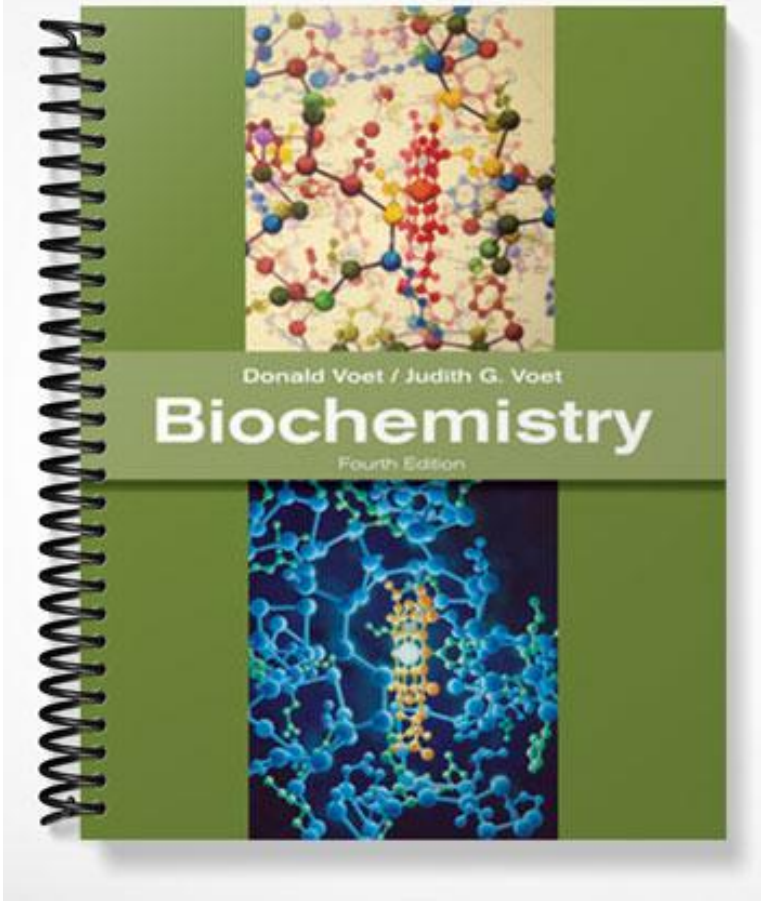
SOLUTIONS MANUAL



Donald Voet / Judith G. Voet

Biochemistry

Fourth Edition

Case 2

Histidine-Proline-rich Glycoprotein as a Plasma pH sensor

1. a. According to the data shown, HPRG binds heparin at high affinity at pH = 5.2, but when the pH increases to 6.8, affinity of HPRG for heparin decreases by half. At pH = 7.2, there is virtually no binding of HPRG to heparin. Since heparin is negatively charged, HPRG will bind to heparin more effectively if the HPRG is positively charged, and ion pairs can form between the two. The pK_a of free histidine is around 6.0; in HPRG the imidazole groups have a pK_R value of about 6.8 (since binding of HPRG to heparin decreased by half at this pH). When $pH < pK_R$, the histidine imidazole group is protonated and positively charged, and the binding of heparin to HPRG is high. But as the pH increases, the imidazole rings become unprotonated and lose their positive charge and bind to the heparin less effectively. Thus binding of HPRG to heparin decreases as pH increases.
   b. The reaction of DEPC to histidine modifies the imidazole ring in such a way that the imidazole ring nitrogens cannot be protonated, no matter what the pH. The modification abolishes the positive charge, so DEPC-modified HPRG cannot form ion pairs with heparin and does not bind to heparin at any pH value.

2. Increasing concentrations of zinc and copper ions increase the binding of HPRG to heparin, as shown in Figure 2.4. At a concentration of 0.l μM of each ion, the binding of HPRG to heparin was measured at about 50 binding units. When the concentrations of the ions were both increased 10-fold, the binding of heparin to HPRG was measured at 300 binding units in the presence of zinc and nearly 400 binding units in the presence of copper. These experiments were carried out at pH 7.3, where binding of HPRG to heparin is negligible, as shown in Figure 2.1. At pH 7.3, the imidazole rings of the histidines of HPRG are unprotonated and thus not positively charged. The presence of the positively charged zinc and copper ions allows the HPRG to bind to these ions and acquire a positive charge, and thus binding to heparin is facilitated in their presence. These statements are supported by the additional data shown in Figure 2.5. The binding curves shift right with increasing concentrations of zinc, indicating that as pH increases, zinc ions play a greater role in facilitating binding. At pH = 6.0, HPRG is protonated and positively charged and the metal ions are not needed to facilitate binding. Thus the binding of HPRG to heparin is the same (about 600 binding units), regardless of whether or not zinc is present. But when the pH is increased to 7.4, the HPRG is not positively charged and the presence of the Zn^{2+} ions facilitates binding. Binding increases from nearly negligible to about 500 binding units when zinc ions are present at this pH. The binding of HPRG to heparin at pH 7.4 in the presence of 33.1 nM zinc is nearly equal to the binding of HPRG to heparin at pH = 6.0 when no zinc is present.

3. At local low pH, the plasma protein HPRG will become protonated on its imidazole rings and will bind to glycosaminoglycans such as heparan sulfate on the surface of endothelial cells. By binding up excess protons, the HPRG removes them from circulation. At pH values greater than 7, the binding of HPRG to heparin will be enhanced in the presence of zinc ions. Binding of protons (and zinc) by HPRG decreases the local proton concentration and leads to an increase in pH to normal values.

4. a. The lysine-rich protein is likely to be positively charged at physiological pH and will be able to bind to the negatively charged glycosaminoglycans by forming ion pairs with the positively charged ε-amino groups of the lysine side chains.
   b. The pK_R of the ε-amino group of lysine is around 10.5 when lysine is a free amino acid. The pKR of lysine incorporated into kininogen is not likely to be substantially different. Thus the lysines of kininogen will remain "permanently" protonated despite small local changes in pH. The pK_R value for histidine is much closer to physiological pH, which means that the binding of protons is more easily reversible. Thus kininogen binding to glycosaminoglycans is not pH-dependent.