

Case 2

Histidine-Proline-rich Glycoprotein as a Plasma pH Sensor

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Focus concept

A histidine-proline-rich glycoprotein may serve as a plasma sensor and regulate local pH in extracellular fluid during ischemia or metabolic acidosis.

Prerequisites

- Acidic/basic properties of amino acids
- Amino acid structure and protein structure

Background

This study focuses on the characteristics of the abundant plasma protein referred to as histidine-proline-rich glycoprotein (HPRG). HPRG is so named because it has a very high content of histidine (13 mol %). The human HPRG contains a pentapeptide GHHPH sequence repeated in tandem twelve times. The authors of this study hypothesized that its high histidine content might allow HPRG to play a role in regulating local pH in the blood. The local pH in blood may drop a half a pH unit during lactic acidosis or even a full pH unit in hypoxia or ischemia. In the case presented here, the binding of HPRG to glycosaminoglycans was investigated. Glycosaminoglycans are anionic polysaccharides that are the major component of the ground substance that forms the matrix of the extracellular spaces of the connective tissue in blood vessel walls. In this study, the binding of HPRG to the glycosaminoglycan heparin was measured. Based on their results, the investigators propose a model which describes how binding of HPRG to glycosaminoglycans may allow HPRG to regulate local blood pH.

Questions

1. Binding studies were carried out in which heparin was immobilized on the surface of cuvettes. The pH dependence of HPRG binding to heparin was investigated and the results are shown in Figure 2.1.
 - a. How is the binding of HPRG to heparin dependent on pH? Give structural reasons for the binding dependence. The structure of heparin is shown in Figure 2.2.
 - b. The same binding studies were carried out in which HPRG was reacted with diethylpyrocarbonate (DEPC), a compound that specifically reacts with histidine residues. The reaction is shown in Figure 2.3. Explain the results.

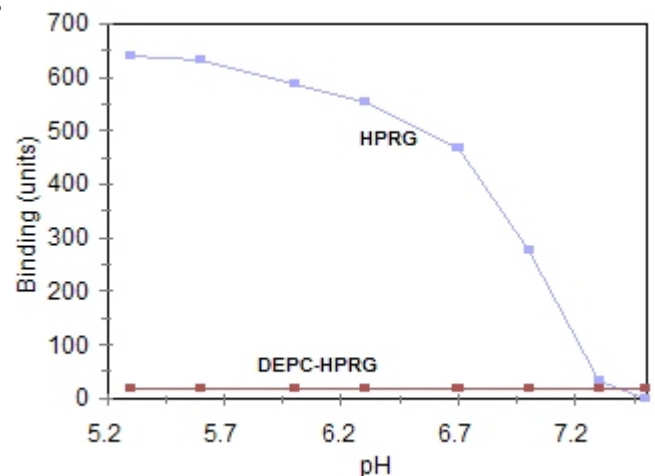


Figure 2.1: The pH-dependence of HPRG binding to unmodified heparin, and heparin modified with DEPC. (Based on Borza and Morgan, 1998.)

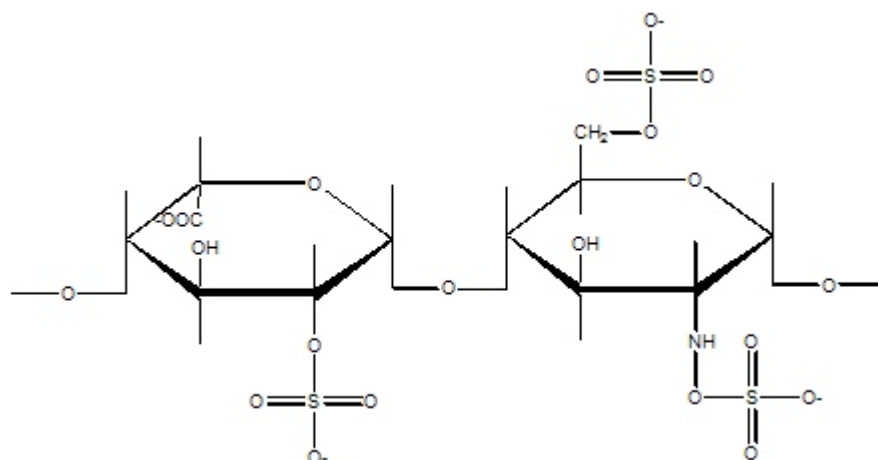


Figure 2.2: Repeating disaccharide unit of heparin.

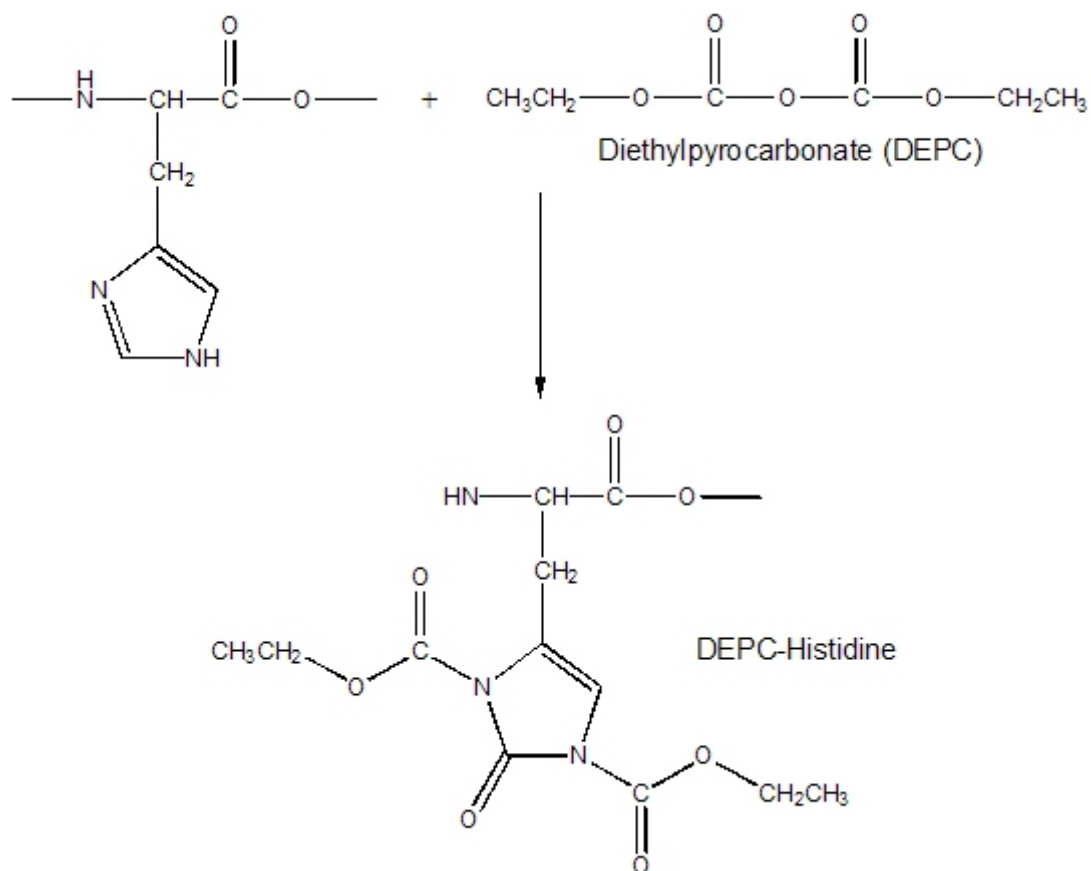


Figure 2.3: Reaction of histidine side chains with diethylpyrocarbonate.

2. The effect of transition metals on the binding of HPRG was investigated next. The ability of increasing concentrations of Cu^{2+} and Zn^{2+} to promote HPRG binding to heparin at $\text{pH} = 7.3$ was measured. The results are shown in Figure 2.4. In addition, the binding of HPRG to heparin in the presence of these ions was compared at various pH's. Figure 2.5 shows the comparison of binding at $\text{pH} = 6.0$ and at $\text{pH} = 7.4$ in the presence of 5.2 nM zinc. What is your interpretation of these results?

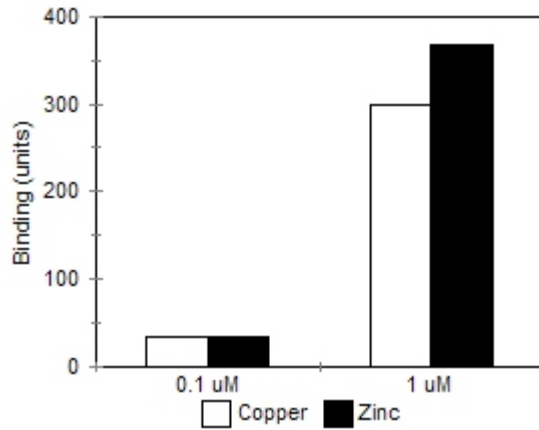


Figure 2.4: Binding of HPRG to heparin in the presence of copper and zinc ions. (Based on Borza and Morgan, 1998.)

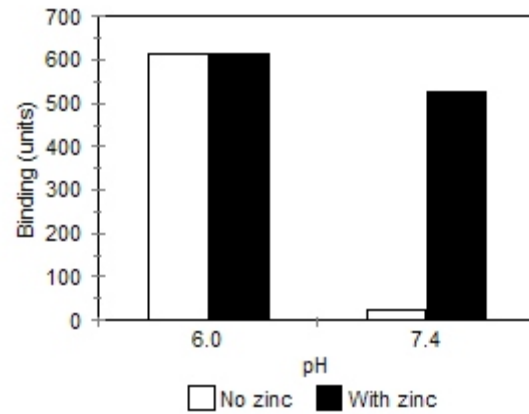


Figure 2.5: Binding of HPRG to heparin in the presence and absence of 5.2 nM zinc ions at two different pH values. (Based on Borza and Morgan, 1998.)

3. Local cellular pH can decrease from one-half to one pH unit depending on a variety of circumstances including ischemia, hypoxia, and inflammation due to lactic acidosis. In addition, metabolic acidosis is often one of the symptoms in complications following surgery. The investigators have proposed that HPRG acts to relieve the acidosis in these circumstances. Propose a model that explains the mechanism of pH regulation by HPRG.
4. Other plasma proteins have been studied for their ability to bind to glycosaminoglycans. One such protein is kininogen, which is a lysine-rich protein. Like HPRG, kininogen is able to bind to glycosaminoglycans, but this binding is far less sensitive to small fluctuations in physiological pH.
- Why does kininogen bind to glycosaminoglycans easily?
 - Why is the binding of kininogen less sensitive to physiological pH changes?

References

Borza, D-B., and Morgan, W. T. (1998) *J. Biol. Chem.*, **273**, pp. 5493-5499.

Lundblad, R. (1995) *Techniques in Protein Modification*, CRC Press, Boca Raton, FL, p. 111.