Heparin inhibits the activity of protein phosphatase-1

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Heparin inhibited the dephosphorylation of rabbit skeletal muscle or liver phosphorylase *a* by protein phosphatase-1. Other glycosaminoglycans (chondroitin sulfates) and their constituents were found to be without effect. The chromatography of a partially purified phosphatase preparation on heparin–Sepharose CL-6B resulted in a fraction that did not bind to the matrix and its activity was not inhibited by heparin or inhibitor-1. The phosphatase bound to heparin–Sepharose was eluted by 0.2 M NaCl and was inhibited by heparin or inhibitor-1.

Glycogen metabolism *Protein phosphatase-1* *Phosphorylase* a *Heparin* *Inhibitor-1* *Heparin–Sepharose*

1. INTRODUCTION

Protein phosphatases (EC 3.1.3._) catalyze the dephosphorylation of various phosphoproteins, and for this reason, there has been much interest in the control of their activity. A very large number of phosphatase activities have been described and the nature of phosphatases has been a subject of controversy. It seems that two classes of the enzymes, termed phosphatase-1 and -2, account for virtually all of the phosphatase activity [1,2]. The dephosphorylation reactions are important regulatory devices of glycogen metabolism. The activity of phosphorylase *a* (EC 2.4.1.1) is controlled by reversible phosphorylation of a serine residue. Ligands and proteins can influence the rate of dephosphorylation with the modification of phosphorylase *a* (for references, see [3–6]).

Also of current interest are two heat-stable protein inhibitors of phosphatase activity. Inhibitor-1 is active only after phosphorylation by cAMP-dependent protein kinase [7] and inhibitor-2 is a constituent of MgATP-dependent phosphatase [8]. Both are specific inhibitors of phosphatase-1 although they do not interact with the enzyme in an identical manner [9]. Heparin, a sulfated polysaccharide, inhibits some protein kinases [10,11], whereas it increases the activity of phosphorylase kinase [12,13].

We have reported that heparin stimulates the activity of skeletal muscle and liver phosphorylase kinase in a Ca^{2+}-dependent manner [14]. We here report the complete inhibition of protein phosphatase-1 by heparin. The purification of phosphatase-1 by chromatography on heparin–Sepharose suggests an interaction between the enzyme and heparin.

2. MATERIALS AND METHODS

Chondroitin sulfates, glucose derivatives and SDS M_r marker proteins were purchased from Sigma, heparin from Serva and heparin–Sepharose CL-6B from Pharmacia. Other chemicals used were analytical grade.

Protein phosphatase-1 was isolated from rabbit muscle [15] as modified in [8]. Liver protein phosphatase was prepared as in [16]. The activity of both preparations was inhibited by inhibitor-1 therefore they are considered as phosphatase-1. Purification and phosphorylation of inhibitor-1 were done as in [7]. Rabbit muscle ^{32}P-labelled phosphorylase *a* and liver phosphorylase *a* were prepared as in [6] and [17], respectively.

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Phosphorylated glycogen synthase was prepared from rabbit muscle [18]. Protein was determined as in [19].

2.1. *Assay of protein phosphatase*

The reactions were carried out at 30°C in 40 mM Tris (pH 7.4) and 10 mM β-mercaptoethanol buffer using 1 mg/ml ^{32}P-labelled phosphosubstrates. Heparin, inhibitor-1 or other effectors tested were preincubated with phosphatase for 5 min and the reaction was started by the addition of phosphosubstrates. The liberation of [^{32}P]orthophosphate was followed as in [5]. One unit of phosphatase activity is defined as 1 nmol phosphate liberated per min under the assay conditions [8].

3. RESULTS

The dephosphorylation of muscle and liver phosphorylase *a* was inhibited by increasing concentration of heparin in an identical manner (fig.1). Five to ten μg/ml of heparin caused 50% inhibition of the dephosphorylation reaction. Furthermore, the heparin inhibition was independent of the origin of phosphatase. Heparin also inhibited the dephosphorylation of glycogen synthase by muscle phosphatase-1 (not shown).

Chondroitin sulfate A and C even in 10-fold higher concentrations than heparin were ineffective in inhibiting phosphatase activity. Monosaccharide constituents of glycosaminoglycans were also tested and found to be without effect (table 1).

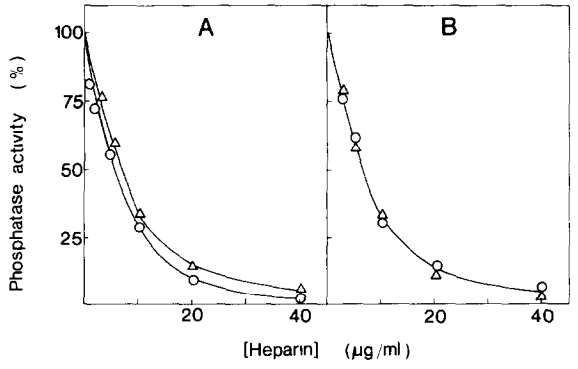

Fig.1. Effect of heparin on the activities of muscle and liver protein phosphatases. Activities of muscle phosphatase-1 (A) and liver phosphatase-1 (B) were assayed with muscle (○) or liver (△) phosphorylase *a*.

Table 1

The effects of glycosaminoglycans and glucose derivatives on phosphorylase phosphatase activity

Effector	Phosphatase activity (%)
None	100
10 μg/ml heparin	34
100 μg/ml chondroitin sulfate A	94
100 μg/ml chondroitin sulfate C	97
10 mM glucosamine	95
10 mM glucuronic acid	96
10 mM Na-sulfate	64

Dephosphorylation of muscle phosphorylase *a* by muscle protein phosphatase-1 was carried out in the presence of the listed ligands. The means of 3 experiments are shown (SD ± 5%)

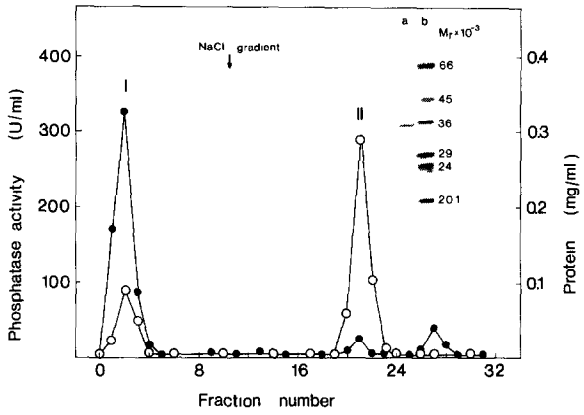

Fig.2. Chromatography of protein phosphatase on heparin–Sepharose. Partially purified muscle protein phosphatase preparation [8], material from step 4 (DEAE–cellulose), was subjected to chromatography on heparin–Sepharose CL-6B. Protein (1.4 mg) was applied to the column (1.2 × 5 cm) equilibrated in 20 mM Tris (pH 7.4), 10 mM β-mercaptoethanol, 0.1 M EGTA (buffer A). The column was washed with buffer A and a linear gradient (80 ml) of 0–0.5 M NaCl in buffer A was developed beginning at fraction 10. The flow rate was 12 ml/h and fractions of 2 ml were collected. Fractions were assayed for phosphatase activity (○) with ^{32}P-labelled phosphorylase *a* and for protein (●). Inset: electrophoresis of peak II on 10% polyacrylamide gels in the presence of SDS [20]. (a) Protein (4 μg) of peak II; (b) M_r standards.

Table 2

Effect of heparin and inhibitor-1 on the activity of phosphatase before and after heparin–Sepharose chromatography

Effector	Specific activity of phosphatase (units/mg)		
	Starting material	Heparin–epharose[a]	
		Peak I	Peak II
None	1080	490	26400
+ 10 μg/ml heparin	626	473	11090
+ 50 μg/ml heparin	305	451	1680
+ 1 μg/ml inhibitor-1	268	460	2544

[a] Peak II accounted for 70–80% of the total phosphatase activity applied to the column and the remaining 20–30% of the activity was contributed by peak I

Phosphatase activities were assayed with muscle phosphorylase *a*. Values are the average of 5 preparations

Sulfate ions caused an inhibition of phosphatase at a high concentration.

The interaction of heparin with phosphatase was also supported by chromatography. It was demonstrated that purified muscle phosphatase-1 was completely adsorbed to heparin–Sepharose and eluted by NaCl (not shown). A partially purified preparation of muscle phosphatase was also loaded on a heparin–Sepharose column and eluted by a linear gradient of NaCl (fig.2). Two peaks of phosphatase were resolved since phosphatase activities appeared in the washing buffer (peak I) and another enzyme eluted at 180–200 mM NaCl (peak II). It is seen that the phosphatase activity of peak I was not inhibited by either heparin or inhibitor-1 (table 2) suggesting the presence of phosphatase-2. The enzyme in peak II was inhibited by heparin. Inhibitor-1 also inhibited the activity of this peak proving that only phosphatase-1 can bind to the immobilized heparin. The gel electrophoretic pattern of peak II showed a major protein band with an M_r of 33000 and a less intense band of higher M_r (inset, fig.2).

4. DISCUSSION

Our results show that the inhibition of phosphatase-1 by heparin is complete and not mimicked by closely related polysaccharides. The inhibition was also observed by using alternative phosphosubstrates (muscle or liver phosphorylase and synthase) suggesting that heparin acts directly on phosphatase-1. The latter was also supported by chromatography on heparin–Sepharose. This single step resolved two phosphatases and the specific activity of matrix-bound enzyme was increased 20–30-fold. Therefore heparin–Sepharose is a useful tool in the purification of phosphatase-1 and in the separation of phosphatase-1 from phosphatase-2. It seems that heparin is able to distinguish between the two types of phosphatases as can the heat-stable inhibitors. This raises the possibility that the negative charges of heparin may cause its inhibitory property since an acidic region (residues 42–54) of inhibitor-1 may also be essential [21].

Experiments are in progress to determine the precise mode of action of heparin and to extend the application of heparin–Sepharose for the purification of phosphatase-1 of high M_r (where ethanol treatment is omitted).

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REFERENCES

[1] Cohen, P. (1982) Nature 296, 613–620.
[2] Ingebritsen, T.S. and Cohen, P. (1983) Science 221, 331–338.
[3] Krebs, E.G. and Beavo, J. (1979) Annu. Rev. Biochem. 48, 923–959.
[4] Chock, P.B., Rhee, S.G. and Stadtman, E.R. (1980) Annu. Rev. Biochem. 49, 813–843.
[5] Gergely, P. and Bot, G. (1981) Acta Biochim. Biophys. Acad. Sci. Hung. 16, 163–178.
[6] Gergely, P., Tóth, B., Dombrádi, V. and Bot, G. (1983) Biochem. Biophys. Res. Commun. 113, 825–831.
[7] Nimmo, G.A. and Cohen, P. (1978) Eur. J. Biochem. 87, 353–365.

[8] Resink, T.J., Hemmings, B.A., Tung, H.Y.L. and Cohen, P. (1983) Eur. J. Biochem. 133, 455–461.
[9] Foulkes, J.G., Strada, S.J., Henderson, P.F.J. and Cohen, P. (1983) Eur. J. Biochem. 132, 309–313.
[10] Hathaway, G.M. and Traugh, J.A. (1982) Curr. Top. Cell. Regul. 21, 101–127.
[11] De Paoli-Roach, A.A. and Roach, P.J. (1982) Arch. Biochem. Biophys. 217, 305–311.
[12] Krebs, E.G., Love, D.S., Bratvold, G.E., Trayser, K.S., Meyer, W.L. and Fischer, E.H. (1964) Biochemistry 3, 1022–1033.
[13] Chrisman, T.D., Jordan, J.E. and Exton, J.H. (1981) J. Biol. Chem. 256, 12981–12985.
[14] Erdődi, F., Gergely, P. and Bot, G. (1984) Int. J. Biochem., in press.
[15] Lee, E.Y.C., Silberman, S.R., Ganapathi, M.K., Paris, H. and Petrovic, S. (1981) Cold Spring Harbor Conf. Cell Proliferation 8, 425–439.
[16] Brandt, H., Capulong, Z.A. and Lee, E.Y.C. (1975) J. Biol. Chem. 250, 8038–8044.
[17] Bot, G., Kovács, E., Tóth, B., Dombrádi, V. and Gergely, P. (1982) Acta Biochim. Biophys. Acad. Sci. Hung. 17, 183–189.
[18] Parker, P.J., Embi, N., Caudwell, F.B. and Cohen, P. (1982) Eur. J. Biochem. 124, 47–55.
[19] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[20] Laemmli, U.K. (1970) Nature 227, 680–685.
[21] Aitken, A. and Cohen, P. (1982) FEBS Lett. 147, 54–58.