**Example 3.1.**

To measure the amount of glucoamylase in a crude enzyme preparation, 1 ml of the crude enzyme preparation containing 8 mg protein is added to 9 ml of a 4.44% starch solution. One unit of activity of glucoamylase is defined as the amount of enzyme which produces 1 μmol of glucose per min in a 4% solution of Lintner starch at pH 4.5 and at 60°C. Initial rate experiments show that the reaction produces 0.6 μmol of glucose/ml-min. What is the specific activity of the crude enzyme preparation?

**Solution** The total amount of glucose made is 10 ml × 0.6 μmol glucose/ml-min or 6 μmol glucose per min. The specific activity is then:

$$\begin{aligned}\text{specific activity} &= \frac{6 \text{ units}}{1 \text{ ml protein solution} \cdot 8 \text{ mg/ml}} \\ &= 6 \text{ units}/8 \text{ mg protein} \\ &= 0.75 \text{ units/mg protein}\end{aligned}$$

$V_m$ must have units such as μmol product/ml-min. Since $V_m = k_2 E_0$, the dimensions of $k_2$ must reflect the definition of units in $E_0$. In the above example we had a concentration of enzyme of 8 mg protein/10 ml solution · 0.75 units/mg protein or 0.6 units/ml. If, for example, $V_m$ = 1 μmol/ml-min, then $k_2$ = 1 μmol/ml-min ÷ 0.6 units/ml or $k_2$ = 1.67 μmol/unit-min.

### 3.3.4. Models for More Complex Enzyme Kinetics

**3.3.4.1. Allosteric enzymes.** Some enzymes have more than one substrate binding site. The binding of one substrate to the enzyme facilitates binding of other substrate molecules. This behavior is known as *allostery* or *cooperative binding,* and regulatory enzymes show this behavior. The rate expression in this case is

$$v = -\frac{d[S]}{dt} = \frac{V_m[S]^n}{K_m'' + [S]^n} \tag{3.18}$$

where $n$ = cooperativity coefficient and $n > 1$ indicates positive cooperativity. Figure 3.8 compares Michaelis–Menten kinetics with allosteric enzyme kinetics, indicating a sigmoidal shape of υ –[S] plot for allosteric enzymes.

The cooperativity coefficient can be determined by rearranging eq. 3.18 as

$$\ln \frac{v}{V_m - v} = n \ln[S] - \ln K_m'' \tag{3.19}$$

and by plotting ln $v/(V_m - v)$ versus ln[S] as depicted in Fig. 3.9.

**3.3.4.2. Inhibited enzyme kinetics.** Certain compounds may bind to enzymes and reduce their activity. These compounds are known to be enzyme inhibitors. Enzyme inhibitions may be irreversible or reversible. Irreversible inhibitors such as heavy metals (lead, cadium, mercury, and others) form a stable complex with enzyme and reduce enzyme activity. Such enzyme inhibition may be reversed only by using chelating agents such as EDTA (ethylenediaminetetraacetic acid) and citrate. Reversible inhibitors may dissociate more easily from the enzyme after binding. The three major classes of re-