STRUCTURE-FUNCTION RELATIONSHIPS FOR HEPARIN-LIKE POLYSACCHARIDES

M. Höök (Uppsala, Sweden)

The dramatic effects of heparin-like polysaccharides in various biological systems are probably often due to interactions between the polysaccharide and a target protein. For example, the action of heparin as an anticoagulant can be explained by the specific interaction of the polysaccharide with antithrombin III, a protease inhibitor acting on several of the serine proteases in the coagulation cascade. Binding of heparin to the protein greatly potentiates the activity of antithrombin III, thus leading to an effective blockage of the selective proteolytic transformation of zymogens to active coagulation factors.

Injection of heparin into the blood of an animal affects not only the coagulation system but also the plasma concentration of triglycerides. This lipolytic effect of heparin is probably caused by the release of lipoprotein lipase, a triglyceride degrading enzyme, from tissue sites to the circulating blood. Lipoprotein lipase readily binds to certain polyanions and is probably released as a complex with heparin.

Heparan sulfate, a polysaccharide structurally related to heparin, occurs associated with the cell surface of several mammalian cell types. Recent work in our laboratory has shown that part of the cell surface heparan sulfate is bound to specific binding sites in the plasma membrane. In the present communication the structural requirements for binding of heparin-like polysaccharides to antithrombin III, lipoprotein lipase and specific sites on the surface of rat liver cells will be discussed.

Binding to antithrombin III. Heparin is the only glycosaminoglycan that binds with high affinity to antithrombin III. Furthermore, not all heparin molecules contain the structural features required for high affinity binding to the protein; and heparin can in fact be separated into two distinct fractions having high and low affinity for antithrombin, respectively. Analysis of chemically modified heparins suggests that the antithrombin-stimulating activity is lost when N-sulfate groups are removed from the polymer and restored on re-N-sulfation but not on N-acetylation of the N-desulfated polysaccharide. A heparin fragment with a molecular weight of about 5 000 containing the antithrombin-binding site and possessing antithrombin-enhancing activity could be isolated after digestion of the heparin-antithrombin complex with a bacterial heparinase. The structure of this fragment will be discussed.

Binding to lipoprotein lipase. Beside heparin also heparan sulfate and dermatan sulfate are capable of binding to lipoprotein lipase. The enzyme appears to bind much stronger to heparin than to the low-sulfated glycosaminoglycans; however, the affinity of the lipase for the polysaccharides does not depend solely on charge density of the glycosaminoglycan as chondroitin sulfate only binds small amount of enzyme although its charge density is similar to that of dermatan sulfate and heparan sulfate. Analysis of chemically modified heparins suggests that N-acetyl groups can be substituted for N-sulfate groups without significantly affecting the affinity for lipoprotein lipase.

After partial degradation of heparin, a fragment with a molecular weight of about 3 500 with appreciable affinity for lipoprotein lipase could be isolated. These results indicate that the binding sites for lipoprotein lipase and antithrombin III in the heparin molecule are different. In support of this conclusion the two heparin species separated by affinity chromatography on antithrombin-Sepharose gave essentially identical elution profiles when chromatographed on immobilized lipoprotein lipase.

Binding to rat liver cells. The heparan sulfate synthetised in rat liver cells ultimately appears at the cell surface where part of the molecules occurs associated with a "receptor". This polysaccharide can be released by heparin and by some heparan sulfates. Polysaccharides capable of releasing endogenous rat liver heparan sulfate from the cells also bind to the cells. Results will be presented suggesting that binding of heparan sulfate to cells depends on the content of ester sulfate but not on the presence of N-sulfate groups in the polymer.

Conclusions: The presented results indicate different structural requirments for binding of heparin-related polysaccharides to antithrombin III, lipoprotein lipase and rat liver cells, respectively.