Vol. 102, No. 1, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 16, 1981 Pages 420-425

DIFFERENT SENSITIVITY OF HUMAN RED CELL CASEIN KINASES TOWARDS GLYCOAMINOGLYCANS

M.C. Lecomte and P. Boivin

Laboratoire d'enzymologie des cellules sanguines
(INSERM U160, CNRS ERA 573) Hôpital Beaujon 92118 Clichy Cedex France.

Received June 16,1981

SUMMARY

Heparin specifically inhibits cytosolic casein kinase from human erythrocyte and has no effect on membrane casein kinase. Other glycoaminoglycans have little or no effect on cytosolic casein kinase activity. Study of inhibition mechanism reveals that heparin acts as a non competitive inhibitor with respect to both substrates : ATP and casein.

INTRODUCTION

It is well known that heparin, a sulfated mucopolysaccharide, acts as an anticoagulant by reacting with antithrombin III. Recent studies showed that heparin is in fact a multifunctional molecule : heparin is able to form complex with many proteins and enzymes and to change the properties of these substances (1) ; it has been found that heparin inhibits the activity of some casein kinases (2, 3).

In human erythrocyte, two casein kinases which phosphorylate some proteins of the cytoskeleton have been described : a membranous casein kinase (MCK) (4) and a cytosolic casein kinase (CCK) (5). In this study, we display that heparin is a potent inhibitor of cytosolic casein kinase and in contrast, has no effect on membranous casein kinase. This inhibitory property is specific of heparin, other aminoglycans being ineffective. The study of inhibition mechanism reveals that heparin acts as a non competitive inhibitor.

MATERIALS AND METHODS

Chemical products

Unlabelled ATP was obtained from Boehringer-Mannheim. γ^{32}P ATP (spec. act. 3-4 Ci/mmol) from Radiochemical Centre Amersham. Partially dephosphorylated casein, histone, chondroitin sulfate (mixed isomers), hyaluronic acid and heparin (from porcine intestinal mucosa) were purchased from Sigma. Other reagents were from Merck.

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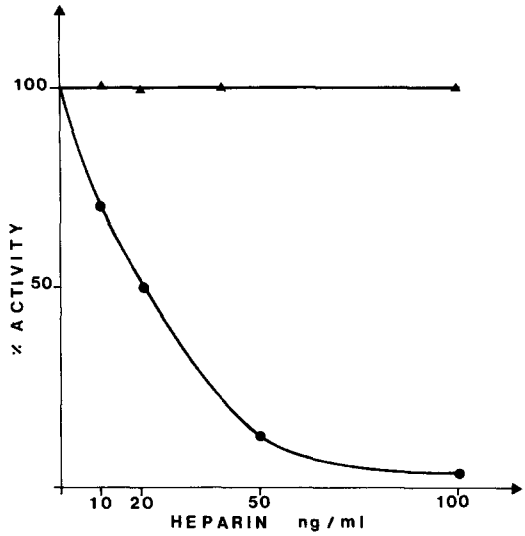

Figure 1 Effect of heparin on activity of casein kinases (2.5 µM ATP ; 1 mg casein/ml)
(▲) membranous casein kinase (●) cytosolic casein kinase

Enzymes

Human erythrocyte casein kinases were purified from membrane and cytosol as previously described, respectively in (6) and (7).

Enzyme assay

The reaction mixture (200 µl) contained : 0.05 M sodium acetate buffer (pH 6.5), 30 mM magnesium acetate, 125 mM KCl, 0.3 mM EGTA for assay of cytosolic casein kinase (CCK) and 0.05 M Tris-HCl buffer (pH 7.5), 45 mM magnesium acetate, 150 mM KCl, 0.3 mM EGTA for membranous casein kinase (MCK) assay.

The concentrations of substrates (casein and ATP) were indicated in the text and figure legends. The reaction was initiated by adding 10 µl enzyme (10 µg/ml). Incubations were done for 10 mn at 30°C. The reactions were stopped by the trichloracetic precipitation and achieved as previously reported (8). Incorporated radioactivity was determined by liquid scintillation spectrometry (Intertechnic XN SL 3000).

RESULTS

Effect of heparin on casein kinases activities

The effect of increasing concentrations of heparin on both casein kinases activities was studied under the above conditions : 1 mg casein/ml, 2.5 µM ATP. The results shown in fig. 1 indicate that heparin is a powerful inhibitor of cytosolic casein kinase. In this experimentation, a concentration of 20 ng heparin/ml is sufficient to inhibit 50% CCK activity. This inhibitory effect is selectively directed toward CCK. No inhibition occurs on MCK with a concentration of heparin producing 95% inhibition on CCK.

Table I Effect of glycoaminoglycans on cytosolic casein kinase activity : I_{50} was the amount of inhibitor which was required to give 50% inhibition.

Compound	I_{50} (ng/ml)
Heparin	20
Chondroitin sulfate	NI
Hyaluronic acid	NI
Dextran sulfate	70

Effects of various glycoaminoglycans on CCK activity

Chemical analogues of heparin were tested on CCK activity under the conditions : 50 µM ATP, 2 mg casein/ml. Hyaluronic acid and chondroitin sulfate (mixed isomers) have no effect on CCK activity at concentrations up to 1 µg/ml (table I). Dextran sulfate is markedly less ineffective than heparin. Thus the inhibitory property of heparin is not only due to its polyanionic sulfate character.

Study of inhibition mechanism

Interaction between casein and heparin was investigated by determining the reaction rate at 4 different heparin concentrations in presence of 50 µM ATP and at 4 different casein concentrations (0.08 mg/ml to 0.5 mg/ml). The lineweaver Burk plot (fig. 2) revealed a variable Vmax and a constant Km, indicating that heparin is a non competitive inhibitor with respect to casein.

Interaction of heparin with ATP was studied in presence of constant casein level (2 mg/ml) and 5 different ATP concentrations (2.5 to 10 µM). Heparin is a non competitive inhibitor with respect to ATP (fig. 3).

DISCUSSION

Different casein kinases activities have been described in various mammalian tissues. These enzymes has been primarily classified according to their order of elution from DEAE cellulose (9), their molecular weight or the type of phosphoryl donor used (ATP or GTP) (10). In human erythrocyte, the MCK is composed of one unit and preferentially uses ATP as phosphoryl donor ; the CCK, composed of two subunits utilizes both ATP and GTP.

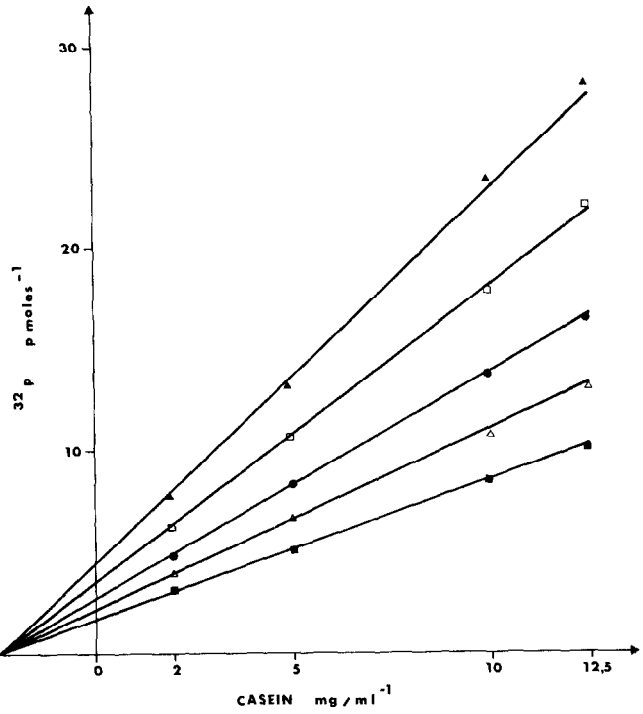

Figure 2 Lineweaver Burk plot of initial rate as function of casein concentrations : 50 µM ATP.

Heparin : (■) none ; (△) 5 ng/ml ; (●) 10 ng/ml ; (□) 15 ng/ml ; (▲) 20 ng/ml.

Our data show that heparin specifically inhibits the CCK activity and has no effect on MCK activity. This inhibitory property is specific of heparin : chondroitin sulfate and hyaluronic acid are inefficient. We cannot only attribuate this effect to the polyanionic characteristic of heparin since dextran sulfate is less efficient than heparin.

Some casein kinases from other source have been found to be inhibited by heparin : a G type casein kinase from bovine adrenal cortex (3) and a type II casein kinase from rabbit reticulocytes (2). These two casein kinases have in common with human erythrocyte CCK the property to use ATP and GTP as phosphoryl donor. So it appears that heparin only acts on casein kinases which share some common characteristics : GTP use, composition of several subunits. But the inhibition mechanism seems to be different according to casein kinase source, and likely the type of enzyme. Our results reveal that heparin acts as a non competitive inhibitor with respect to both substrates (ATP and casein) of the human erythrocyte

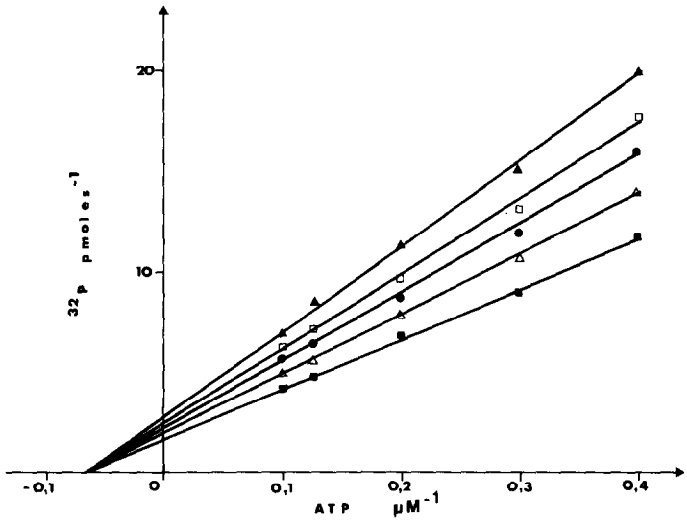

Figure 3 Lineweaver Burk plot of initial rate as function of ATP concentrations : 1 mg casein/ml.

Heparin : (■) none ; (△) 5 ng/ml ; (●) 10 ng/ml ; (□) 15 ng/ml ; (▲) 20 ng/ml.

cytosolic casein kinase. By contrast, it has to be pointed out that heparin is a competitive inhibitor with respect to casein for a casein kinase obtained by Hathaway et al from post ribosomal supernatant of rabbit reticulocytes. It seems to us that the difference in inhibition mechanism is another clue of the differences in the two enzyme preparations: one isolated from rabbit reticulocytes, the other one from human erythrocytes which no longer synthesize proteins. The inhibition specificity by heparin versus other glycoaminoglycans for the erythrocyte CCK allows to further distinguish this enzyme from the casein kinase of bovine adrenal cortex, even this later usesalso ATP and GTP. These differences are arguments for the multiplicity of casein kinases according to this source.

Acknowledgments

This work was supported by a grant of the University Paris 7 (UER de Médecine Xavier-Bichat).

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