

## chapter

## 5

## Protein Function

- 1. Relationship between Affinity and Dissociation Constant** Protein A has a binding site for ligand X with a  $K_d$  of  $10^{-6}$  M. Protein B has a binding site for ligand X with a  $K_d$  of  $10^{-9}$  M. Which protein has a higher affinity for ligand X? Explain your reasoning. Convert the  $K_d$  to  $K_a$  for both proteins.

**Answer** Protein B has a higher affinity for ligand X. The lower  $K_d$  indicates that protein B will be half-saturated with bound ligand X at a much lower concentration of X than will protein A. Because  $K_a = 1/K_d$ , protein A has  $K_a = 10^6 \text{ M}^{-1}$ ; protein B has  $K_a = 10^9 \text{ M}^{-1}$ .

- 2. Negative Cooperativity** Which of the following situations would produce a Hill plot with  $n_H < 1.0$ ? Explain your reasoning in each case.
- (a) The protein has multiple subunits, each with a single ligand-binding site. Binding of ligand to one site decreases the binding affinity of other sites for the ligand.
  - (b) The protein is a single polypeptide with two ligand-binding sites, each having a different affinity for the ligand.
  - (c) The protein is a single polypeptide with a single ligand-binding site. As purified, the protein preparation is heterogeneous, containing some protein molecules that are partially denatured and thus have a lower binding affinity for the ligand.

**Answer** All three situations would produce  $n_H < 1.0$ . An  $n_H$  (Hill coefficient) of  $< 1.0$  generally suggests situation (a)—the classic case of negative cooperativity. However, closer examination of the properties of a protein exhibiting apparent negative cooperativity in ligand binding often reveals situation (b) or (c). When two or more types of ligand-binding sites with different affinities for the ligand are present on the same or different proteins in the same solution, apparent negative cooperativity is observed. In (b), the higher-affinity ligand-binding sites bind the ligand first. As the ligand concentration is increased, binding to the lower-affinity sites produces an  $n_H < 1.0$ , even though binding to the two ligand-binding sites is completely independent. Even more common is situation (c), in which the protein preparation is heterogeneous. Unsuspected proteolytic digestion by contaminating proteases and partial denaturation of the protein under certain solvent conditions are common artifacts of protein purification. There are few well-documented cases of *true* negative cooperativity.

- 3. Affinity for Oxygen of Hemoglobin** What is the effect of the following changes on the  $O_2$  affinity of hemoglobin? (a) A drop in the pH of blood plasma from 7.4 to 7.2. (b) A decrease in the partial pressure of  $CO_2$  in the lungs from 6 kPa (holding one's breath) to 2 kPa (normal). (c) An increase in the BPG level from 5 mM (normal altitudes) to 8 mM (high altitudes). (d) An increase in CO from 1.0 parts per million (ppm) in a normal indoor atmosphere to 30 ppm in a home that has a malfunctioning or leaking furnace.

**Answer** The affinity of hemoglobin for  $O_2$  is regulated by the binding of the ligands  $H^+$ ,  $CO_2$ , and BPG. The binding of each ligand shifts the  $O_2$ -saturation curve to the right—that is, the  $O_2$  affinity of hemoglobin is reduced in the presence of ligand. **(a)** decreases the affinity; **(b)** increases the affinity; **(c)** decreases the affinity; **(d)** decreases the affinity.

- 4. Reversible Ligand Binding** The protein calcineurin binds to the protein calmodulin with an association rate of  $8.9 \times 10^3 \text{ M}^{-1}\text{s}^{-1}$  and an overall dissociation constant,  $K_d$ , of 10 nM. Calculate the dissociation rate,  $k_d$ , including appropriate units.

**Answer**  $K_d$ , the dissociation constant, is the ratio of  $k_d$ , the rate constant for the dissociation reaction, to  $k_a$ , the rate constant for the association reaction.

$$K_d = k_d/k_a$$

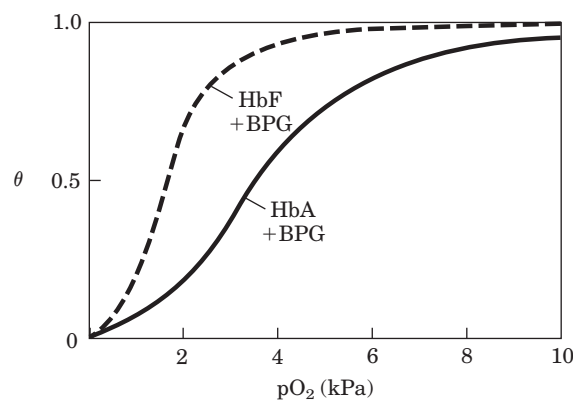
Rearrange to solve for  $k_d$  and substitute the known values.

$$k_d = K_d \times k_a = (10 \times 10^{-9} \text{ M})(8.9 \times 10^3 \text{ M}^{-1}\text{s}^{-1}) = 8.9 \times 10^{-5} \text{ s}^{-1}$$

- 5. Cooperativity in Hemoglobin** Under appropriate conditions, hemoglobin dissociates into its four subunits. The isolated  $\alpha$  subunit binds oxygen, but the  $O_2$ -saturation curve is hyperbolic rather than sigmoid. In addition, the binding of oxygen to the isolated  $\alpha$  subunit is not affected by the presence of  $H^+$ ,  $CO_2$ , or BPG. What do these observations indicate about the source of the cooperativity in hemoglobin?

**Answer** These observations indicate that the cooperative behavior—the sigmoid  $O_2$ -binding curve and the positive cooperativity in ligand binding—of hemoglobin arises from interaction between subunits.

- 6. Comparison of Fetal and Maternal Hemoglobins** Studies of oxygen transport in pregnant mammals show that the  $O_2$ -saturation curves of fetal and maternal blood are markedly different when measured under the same conditions. Fetal erythrocytes contain a structural variant of hemoglobin, HbF, consisting of two  $\alpha$  and two  $\gamma$  subunits ( $\alpha_2\gamma_2$ ), whereas maternal erythrocytes contain HbA ( $\alpha_2\beta_2$ ).

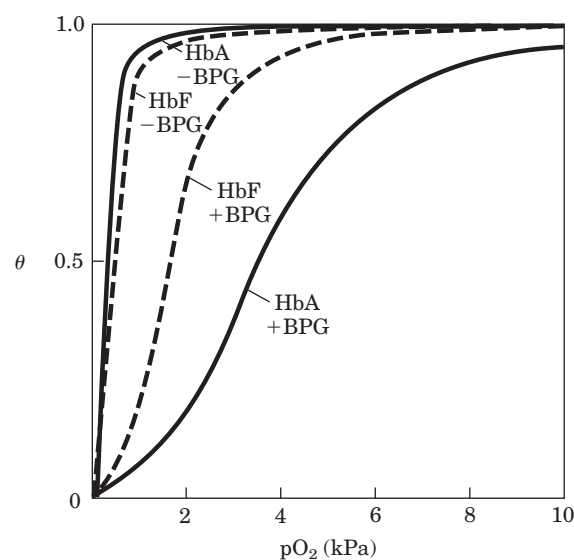


- (a)** Which hemoglobin has a higher affinity for oxygen under physiological conditions, HbA or HbF? Explain.
- (b)** What is the physiological significance of the different  $O_2$  affinities?
- (c)** When all the BPG is carefully removed from samples of HbA and HbF, the measured  $O_2$ -saturation curves (and consequently the  $O_2$  affinities) are displaced to the left. However, HbA now has a greater affinity for oxygen than does HbF. When BPG is reintroduced, the  $O_2$ -saturation curves return to normal, as shown in the graph. What is the effect of BPG on the  $O_2$  affinity of hemoglobin? How can the above information be used to explain the different  $O_2$  affinities of fetal and maternal hemoglobin?

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**Answer**

- (a) The observation that hemoglobin A (HbA; maternal) is about 60% saturated at  $pO_2 = 4$  kPa (the  $pO_2$  in tissues), whereas hemoglobin F (HbF; fetal) is more than 90% saturated under the same physiological conditions, indicates that HbF has a higher  $O_2$  affinity than HbA. In other words, at identical  $O_2$  concentrations, HbF binds more oxygen than does HbA. Thus, HbF must bind oxygen more tightly (with higher affinity) than HbA under physiological conditions.
- (b) The higher  $O_2$  affinity of HbF ensures that oxygen will flow from maternal blood to fetal blood in the placenta. For maximal  $O_2$  transport, the oxygen pressure at which fetal blood approaches full saturation must be in the region where the  $O_2$  affinity of HbA is low. This is indeed the case.
- (c)



Binding of BPG to hemoglobin reduces the affinity of hemoglobin for  $O_2$ , as shown in the graph. The  $O_2$ -saturation curve for HbA shifts far to the right when BPG binds (solid curves)—that is, the  $O_2$  affinity is dramatically lowered. The  $O_2$ -saturation curve for HbF also shifts to the right when BPG binds (dashed curves), but not as far. Because the  $O_2$ -saturation curve of HbA undergoes a larger shift on BPG binding than does that of HbF, we can conclude that HbA binds BPG more tightly than does HbF. Differential binding of BPG to the two hemoglobins may determine the difference in their  $O_2$  affinities.



**7. Hemoglobin Variants** There are almost 500 naturally occurring variants of hemoglobin. Most are the result of a single amino acid substitution in a globin polypeptide chain. Some variants produce clinical illness, though not all variants have deleterious effects. A brief sample follows:

HbS (sickle-cell Hb): substitutes a Val for a Glu on the surface

Hb Cowtown: eliminates an ion pair involved in T-state stabilization

Hb Memphis: substitutes one uncharged polar residue for another of similar size on the surface

Hb Bibba: substitutes a Pro for a Leu involved in an  $\alpha$  helix

Hb Milwaukee: substitutes a Glu for a Val

Hb Providence: substitutes an Asn for a Lys that normally projects into the central cavity of the tetramer

Hb Philly: substitutes a Phe for a Tyr, disrupting hydrogen bonding at the  $\alpha_1\beta_1$  interface

Explain your choices for each of the following:

- (a) The Hb variant *least* likely to cause pathological symptoms.
- (b) The variant(s) most likely to show pI values different from that of HbA on an isoelectric focusing gel.

- (c) The variant(s) most likely to show a decrease in BPG binding and an increase in the overall affinity of the hemoglobin for oxygen.

**Answer**

- (a) Hb Memphis; it has a conservative substitution that is unlikely to have a significant effect on function.
- (b) HbS, Hb Milwaukee, and Hb Providence; all have substitutions that alter the net charge on the protein, which will change the pI. The loss of an ion pair in Hb Cowtown may indicate loss of a charged residue, which would also change the pI, but there is not enough information to be sure.
- (c) Hb Providence; it has an Asn residue in place of a Lys that normally projects into the central cavity of hemoglobin. Loss of the positively charged Lys that normally interacts with the negative charges on BPG results in Hb Providence having lower affinity for BPG and thus higher affinity for O<sub>2</sub>.

- 8. Oxygen Binding and Hemoglobin Structure** A team of biochemists uses genetic engineering to modify the interface region between hemoglobin subunits. The resulting hemoglobin variants exist in solution primarily as  $\alpha\beta$  dimers (few, if any,  $\alpha_2\beta_2$  tetramers form). Are these variants likely to bind oxygen more weakly or more tightly? Explain your answer.

**Answer** More tightly. An inability to form tetramers would limit the cooperativity of these variants, and the binding curve would become more hyperbolic. Also, the BPG-binding site would be disrupted. Oxygen binding would probably be tighter, because the default state in the absence of bound BPG is the tight-binding R state.

- 9. Reversible (but Tight) Binding to an Antibody** An antibody binds to an antigen with a  $K_d$  of  $5 \times 10^{-8}$  M. At what concentration of antigen will  $\theta$  be (a) 0.2, (b) 0.5, (c) 0.6, (d) 0.8?

**Answer** (a)  $1 \times 10^{-8}$  M, (b)  $5 \times 10^{-8}$  M, (c)  $8 \times 10^{-8}$  M, (d)  $2 \times 10^{-7}$  M. These are calculated from a rearrangement of Equation 5-8 to give  $[L] = \theta K_d / (1 - \theta)$ , and for this antigen-antibody binding,  $[L] = \theta(5 \times 10^{-8} \text{ M}) / (1 - \theta)$ . For example, for (a)  $[L] = 0.2(5 \times 10^{-8} \text{ M}) / (0.8) = 1 \times 10^{-8}$  M.

- 10. Using Antibodies to Probe Structure-Function Relationships in Proteins** A monoclonal antibody binds to G-actin but not to F-actin. What does this tell you about the epitope recognized by the antibody?

**Answer** The epitope is likely to be a structure that is buried when G-actin polymerizes to form F-actin.

- 11. The Immune System and Vaccines** A host organism needs time, often days, to mount an immune response against a new antigen, but memory cells permit a rapid response to pathogens previously encountered. A vaccine to protect against a particular viral infection often consists of weakened or killed virus or isolated proteins from a viral protein coat. When injected into a human patient, the vaccine generally does not cause an infection and illness, but it effectively “teaches” the immune system what the viral particles look like, stimulating the production of memory cells. On subsequent infection, these cells can bind to the virus and trigger a rapid immune response. Some pathogens, including HIV, have developed mechanisms to evade the immune system, making it difficult or impossible to develop effective vaccines against them. What strategy could a pathogen use to evade the immune system? Assume that a host’s antibodies and/or T-cell receptors are available to bind to any structure that might appear on the surface of a pathogen and that, once bound, the pathogen is destroyed.

**Answer** Many pathogens, including HIV, have evolved mechanisms by which they can repeatedly alter the surface proteins to which immune system components initially bind. Thus the host organism regularly faces new antigens and requires time to mount an immune response to each one. As the immune system responds to one variant, new variants are created. Some

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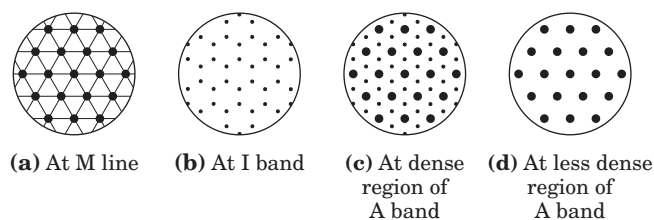
molecular mechanisms that are used to vary viral surface proteins are described in Part III of the text. HIV uses an additional strategy to evade the immune system: it actively infects and destroys immune system cells.

- 12. How We Become a “Stiff”** When a vertebrate dies, its muscles stiffen as they are deprived of ATP, a state called rigor mortis. Explain the molecular basis of the rigor state.

**Answer** Binding of ATP to myosin triggers dissociation of myosin from the actin thin filament. In the absence of ATP, actin and myosin bind tightly to each other.

- 13. Sarcomeres from Another Point of View** The symmetry of thick and thin filaments in a sarcomere is such that six thin filaments ordinarily surround each thick filament in a hexagonal array. Draw a cross section (transverse cut) of a myofibril at the following points: **(a)** at the M line; **(b)** through the I band; **(c)** through the dense region of the A band; **(d)** through the less dense region of the A band, adjacent to the M line (see Fig. 5–29b, c).

**Answer**



The less dense region of the A band, also known as the H zone (not shown in Fig. 5–29b), is the region in which the myosin thick filaments do not overlap the actin thin filaments. When the sarcomere contracts (see Fig. 5–29c), the H zone and the I band decrease in width.

### Biochemistry on the Internet

- 14. Lysozyme and Antibodies** To fully appreciate how proteins function in a cell, it is helpful to have a three-dimensional view of how proteins interact with other cellular components. Fortunately, this is possible using Web-based protein databases and three-dimensional molecular viewing utilities. Some molecular viewers require that you download a program or plug-in; some can be problematic when used with certain operating systems or browsers; some require the use of command-line code; some have a more user-friendly interface. We suggest you go to [www.umass.edu/microbio/rasmol](http://www.umass.edu/microbio/rasmol) and look at the information about RasMol, Protein Explorer, and Jmol FirstGlance. Choose the viewer most compatible with your operating system, browser, and level of expertise. Then download and install any software or plug-ins you may need.

In this exercise you will examine the interactions between the enzyme lysozyme (Chapter 4) and the Fab portion of the anti-lysozyme antibody. Use the PDB identifier 1FDL to explore the structure of the IgG1 Fab fragment–lysozyme complex (antibody–antigen complex). To answer the following questions, use the information on the Structure Summary page at the Protein Data Bank ([www.rcsb.org](http://www.rcsb.org)), and view the structure using RasMol, Protein Explorer, or FirstGlance in Jmol.

- (a) Which chains in the three-dimensional model correspond to the antibody fragment and which correspond to the antigen, lysozyme?
- (b) What type of secondary structure predominates in this Fab fragment?
- (c) How many amino acid residues are in the heavy and light chains of the Fab fragment? In lysozyme? Estimate the percentage of the lysozyme that interacts with the antigen-binding site of the antibody fragment.
- (d) Identify the specific amino acid residues in lysozyme and in the variable regions of the Fab heavy and light chains that are situated at the antigen–antibody interface. Are the residues contiguous in the primary sequence of the polypeptide chains?

**Answer**

- (a) Chain L is the light chain and chain H is the heavy chain of the Fab fragment of this antibody molecule. Chain Y is lysozyme.
- (b) At the PDB, the SCOP and CATH data show that the proteins have predominantly  $\beta$  secondary structure forming immunoglobulin-like  $\beta$ -sandwich folds. Use the Jmol viewing utility at the PDB to view the complex. You should be able to identify the  $\beta$  structures in the variable and constant regions of both the light and heavy chains.
- (c) The heavy chain of the Fab fragment has 218 amino acid residues, the light chain fragment has 214, and lysozyme has 129. Viewing the structure in the spacefill mode shows that less than 15% of the total lysozyme molecule is in contact with the combined  $V_L$  and  $V_H$  domains of the antibody fragment.
- (d) To answer this question you may wish to use FirstGlance in Jmol (<http://firstglance.jmol.org>). Enter the PDB ID 1FDL. When the molecule appears, check the “Spin” box to stop the molecule from spinning. Next, click “Contacts.” With “Chains” selected as the target, click on the lysozyme portion of the complex (Chain Y). The atoms will have asterisks when they are selected. Click “Show Atoms Contacting Target.” Only the atoms (in the immunoglobulin chains) that are in contact with lysozyme will remain in space-filling mode. A quick click on each atom will bring up identifying information. Repeat the process with each of the immunoglobulin chains selected to find the lysozyme residues at the interface. In the H chain these residues include Gly<sup>31</sup>, Tyr<sup>32</sup>, Asp<sup>100</sup>, and Tyr<sup>101</sup>; in the L chain, Tyr<sup>32</sup>, Tyr<sup>49</sup>, Tyr<sup>50</sup>, and Trp<sup>92</sup>. In lysozyme, residues Asn<sup>19</sup>, Gly<sup>22</sup>, Tyr<sup>23</sup>, Ser<sup>24</sup>, Lys<sup>116</sup>, Gly<sup>117</sup>, Thr<sup>118</sup>, Asp<sup>119</sup>, Gln<sup>121</sup>, and Arg<sup>125</sup> appear to be situated at the antigen-antibody interface. Not all these residues are adjacent in the primary structure. In any antibody, the residues in the  $V_L$  and  $V_H$  domains that come into contact with the antigen are located primarily in the loops connecting the  $\beta$  strands of the  $\beta$ -sandwich supersecondary structure. Folding of the polypeptide chain into higher levels of structure brings the nonconsecutive residues together to form the antigen-binding site.

**15. Exploring Reversible Interactions of Proteins and Ligands with Living Graphs**  Use the living graphs for Equations 5–8, 5–11, 5–14, and 5–16 to work through the following exercises.

- (a) Reversible binding of a ligand to a simple protein, without cooperativity. For Equation 5–8, set up a plot of  $\theta$  versus [L] (vertical and horizontal axes, respectively). Examine the plots generated when  $K_d$  is set at 5, 10, 20, and 100  $\mu\text{M}$ . Higher affinity of the protein for the ligand means more binding at lower ligand concentrations. Suppose that four different proteins exhibit these four different  $K_d$  values for ligand L. Which protein would have the highest affinity for L?
- Examine the plot generated when  $K_d = 10 \mu\text{M}$ . How much does  $\theta$  increase when [L] increases from 0.2 to 0.4  $\mu\text{M}$ ? How much does  $\theta$  increase when [L] increases from 40 to 80  $\mu\text{M}$ ?
- You can do the same exercise for Equation 5–11. Convert [L] to  $p\text{O}_2$  and  $K_d$  to  $P_{50}$ . Examine the curves generated when  $P_{50}$  is set at 0.5, 1, 2, and 10 kPa. For the curve generated when  $P_{50} = 1 \text{ kPa}$ , how much does  $\theta$  change when the  $p\text{O}_2$  increases from 0.02 to 0.04 kPa? From 4 to 8 kPa?
- (b) Cooperative binding of a ligand to a multisubunit protein. Using Equation 5–14, generate a binding curve for a protein and ligand with  $K_d = 10 \mu\text{M}$  and  $n = 3$ . Note the altered definition of  $K_d$  in Equation 5–16. On the same plot, add a curve for a protein with  $K_d = 20 \mu\text{M}$  and  $n = 3$ . Now see how both curves change when you change to  $n = 4$ . Generate Hill plots (Eqn 5–16) for each of these cases. For  $K_d = 10 \mu\text{M}$  and  $n = 3$ , what is  $\theta$  when [L] = 20  $\mu\text{M}$ ?
- (c) Explore these equations further by varying all the parameters used above.

**Answer**

- (a) The plots should be a series of hyperbolic curves, with  $\theta = 1.0$  as the limit. Each curve passes through  $\theta = 0.5$  at the point on the  $x$  axis where [L] =  $K_d$ . The protein with  $K_d = 5 \mu\text{M}$  has the highest affinity for ligand L. When  $K_d = 10 \mu\text{M}$ , doubling [L] from 0.2 to 0.4  $\mu\text{M}$  (values well below  $K_d$ ) nearly doubles  $\theta$  (the actual increase factor is 1.96).

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This is a property of the hyperbolic curve; at low ligand concentrations,  $\theta$  is an almost linear function of  $[L]$ . By contrast, doubling  $[L]$  from 40 to 80  $\mu\text{M}$  (well above  $K_d$ , where the binding curve is approaching its asymptotic limit) increases  $\theta$  by a factor of only 1.1. The increase factors are identical for the curves generated from Equation 5-11.

- (b) The curves generated from Equation 5-14 should be sigmoidal. Increasing the Hill coefficient ( $n$ ) increases the slope of the curves at the inflection point. Using Equation 5-14, with  $[L] = 20 \mu\text{M}$ ,  $K_d = 10 \mu\text{M}$ , and  $n = 3$ , you will find that  $\theta = 0.998$ .
- (c) A variety of answers will be obtained depending on the values entered for the different parameters.

## Data Analysis Problem

**16. Protein Function** During the 1980s, the structures of actin and myosin were known only at the resolution shown in Figure 5-28a, b. Although researchers knew that the S1 portion of myosin binds to actin and hydrolyzes ATP, there was a substantial debate about where in the myosin molecule the contractile force was generated. At the time, two competing models were proposed for the mechanism of force generation in myosin.

In the “hinge” model, S1 bound to actin, but the pulling force was generated by contraction of the “hinge region” in the myosin tail. The hinge region is in the heavy meromyosin portion of the myosin molecule, near where trypsin cleaves off light meromyosin (see Fig. 5-27b). This is roughly the point labeled “Two supercoiled  $\alpha$  helices” in Figure 5-27a. In the “S1” model, the pulling force was generated in the S1 “head” itself and the tail was just for structural support.

Many experiments had been performed but provided no conclusive evidence. In 1987, James Spudich and his colleagues at Stanford University published a study that, although not conclusive, went a long way toward resolving this controversy.

Recombinant DNA techniques were not sufficiently developed to address this issue in vivo, so Spudich and colleagues used an interesting in vitro motility assay. The alga *Nitella* has extremely long cells, often several centimeters in length and about 1 mm in diameter. These cells have actin fibers that run along their long axes, and the cells can be cut open along their length to expose the actin fibers. Spudich and his group had observed that plastic beads coated with myosin would “walk” along these fibers in the presence of ATP, just as myosin would do in contracting muscle.

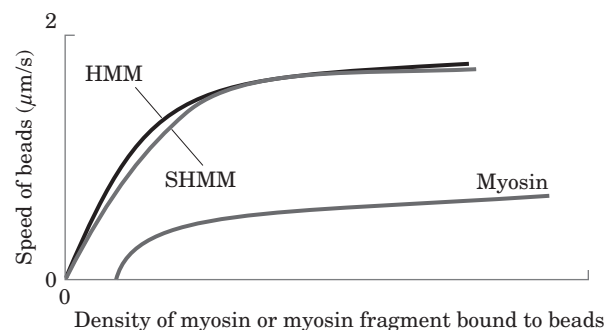
For these experiments, they used a more well-defined method for attaching the myosin to the beads. The “beads” were clumps of killed bacterial (*Staphylococcus aureus*) cells. These cells have a protein on their surface that binds to the Fc region of antibody molecules (Fig. 5-21a). The antibodies, in turn, bind to several (unknown) places along the tail of the myosin molecule. When bead-antibody-myosin complexes were prepared with intact myosin molecules, they would move along *Nitella* actin fibers in the presence of ATP.

- (a) Sketch a diagram showing what a bead-antibody-myosin complex might look like at the molecular level.
- (b) Why was ATP required for the beads to move along the actin fibers?
- (c) Spudich and coworkers used antibodies that bound to the myosin tail. Why would this experiment have failed if they had used an antibody that bound to the part of S1 that normally binds to actin? Why would this experiment have failed if they had used an antibody that bound to actin?

To help focus in on the part of myosin responsible for force production, Spudich and his colleagues used trypsin to produce two partial myosin molecules (see Fig. 5-27): (1) heavy meromyosin (HMM), made by briefly digesting myosin with trypsin; HMM consists of S1 and the part of the tail that includes the hinge; and (2) short heavy meromyosin (SHMM), made from a more extensive digestion of HMM with trypsin; SHMM consists of S1 and a shorter part of the tail that does not include the hinge. Brief digestion of myosin with trypsin produces HMM and light meromyosin (Fig. 5-27), by cleavage of a single specific peptide bond in the myosin molecule.

(d) Why might trypsin attack this peptide bond first rather than other peptide bonds in myosin?

Spudich and colleagues prepared bead-antibody-myosin complexes with varying amounts of myosin, HMM, and SHMM, and measured their speeds along *Nitella* actin fibers in the presence of ATP. The graph below sketches their results.



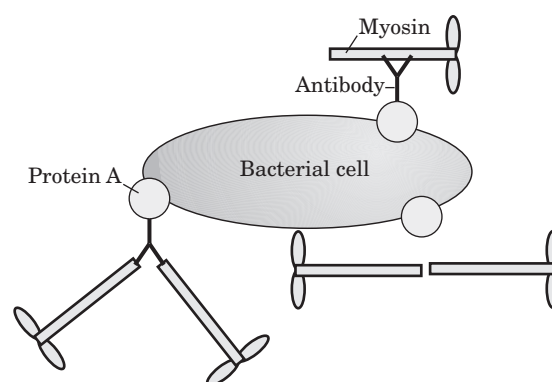
- (e) Which model (“S1” or “hinge”) is consistent with these results? Explain your reasoning.  
 (f) Provide a plausible explanation for why the speed of the beads increased with increasing myosin density.  
 (g) Provide a plausible explanation for why the speed of the beads reached a plateau at high myosin density.

The more extensive trypsin digestion required to produce SHMM had a side effect: another specific cleavage of the myosin polypeptide backbone in addition to the cleavage in the tail. This second cleavage was in the S1 head.

- (h) Based on this information, why is it surprising that SHMM was still capable of moving beads along actin fibers?  
 (i) As it turns out, the tertiary structure of the S1 head remains intact in SHMM. Provide a plausible explanation of how the protein remains intact and functional even though the polypeptide backbone has been cleaved and is no longer continuous.

### Answer

(a)



The drawing is not to scale; any given cell would have many more myosin molecules on its surface.

- (b) ATP is needed to provide the chemical energy to drive the motion (see Chapter 13).  
 (c) An antibody that bound to the myosin tail, the actin-binding site, would block actin binding and prevent movement. An antibody that bound to actin would also prevent actin-myosin interaction and thus movement.

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- (d) There are two possible explanations: (1) Trypsin cleaves only at Lys and Arg residues (see Table 3–7) so would not cleave at many sites in the protein. (2) Not all Arg or Lys residues are equally accessible to trypsin; the most-exposed sites would be cleaved first.
- (e) The S1 model. The hinge model predicts that bead-antibody-HMM complexes (with the hinge) would move, but bead-antibody-SHMM complexes (no hinge) would not. The S1 model predicts that because both complexes include S1, both would move. The finding that the beads move with SHMM (no hinge) is consistent only with the S1 model.
- (f) With fewer myosin molecules bound, the beads could temporarily fall off the actin as a myosin let go of it. The beads would then move more slowly, as time is required for a second myosin to bind. At higher myosin density, as one myosin lets go another quickly binds, leading to faster motion.
- (g) Above a certain density, what limits the rate of movement is the intrinsic speed with which myosin molecules move the beads. The myosin molecules are moving at a maximum rate and adding more will not increase speed.
- (h) Because the force is produced in the S1 head, damaging the S1 head would probably inactivate the resulting molecule, and SHMM would be incapable of producing movement.
- (i) The S1 head must be held together by noncovalent interactions that are strong enough to retain the active shape of the molecule.

**Reference**

**Hynes, T.R., Block, S.M., White, B.T., & Spudich, J.A.** (1987) Movement of myosin fragments in vitro: domains involved in force production. *Cell* **48**, 953–963.