Lipid Res.*, 10 (1969) 374.
[22] I. Posner and J. DeSanctis, *Arch. Biochem. Biophys.*, 253 (1987) 475.
[23] J. Le Petit, O. Nobili and J. Boyer, *Pharmacol. Biochem. Behav.*, 24 (1986) 1543.
[24] M.B. Diccianni, L.R. McLean, W.D. Stuart, M.J. Mistry, C.M. Gil and J.A.K. Harmony, *Biochim. Biophys. Acta*, 1082 (1991) 85.
[25] T.P. Parks, S. Lukas and A.F. Hoffman, *Adv. Exp. Med. Biol.*, 275 (1990) 55.
[26] H.-C. Yang, A.A. Farooqui and L.A. Horrocks, *Biochem. J.*, 299 (1994) 91.

[27] W.B. Weglicki, R.C. Ruth, K. Owens, H.D. Griffin and B.M. Waite, *Biochim. Biophys. Acta*, 150 (1973) 145.
[28] S.L. Drake, D.J. Klein, D.J. Mickelson, T.R. Oegama, L.T. Furcht and J.B. McCarthy, *J. Cell Biol.*, 117 (1992) 1331.
[29] P.H. Mäenpää, *Biochim. Biophys. Acta*, 498 (1977) 294.
[30] G.M. Hathaway, T.H. Lubben and J.A. Traugh, *J. Biol. Chem.*, 255 (1980) 8038.
[31] Q.G. Medley, W.L. Bagshaw, T. Truong and G.P. Côté, *Biochim. Biophys. Acta*, 1175 (1992) 7.
[32] J.-M. Herbert and J.-P. Maffrand, *Biochim. Biophys. Acta*, 1091 (1991) 432.
[33] L.A. Pukac, M.E. Ottlinger and M.J. Karnovsky, *J. Biol. Chem.*, 267 (1992) 3707.
[34] J.L. Benovic, W.C. Stone, M.G. Caron and R.J. Lefkowitz, *J. Biol. Chem.*, 264 (1989) 6707.
[35] C. Cochet and E.M. Chambaz, *Biochem. J.*, 237 (1986) 25.
[36] G.H. Jenkins, G. Subrahmanyam and R.A. Anderson, *Biochim. Biophys. Acta*, 1080 (1991) 11.
[37] R.L. Schnaar, *Anal. Biochem.*, 143 (1984) 1.
[38] H. Sasaki, A. Hayashi, H. Kitagaki-Ogawa, I. Matsumo-To and N. Seno, *J. Chromatogr.*, 400 (1987) 123.
[39] S.R. Narayanan, S.V. Kakodkar and L.J. Crane, *Anal. Biochem.*, 188 (1990) 278.
[40] M.Ya. Varshavskaya, A.L. Klibanov, V.S. Goldmacher and V.P. Torchilin, *Anal. Biochem.*, 95 (1979) 449.
[41] P.K. Smith, A.K. Mallia and G.T. Hermanson, *Anal. Biochem.*, 109 (1980) 466.
[42] M.Y. Khan and S.A. Newman, *Anal. Biochem.*, 187 (1990) 124.
[43] P.H. Frost, V.G. Shore and R.J. Havel, *Biochim. Biophys. Acta*, 712 (1982) 71.
[44] A.A. Farooqui, K.W. Rammohan and L.A. Horrocks, *Ann. N.Y. Acad. Sci.*, 559 (1989) 25.
[45] D.K. Kim, P.G. Suh and S.H. Ryu, *Biochem. Biophys. Res. Commun.*, 174 (1991) 189.
[46] D.K. Kim, I. Kudo and K. Inoue, *Biochim. Biophys. Acta*, 1083 (1991) 80.
[47] M. Murakami, I. Kudo, M. Umeda, A. Matsuzawa, M. Takeda, M. Komada, Y. Fujimori, K. Takahashi and K. Inoue, *J. Biochem.*, 111 (1992) 175.
[48] W. Rehfeldt, K. Resch and M. Goppelt-Struebe, *Biochem. J.*, 293 (1993) 255.
[49] J.J. Baldassare, P.A. Henderson and G.J. Fisher, *Biochemistry*, 28 (1989) 6010.
[50] Y. Homma, Y. Emori, F. Shibasaki, K. Suzuki and T. Takenawa, *Biochem. J.*, 269 (1990) 13.
[51] H. Nakanishi and J.H. Exton, *J. Biol. Chem.*, 267 (1992) 16347.
[52] J.S. Sanghera, L.A. Charlton, H.B. Paddon and S.L. Pelech, *Biochem. J.*, 283 (1992) 829.
[53] H. Kawaguchi and H. Yasuda, *Circ. Res.*, 62 (1988) 1175.
[54] A. Faber and I. Aviram, *Biochim. Biophys. Acta*, 1128 (1992) 8.
[55] Y. Banno, T. Nakashima, T. Kumada, K. Ebisawa, Y. Nonomura and Y. Nozawa, *J. Biol. Chem.*, 267 (1992) 6488.
[56] D.J. Winzor, *J. Chromatogr.*, 597 (1992) 67.
[57] A.A. Farooqui, unpublished results.
[58] A.D. Elbein and M. Mitchell, *Arch. Biochem. Biophys.*, 168 (1975) 369.
[59] P.N. Srivastava and A.A. Farooqui, *Biochem. J.*, 183 (1979) 531.
[60] N. Hack, B.L. Margolis, A. Ullrich, J. Schlessinger and K.L. Skorecki, *Biochem J.*, 275 (1991) 563.
[61] S. Vannucchi, F. Pasquali, G. Fiorelli, P. Bianchini and M. Ruggiero, *FEBS Lett.*, 263 (1990) 137.
[62] D. Gospodarowicz and J. Cheng, *J. Cell. Physiol.*, 128 (1986) 475.
[63] G. Neufeld, D. Gospodarowicz, L. Dodge and D.K. Fujii, *J. Cell. Physiol.*, 131 (1987) 131.
[64] A. Górski, M. Wasik, M. Nowaczyk and G. Korczak-Kowalska, *FASEB J.*, 5 (1991) 2287.
[65] D.R. Coombe, C.R. Parish, I.A. Ramshaw and J.M. Snowden, *Int. J. Cancer*, 39 (1987) 82.
[66] T. Saito, E. Sumithran, E.F. Glasgow and R.C. Atkins, *Kidney Int.*, 32 (1987) 691.
[67] D.L. Friedman, N.J. Kleiman and F.E. Campbell, Jr., *Biochim. Biophys. Acta*, 847 (1985) 165.