Vol. 90, No. 1, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 12, 1979 Pages 234-239

EFFECT OF SULFATED POLYSACCHARIDES AND SULFATE ANIONS ON THE AMP-DEPENDENT ACTIVITY OF PHOSPHORYLASE b

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Received July 9,1979

SUMMARY

The effect of sulfated polysaccharides on the AMP-dependent activity of rabbit muscle phosphorylase b as compared with that of Na_2SO_4 has been studied. It has been shown that sulfated polysaccharides and Na_2SO_4 greatly stimulated AMP-activation of the enzyme at low AMP concentrations. Dextran sulfate and Na_2SO_4 desensitized the allosteric interactions of the enzyme towards the nucleotide activator and reversed the enzyme inhibition caused by glucose-6-phosphate and glucose. Furthermore, it was found that while dextran sulfate decreased the K_m value for both substrates, glucose-1-phosphate and glycogen, sulfate anions decreased only the K_m value for glycogen.

INTRODUCTION

Phosphorylase b from rabbit skeletal muscle (EC 2.4.1.1) requires AMP for catalytic activity (1). The nucleotide induced activation is enhanced by a variety of factors such as fluoride, substrate anions, divalent metal ions and polyamines (reviewed (2)). A phosphopeptide derived from the NH_2-terminal region of phosphorylase a (3), phenothiazines (4) and polycarboxylates (5) were also reported as stimulators of AMP-activation of the enzyme.

Na_2SO_4 is known to stimulate rabbit muscle phosphorylase b in absence of AMP (6), while the activity of various phosphorylase preparations as lobster muscle, bovine adrenal, pig and rabbit livers, bovine corpus luteum (reviewed (7)), rat placental (8), transplantable rat hepatomas (9) and cow uterus (10), has been shown to be enhanced by high concentrations of sulfate anions.

In view of the general property of sulfates to stimulate phosphorylase activity, we have investigated the action of sulfated polysaccharides on the AMP-dependent activity of rabbit muscle phosphorylase b as compared with the effect of Na_2SO_4.

MATERIALS AND METHODS

Crystalline rabbit muscle glycogen phosphorylase b was prepared as in (5). Oyster glycogen was purchased from BDH and it was freed of AMP as described in (11). Glucose-1-phosphate, AMP, UDPG and

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D-glucose were products of BDH.

Phosphorylase b was assayed in the direction of glycogen synthesis as in (5) except that 32 mM glucose-1-phosphate were used. The enzyme concentration was measured spectrophotometrically using an extinction coefficient ($E1^{\%}_{cm}$) at 280 nm of 13.2 (12).

Dextran sulfate, chondroitin sulfate and heparin were purchased from Serva. Before use, they were dissolved in 1 mM EDTA, 30 mM 2-mercaptoethanol, 40 mM glycerol-2-phosphate buffer, pH 6.8, in an appropriate concentration and were dialysed extensively against the same buffer at 4°C. The determination of the sulfate content of the sulfated polysaccharides involved acid hydrolysis (1 M HCl at 105-110°C for 5 h) followed by determination of liberated inorganic sulfate by the turbidimetric method of (13). The analysis gave a sulfate content (% of sulfate ion) of polysaccharides: dextran sulfate, 46.6%; chondroitin sulfate, 20.2%; heparin, 32.6%.

RESULTS AND DISCUSSION

We have found in our studies that sulfated polysaccharides and Na_2SO_4 in glycerol-2-phosphate buffer greatly stimulated AMP activation of phosphorylase b at suboptimal concentrations of the nucleotide. The effect of increasing concentrations of sulfated polysaccharides (expressed as SO_4^{2-} content) and sulfate anions on the activity of phosphorylase b at 10 µM AMP is shown Fig. 1. In

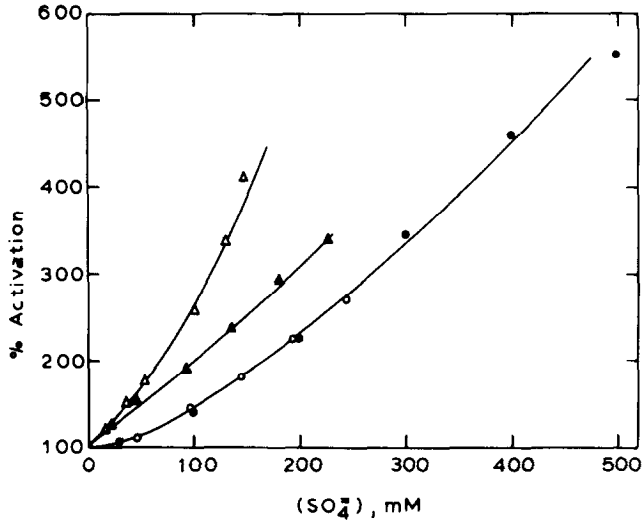

Fig. 1. Effect of Na_2SO_4 and sulfated polysaccharides on the activity of phosphorylase b at low AMP concentration. The enzyme (20 µg/ml) was assayed at 30°C in 1 mM EDTA, 30 mM 2-mercaptoethanol, 40 mM glycerol-2-phosphate buffer, pH 6.8, with 32 mM glucose-1-phosphate, 10 µM AMP, 1% glycogen and various concentrations of Na_2SO_4 or sulfated polysaccharides mentioned in the figure. The concentration of sulfated polysaccharides was expressed in mM of inorganic sulfates. Enzyme activity in absence of sulfates (4 µmol P_i/mg/min) was taken as 100%. The activity induced by Na_2SO_4 in absence of AMP was deducted from each rate measurement in order to evaluate the stimulation induced by the salt. (●) Na_2SO_4; (o) dextran sulfate; (▲) heparin from hog intestinal mucosa; (Δ) chondroitin sulfate from bovine trachea.

Table 1

Effect of dextran sulfate and Na_2SO_4 on the kinetic parameters of phosphorylase b

Enzyme system	Glucose-1-phosphate $K_m X10^3$	Glycogen K_m	AMP n	AMP $K_m X10^5$
	M	%		M
Phosphorylase b	7.6 ± 0.3	0.024 ± 0.0015	1.7 ± 0.06	6.2 ± 0.3
Phosphorylase b + dextran sulfate (0.1 mM)	3.2 ± 0.2	0.015 ± 0.001	1.5 ± 0.06	2.4 ± 0.2
Phosphorylase b + Na_2SO_4 (0.25 M)	7.8 ± 0.3	0.014 ± 0.001	1.4 ± 0.05	2.3 ± 0.2

Phosphorylase b (10 µg/ml) was assayed at 30°C with various concentrations of AMP, glucose-1-phosphate and glycogen in a 40 mM glycerol-2-phosphate, 30 mM 2-mercaptoethanol, 1 mM EDTA buffer, pH 6.8. Glucose-1-phosphate was varied from 4 to 32 mM at constant concentrations of AMP (1 mM) and glycogen (1%); glycogen was varied from 0.005 to 1% at constant concentrations of AMP (1 mM) and glucose-1-phosphate (32 mM); AMP was varied from 0.02 to 1 mM at constant concentrations of glycogen (1%) and glucose-1-phosphate (32 mM). The data represent the average values from five experiments. The mean values are quoted together with the standard deviation of the mean. n=Hill coefficient.

the range of SO_4^{2-} concentrations tested, dextran sulfate exhibits stimulation similar to that of Na_2SO_4, while chondroitin sulfate is the most effective stimulator. The enhanced stimulation observed by chondroitin sulfate and heparin as compared with that of dextran sulfate can be explained probably on the basis of the polycarboxylate nature of the first two polysaccharides (5). Although Na_2SO_4 induces a small activation in phosphorylase b in absence of AMP (6), sulfated polysaccharides tested under the same experimental conditions did not substitute for AMP.

In order to compare the effect of sulfated polysaccharides on the catalytic properties of phosphorylase b with that of sulfate anions, we studied the influence of dextran sulfate and Na_2SO_4 at the same SO_4^{2-} concentration (0.25 M) on the kinetic parameters of the enzyme. Dextran sulfate was chosen because it is a polyanion with only sulfates as anionic groups. A summary of the kinetic data obtained is presented in Table 1. It can be seen that both dextran sulfate and Na_2SO_4 enhance equally the affinity of the enzyme for AMP and glycogen. The maximum velocity of the enzyme for the substrates and effector are not significantly altered (not shown), while the Hill coefficient for AMP is decreased. The only difference observed between the effect of dextran sulfate and Na_2SO_4 is that sulfated polysaccharide increases enzyme affinity for glucose-1-phosphate, while in this respect inorganic sulfates are ineffective.

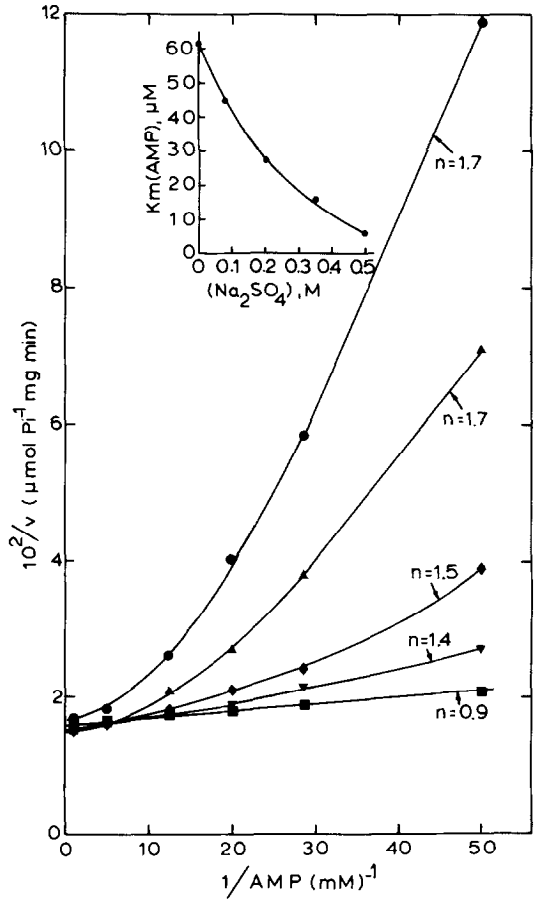

Fig. 2. Effect of Na_2SO_4 on the kinetics of activation of phosphorylase b by AMP. The assay mixture contained in addition to AMP, 10 µg/ml of enzyme, 32 mM glucose-1-phosphate, 1% glycogen and (●) 0; (▲) 0.08; (◆) 0.2; (▼) 0.35 or (■) 0.5 M Na_2SO_4. Reactions were carried out at 30°C and buffered as in Fig. 1. The activity induced by the salt in absence of AMP was deducted from each rate measurement. n=Hill coefficient. Insert: The K_m value for AMP as a function of the Na_2SO_4 concentration present in the assay system. K_m values for AMP were obtained by replotting the data of Fig. 2 in the form of a Hill plot.

This result suggests that the mechanism of salt activation of phosphorylase b by Na_2SO_4 is different from that of dextran sulfate.

The possibility that the apparent stimulation of AMP activation of the enzyme by sulfates is a result of the elimination of the interactions between the nucleotide binding sites has been examined. The Lineweaver-Burk plot presented in Fig. 2 shows the expected cooperative binding of AMP on phosphorylase b with a Hill coefficient n=1.7 . When increasing concentrations of Na_2SO_4 are added, n decreases gradually and the kinetics change to simple Michaelis-Menten type. At the meantime the affinity of the enzyme for the nucleotide

Table 2

Effect of dextran sulfate and Na_2SO_4 on the inhibition of phosphorylase b by glucose-6-phosphate, glucose and UDPG

Additions to reaction mixture	% Activity
none	100
Na_2SO_4	104
dextran sulfate	128
glucose-6-phosphate (10 mM)	53
glucose-6-phosphate (10 mM) + Na_2SO_4	103
glucose-6-phosphate (10 mM) + dextran sulfate	120
glucose (40 mM)	37
glucose (40 mM) + Na_2SO_4	115
glucose (40 mM) + dextran sulfate	100
UDPG (10 mM)	54
UDPG (10 mM) + Na_2SO_4	63
UDPG (10 mM) + dextran sulfate	75

Reaction mixtures at 30°C contained 10 µg/ml of enzyme, 16 mM glucose-1-phosphate, 1 mM AMP, 1% glycogen and buffered as in Fig. 1. The concentrations of dextran sulfate and Na_2SO_4 were 0.1 mM and 0.5 M respectively. Other components were added as indicated.

is highly enhanced (Fig. 2, insert) without significant change of the maximum velocity. The enhancement of the enzyme affinity for AMP is in accordance with the finding that high concentrations of K_2SO_4 in presence of 1 mM AMP, in glycerol-2-phosphate buffer, promote complete conversion of the dimeric form of phosphorylase b into the tetrameric form (14, 15). It has been reported that the binding affinity of rabbit muscle phosphorylase b for AMP determined by Sephadex gel filtration technique (16), is decreased in presence of K_2SO_4 in glycerol-2-phosphate buffer. This inconsistency between binding and catalytic studies is possibly due to the different conditions used in the two kinds of experiments.

To obtain additional information for the effect of sulfates on the allosteric interactions of phosphorylase b, we studied the influence of dextran sulfate and Na_2SO_4 on the inhibition of the enzyme by allosteric inhibitors. The data presented in Table 2 show that while sulfates are able to reverse the inhibition of phosphorylase b caused by glucose-6-phosphate and glucose, they fail to remove the inhibition caused by UDPG. The ability of sulfates to desensitize the allosteric interactions towards glucose-6-phosphate and glucose

in connection with their inability to remove the inhibition caused by UDPG, reflects probably that the binding of the first two inhibitors occurs at binding sites different from that of UDPG (17-19).

Although the concentrations of sulfates used in our experiments are rather high the observed phenomena appear to be specific since analogous concentrations of chlorides or acetates could not substitute for sulfates.

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