Peptidase Activity Assays Using Protein Substrates

The International Union of Biochemistry and Molecular Biology recommends that the term "peptidase" be used synonymously with the term "peptide hydrolase" (IUBMB, 1992). Thus, in this unit the term peptidase is used in reference to any enzyme that catalyzes the hydrolysis of peptide bonds, without distinguishing between exo- and endopeptidase activities. Peptidases may be assayed using native or modified proteins, peptides, or synthetic substrates. In this unit, the focus is on assays based on the hydrolysis of common, commercially available, protein substrates. Thus, the assays are not intended to be selective for a given peptidase; they are designed to provide estimates of overall peptidase activity. Other units in this publication focus on synthetic or "model" substrates, which can be designed for the measurement of specific endo- and/or exopeptidase activities.

STRATEGIC PLANNING

The following protocols may be viewed as comprising three operations. The first is the enzymatic reaction itself, the second is the simultaneous termination of the reaction and separation of enzyme-generated products from other protein/peptide components, and the third is product quantification. Variables that must be defined in the first operation include pH, buffer composition, temperature, and protein substrate. Traditional substrates for these assays are the caseins and denatured hemoglobin, or conjugates thereof (Stauffer, 1989). These proteins are convenient substrates for monitoring peptidase activity because fairly reproducible preparations are commercially available at relatively low cost and, in general, they are readily susceptible to proteolysis by a wide range of peptidases. The protocols in this unit, regardless of the substrate employed, are dependent on monitoring the generation of short peptides resulting from peptidase activity. Separation, prior to peptide quantification, is in all cases based on the differential solubility of peptides and proteins in trichloroacetic acid (TCA) solutions. Lower-molecular-weight peptides are more soluble in 3% to 5% TCA than are higher-molecular-weight peptides and proteins (Yvon et al., 1989). Quantitative estimates of the TCA-soluble peptides resulting from peptidase activity can be obtained by several methods (for methods to measure soluble protein, see Chapter B1).

In this unit, Basic Protocol 1 presents a procedure using casein as substrate. The Alternate Protocol describes the modification of this procedure for use with a denatured hemoglobin substrate. Basic Protocol 2 presents a procedure using a chromaphore-conjugated casein derivative, azocasein. For quantitation, the authors have chosen to use either the BCA-based colorimetric assay (*UNIT B1.1*) for soluble protein/peptides (in Basic Protocol 1) or the intrinsic absorbance of the chromaphore-conjugated peptide products (in Basic Protocol 2).

The assays presented in this unit assume that the analyst is working with a soluble enzyme preparation. Enzyme preparations of this type are often made by rupturing cell walls and/or membranes of the target tissue followed by enzyme extraction with an appropriately buffered solution. The extraction buffer should maintain pH and ionic strength in a range that is compatible with the enzyme's stability. Tissue disruption and enzyme extraction are often done simultaneously by homogenizing the target tissue in the extraction buffer and then separating the solids fraction by either centrifugation or filtration. It is expected that all enzyme preparations will be kept cold to minimize activity loss. In some cases it may be beneficial to add stabilizing agents to the extraction buffer,

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like a metal chelator (such as 1 mM EDTA) or a reducing agent (such as 1 mM dithiothreitol). It is important to make sure that all components included in the extraction buffer are compatible with each phase of the assay. A sample protocol for enzyme preparation from kiwi fruit is presented in the Support Protocol.

BASIC PROTOCOL 1

DETERMINATION OF PEPTIDASE ACTIVITY USING A CASEIN SUBSTRATE

This traditional assay is based on the ability of peptidases to generate 5% trichloroacetic acid (TCA)–soluble peptides from casein. Casein preparations suitable for use as peptidase substrates are commercially available (e.g., Hammerstein grade, ICN Biomedicals). They are typically prepared by acid precipitation of bovine skim milk, the proteinaceous precipitate being casein. The different casein preparations are purified, to a greater or lesser extent, by cycles of dissolving in alkali and reprecipitating. Acidulants commonly used for these processes include lactic, hydrochloric, and sulfuric acids (Walstra et al., 1999); the original "Hammerstein casein" method used acetic acid as the acidulant (McMeekin, 1954). Casein preparations typically consist of four principal gene products, α_{s1}, α_{s2}, β, and κ, in relative amounts of 40:10:35:12, respectively (Mulvihill, 1994).

Enzyme activities are based on rates of casein hydrolysis under defined conditions. The products of casein hydrolysis, as defined in this protocol, are those peptides soluble in 5% TCA that can be detected by the bicinchoninic acid (BCA) protein assay (*UNIT B1.1*). The amount of TCA-soluble peptide generated during the course of the reaction can actually be quantified by any one of several protein/peptide assays. The color yield in these assays is assumed to be proportional to the amount of peptide in solution. The amount of product/peptide in the reaction mixture is often reported as bovine serum albumin (BSA) equivalents—since standard curves based on this protein may be used to calibrate the assay. Thus, activity units can be expressed as the amount of BSA equivalents generated per unit time.

Materials

Enzyme preparation (see Strategic Planning and Support Protocol)
1% (w/v) casein substrate solution (see recipe)
10% (w/v) trichloroacetic acid (TCA)

30°C water bath
10-ml glass test tubes

Additional reagents and equipment for the bicinchoninic acid (BCA) protein assay (*UNIT B1.1*)

Perform enzymatic reaction

1. Bring 1% casein substrate solution to reaction temperature (30°C).
2. Set up a series of eighteen 10-ml glass test tubes for each enzyme preparation to be analyzed (i.e., enough for triplicate sample tubes and triplicate enzyme-substrate blank tubes for each of the three enzyme concentrations tested).
3. Add 0.1 ml of appropriate enzyme preparation to each of the eighteen test tubes. Place the nine sample tubes in the 30°C water bath for temperature equilibration (~5 min). Leave the nine enzyme-substrate blank tubes on ice.
4. Once equilibrated, initiate reactions in just the nine sample tubes by adding 0.9 ml of 1% casein substrate solution, with gentle mixing. Incubate 10 min at 30°C. After the sample tubes have incubated 5 min, place the enzyme-substrate blank tubes in the 30°C water bath to equilibrate.

Terminate reaction and separate components

5. At the completion of the 10-min reaction period, terminate the reactions by adding 1 ml of 10% TCA to each of the eighteen (sample and blank) tubes.

   This will inactivate the enzyme and thus terminate the reaction. TCA also terminates potential autolysis, which may occur in the blanks.

6. Immediately after TCA addition, add 0.9 ml casein substrate solution to each of the nine enzyme-substrate blank tubes.

   Peptidase activity is prevented in the blanks by inactivating the enzyme prior to adding substrate.

7. Remove all tubes from water bath and allow to stand 20 min at room temperature.
8. Pellet the precipitated material by centrifuging 5 min at $8000 \times g$, room temperature.

Quantify products

9. Remove sufficient supernatant to allow measurement of the soluble peptide by the BCA protein assay (*UNIT B1.1*), using bovine serum albumin (BSA; 20 to 120 µg/assay) as a standard.

   Many methods are available for measuring TCA-soluble peptides. Possibly the easiest is to measure the absorbance of the solution at 280 nm, as the absorbance at this wavelength is a function of the aromatic amino acid content of the solution. This approach requires a UV spectrophotometer, and the sensitivity of the assay is likely to be lower than that of some of the colorimetric assays. There are also several colorimetric peptide assays that can be applied to this type of peptidase assay, such as the Biuret, Lowry, and Bradford dye-binding methods (for comparison see Piyachomkwan and Penner, 1995). All of these methods measure a relative value rather than an absolute amount of peptide in solution. The results should thus be reported in terms of "equivalents," such as BSA equivalents when using a calibration curve prepared using a BSA standard solution.

10. Subtract appropriate mean blank values from mean sample values of the same enzyme concentration to obtain the average amount of product peptide produced during the 10-min reaction period.
11. Report activity as BSA equivalents generated per unit time.

    A "unit" could thus be defined as the amount of enzyme that produces 1 µg BSA equivalents per minute, under the conditions defined for a particular assay.

DETERMINATION OF PEPTIDASE ACTIVITY USING A HEMOGLOBIN SUBSTRATE

ALTERNATE PROTOCOL

Hemoglobin is also widely used as a substrate for monitoring general peptidase activity. It is particularly useful for measuring activities at pH <6, since casein is insoluble in that pH region. The hemoglobin used for such assays is almost always referred to as "denatured." Denatured hemoglobin is used because the native form of the protein is relatively resistant to peptidase activity. Denatured hemoglobin can be prepared in several ways; the different methods of preparation will not necessarily result in substrates that are equally susceptible to proteolytic activity (Schlamowitz and Peterson, 1959). The most common of these substrates is urea-denatured hemoglobin (Anson, 1938). It is typically prepared under alkaline conditions (see Reagents and Solutions; adapted from Worthington, 1993). Assays using this substrate are, however, complicated by the presence of urea in the reaction mixture, which can lead to denaturation of the peptidase itself. Thus, assays using urea-denatured hemoglobin should be completed in as short a time as possible to minimize the amount of time the enzyme is exposed to urea. It has been suggested that assays not exceed 10 min (Sarath et al., 2001).

BASIC PROTOCOL 2

DETERMINATION OF PEPTIDASE ACTIVITY USING AN AZOCASEIN SUBSTRATE

Reporter group–labeled proteins are often effective substrates for detecting peptidase activity (Sarath et al., 2001). Azoproteins, which are chromophore-labeled proteins, have been used for this purpose for many years (Charney and Tomarelli, 1947). The term azoprotein, when used in relation to generic peptidase substrates, most commonly refers to the sulfanilic-acid-azo or the sulfanilamide-azo derivative of the protein. They are prepared by coupling the corresponding diazo compound with the native protein under alkaline conditions (for examples, see Hazen et al., 1965, or Jones et al., 1998). The resulting modified protein is expected to have azo-derivatized tyrosine and histidine residues; other amino acids are also susceptible to modification (Means and Feeney, 1971). If diazobenzenesulfonic acid, prepared by diazotization of sulfanilic acid, couples with the phenolic moieties of a protein's tyrosyl residues, then the resulting moieties will be substituted by azophenylsulfonate groups (a bis-coupled phenolic group is shown in Fig. C2.2.1; Haurowitz, 1963).

Azoproteins are particularly useful for detecting peptidase activity due to their rather intense color (in the orange range). Azocasein is one of the most widely used azoproteins for this purpose. It is commercially available (e.g., Sigma) and easy to use. The major advantage of using the azo-modified protein is that the peptide-quantifying (color-generating) reaction, following TCA precipitation in the traditional assay, is not necessary. Instead, the absorbance inherent in the TCA-soluble chromophore-labeled peptides is the basis of quantification. Thus, a simple visible-wavelength spectrophotometer (colorimeter) can be used to monitor the extent of proteolysis without the need for a color-generating reaction (although the pH of the TCA-containing soluble phase is typically raised to increase the assay's sensitivity at 440 nm; Planter, 1991).

Materials

Enzyme preparation (see Strategic Planning and Support Protocol)
1.5% (w/v) azocasein substrate solution (see recipe)
10% (w/v) trichloroacetic acid
Color generating solution: 1 M NaOH

30°C water bath
5- and 10-ml glass test tubes
Spectrophotometer with 1-cm glass or plastic cuvettes

Perform enzymatic reaction

1. Bring 1.5% azocasein substrate solution to reaction temperature (30°C).
2. Set up a series of eighteen 10-ml glass test tubes for each enzyme preparation to be analyzed (enough for triplicate sample tubes and triplicate enzyme-substrate blank tubes for each of the three enzyme concentrations tested).

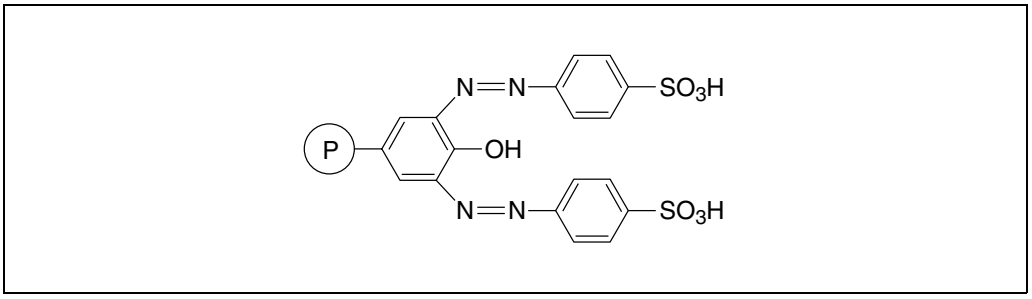

Figure C2.2.1 Azophenylsulfonate substitution of protein tyrosyl side chain.

3. Add 0.2 ml of appropriate enzyme preparation to each of the eighteen test tubes. Place the nine sample tubes in the 30°C water bath for temperature equilibration (~5 min). Leave the nine enzyme-substrate blank tubes on ice.

4. Once equilibrated, initiate reactions in just the nine sample tubes by adding 0.80 ml of 1% azocasein substrate solution and mixing gently. Incubate 10 min at 30°C. After the sample tubes have incubated 5 min, place the enzyme-substrate blank tubes in the 30°C water bath to equilibrate.

Terminate reaction and separate components

5. At the completion of the 10-min reaction period, terminate the reactions by adding 1 ml of 10% TCA to each of the eighteen (sample and blank) tubes.

   This will inactivate the enzyme and thus terminate the reaction. TCA addition also terminates potential autolysis, which may occur in the blanks.

6. Immediately after TCA addition, add 0.8 ml of azocasein substrate solution to each of the nine enzyme-substrate blank tubes.

   Peptidase activity is prevented in the blanks by inactivating the enzyme prior to adding substrate.

7. Remove all tubes from water bath and allow to stand 20 min at room temperature.

8. Pellet the precipitated material by centrifuging 5 min at 8000 × *g*, room temperature.

Quantify products

9. Transfer 1 ml of each resulting supernatant to a 5-ml glass test tube containing 1 ml of 1.0 N NaOH.

10. Measure absorbance at 440 nm against water as a reference.

11. Subtracting the appropriate mean blank value from the mean sample value for each of the enzyme concentrations tested to give the amount of peptide product produced during the 10-min reaction period.

12. Determine the activity units, where a unit is defined as that amount of enzyme required to produce an absorbance change of 1.0 per min in a 1-cm cuvette.

ENZYME PREPARATION EXAMPLE: KIWI FRUIT

SUPPORT PROTOCOL

In this protocol, enzyme is prepared from a single kiwi fruit, the fruit of the Chinese gooseberry (*Actinidia chinensis*), purchased at a local grocery store. The predominant peptidase of the kiwi fruit is a cysteine peptidase called actinidain.

Materials

Kiwi fruit
0.1 M sodium phosphate buffer, pH 7.0 (*APPENDIX 2A*)

Kitchen spoon
Freezer storage bags
Cheesecloth

1. Cut fruit in half and scoop out the flesh using a kitchen spoon.

2. Place tissue in a preweighed freezer storage bag, seal, and then weigh to determine the weight of the flesh (by difference).

3. Pulp the flesh by hand squeezing and place the sealed bag in a freezer until ready for use (typically overnight).
4. Allow pulp to thaw at room temperature for ~30 min.
5. Add an equal weight of 0.1 M sodium phosphate buffer, pH 7.0, to the pulp and homogenize the mixture by hand (~5 min).
6. Filter the resulting suspension through cheesecloth.
7. Centrifuge the cloudy filtrate 20 min at 10,000 × *g*, 4°C, and collect the supernatant as the enzyme preparation.

REAGENTS AND SOLUTIONS

Use deionized or distilled water in all recipes and protocol steps. For common stock solutions, see ***APPENDIX 2A****; for suppliers, see* ***SUPPLIERS APPENDIX****.*

Azocasein substrate solution, 1.5% (w/v)

Dissolve 1.5 g azocasein (Sigma) in 0.05 M sodium phosphate buffer, pH 7 (*APPENDIX 2A*) by stirring and, if necessary, gentle heating (to ~50°C). Bring the final volume to 100 ml with the same buffer. Store up to 5 days at 4°C.

Casein substrate solution, 1% (w/v)

Dissolve 1 g casein (Hammerstein grade, ICN Biomedicals) in 50 ml of 0.01 N NaOH by stirring and, if necessary, gentle heating (to ~50°C). With continued stirring, add 40 ml of 0.25 M sodium phosphate buffer, pH 7.0 (*APPENDIX 2A*) and bring to 100 ml with deionized water. Store up to 5 days at 4°C.

The pH of this solution will be ~7.1. If a different pH is desired, then adjust the pH to the desired value prior to bringing the final volume to 100 ml. The pH of the reaction mixture should be kept above 6 to prevent precipitation of casein.

Preparing a 1% casein solution is somewhat tricky because dried casein preparations are typically only sparingly soluble in neutral aqueous systems. This method, which uses alkaline conditions to prepare a casein sol, should work for most purposes. The casein substrate solutions used for peptidase assays are often more accurately described as substrate suspensions, since these preparations are typically not true solutions—the casein component being of a colloidal nature.

Denatured hemoglobin, 2% (w/v)

Dissolve 2.0 g bovine hemoglobin (Sigma) in 35 ml reagent-grade water. Add 36.0 g urea and 16 ml of 0.5 M NaOH. Stir 30 min at room temperature. Add buffer (typically sodium phosphate; see *APPENDIX 2A*), adjust pH to that appropriate for the enzyme system under investigation, and then bring to 100 ml with water. Store up to 5 days at 4°C.

COMMENTARY

Background Information

Peptidases have been referred to as "the most important group of enzymes in the food processing industry" (Whitaker, 1994). Their integral role is in catalyzing the depolymerization of proteins, which are themselves significant contributors to the functional properties of foods. Endogenous and supplemental peptidases are both important; the former affecting such properties as gel strength, foam and emulsion stability, and flavor profiles; the latter being used in applications such as meat tenderization and the initiation of curd formation in cheese manufacture.

All peptidases catalyze the general reaction depicted in Figure C2.2.2, the hydrolysis of a peptide bond. The different peptidases are unique with respect to their specificity; that is, their ability to accommodate particular sets of amino acids in the vicinity of a potentially scissile peptide bond. Some peptidases have very broad specificities, such as papain, which has few limi-

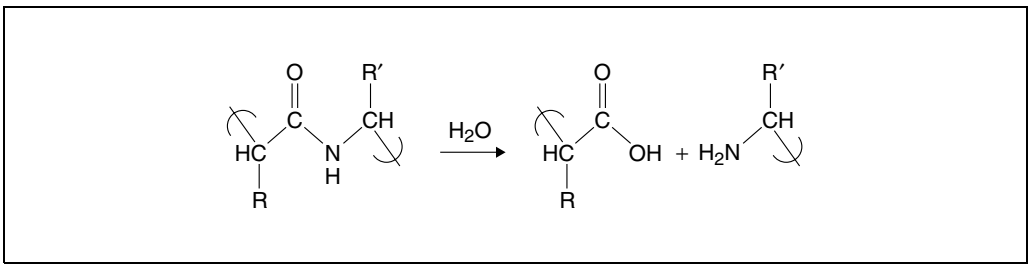

Figure C2.2.2 Peptidase reaction: hydrolysis of peptide bond.

tations on the amino acids in the vicinity of the scissile bond (Keil, 1992; Rawlings and Barrett, 1994). Others are highly specific, such as renin, which is specific for a particular locus in the protein angiotensin, its natural substrate (Slater, 1981; Keil, 1992).

Peptidases are often classified as either exopeptidases or endopeptidases, depending on the positional specificity of the bonds they hydrolyze. Exopeptidases act at peptide bonds located at either the N or C terminus of the protein. Those acting at the C terminus are referred to as carboxypeptidases, those acting at the N terminus as aminopeptidases. Endopeptidases, on the other hand, act at peptide bonds internal to the polypeptide chain.

Peptidases are further classified based on their catalytic mechanism. The following four mechanistic classes are well established (Dunn, 2001).

1. Serine peptidases: the catalytic apparatus includes a nucleophilic serine residue.

2. Cysteine peptidases: the catalytic apparatus includes a nucleophilic cysteine residue.

3. Metallopeptidases: the catalytic apparatus includes a metal ion.

4. Aspartic proteases: the catalytic apparatus includes two aspartic acid residues.

This nomenclature scheme, based on active site chemistry, is complimentary to the endo/exo nomenclature described above. Thus, the two schemes are frequently combined. For example, trypsin (a digestive enzyme) is often descriptively referred to as a serine endopeptidase.

The assays presented in this unit are far more sensitive for endopeptidases than for exopeptidases, because the assays are not designed to measure the actual number of catalytic events resulting from peptidase activity. Instead, the assays attempt to measure the amount of TCA-soluble peptide generated during a given reaction period, assuming that this value will be a function of the amount of active endopeptidase present in the reaction mixture. These TCA-soluble peptides can range from 2 to >20 amino acids in length (Yvon et al., 1989). The quantification methods used to detect these peptides, such as the BCA peptide/protein assay, are not well suited for the measurement of free (albeit TCA-soluble) amino acids, which are (along with dipeptides) the expected products resulting from exopeptidase activity. Hence, exopeptidase activity may go largely undetected with this type of assay. The azosubstrates present yet another barrier for exopeptidase activity, because exopeptidases are not expected to process the azo-derivatized amino acids, and hence should generate little to no color. The endopeptidases apparently work by hydrolyzing peptide linkages on either side of the derivatized amino acid, thus generating a chromaphore-labeled peptide from the azosubstrate.

Critical Parameters

Quantitative measurements of enzyme activity are based on rate assays. Hence, all experimental parameters that may affect the rate of an enzyme-catalyzed reaction, including pH, ionic strength, buffer composition, and temperature, are to be defined. These parameters are typically chosen to coincide with optimum or physiologically/technologically relevant conditions. Furthermore, it is generally assumed that quantitative activity measurements reflect initial-velocity kinetics. This means that the analyst must establish, for all experimental permutations, that initial rates are indeed being measured. These topics, as well as other information related to the design of enzyme assays, are discussed in *UNIT C1.1*.

A unique aspect of using protein substrates is that the substrates themselves are so complex. Even a relatively small protein, having say 100 amino acids, has 99 potentially susceptible peptide bonds—and each of these bonds is expected to be unique. This hypothetical protein thus has 99 structurally unique substrates (potential sites for catalysis). The environment surrounding each of the protein's peptide bonds will be dependent on the tertiary structure of the protein, so one must consider, for example, native

versus denatured forms of the protein. Furthermore, initially unavailable (e.g., buried) peptide bonds may become accessible as a protein is depolymerized; at a minimum the physicochemical environment of the different peptide linkages will change as the protein is depolymerized. Thus, one can expect that the substrate profile will change over the course of the reaction. When considering the azoproteins, the substrate profile will also be a function of the extent of azo-coupling. Here the rational assumption is made that a derivatized amino acid, in the vicinity of a peptide bond, will perturb the natural protein-substrate interactions that occur at that peptide bond.

The peptide/protein quantification methods used in these assays are somewhat imprecise, because the color yield per mole or per milligram peptide is not consistent between peptides or proteins of different composition (Davis, 1988). Amounts of product are thus typically reported in terms of BSA equivalents, because calibration curves are typically prepared with this protein, and, again, absolute mole or absolute mass values are not readily obtained. This same limitation applies to the azosubstrates, since the color yield per mass peptide will be dependent on that given peptide's extent of derivitization.

The assays presented in this unit are best used for the detection of peptidase activity and for estimating the relative activity of test samples having the same peptidase composition. The authors do not recommend using these assays for quantitative comparisons of the molar amounts of different peptidases, because different peptidases, due to their unique specificities, may have vastly different activities on different substrates. It is not inconceivable for preparation "A," consisting of peptidase "A," to show higher activity than preparation "B," consisting of peptidase "B," when assayed with protein substrate "1" (say, casein), but lower activity than preparation "B" when assayed with protein substrate "2" (say, hemoglobin). This limitation suggests that comparative studies with different enzymes should include assays with different substrates, and, even then, be interpreted with caution.

Anticipated Results

Experimental conditions resulting in a linear or near-linear relationship between rate of product formation and enzyme concentration are advantageous. Such a relationship allows the analyst to back-calculate relative enzyme activities by simply accounting for dilution factors. Figure C2.2.3, using a commercial peptidase preparation, shows that such a linear relationship can be expected at lower enzyme concentrations.

A model experiment using a "crude" enzyme preparation from kiwi fruit (see Support Protocol) is presented in the hope that the approach may serve as a template for the design of other assays, and the results provide an example of what may be expected with raw biological materials, albeit the kiwi fruit is a raw product with relatively high peptidase activity (Lewis and Luh, 1988). Assay conditions and substrate preparation were performed as in Basic Protocol 1, with the pH of the substrate adjusted to 7.0. Enzyme preparation was performed as in the Support Protocol. The same

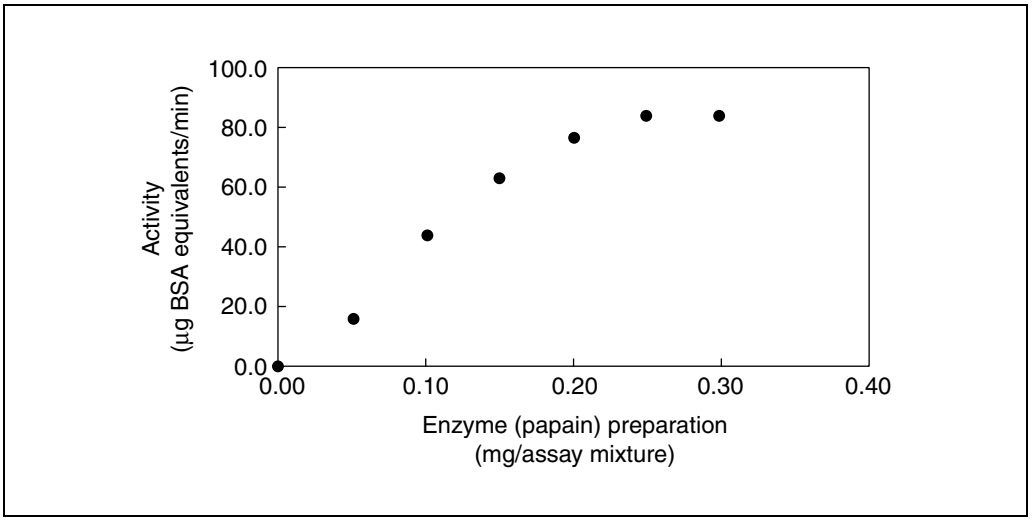

Figure C2.2.3 Relationship between amount of enzyme in reaction mixture (mg papain preparation from Sigma) and measured activity. Results were obtained using Basic Protocol 1 with Hammerstein casein substrate (ICN Biomedicals).

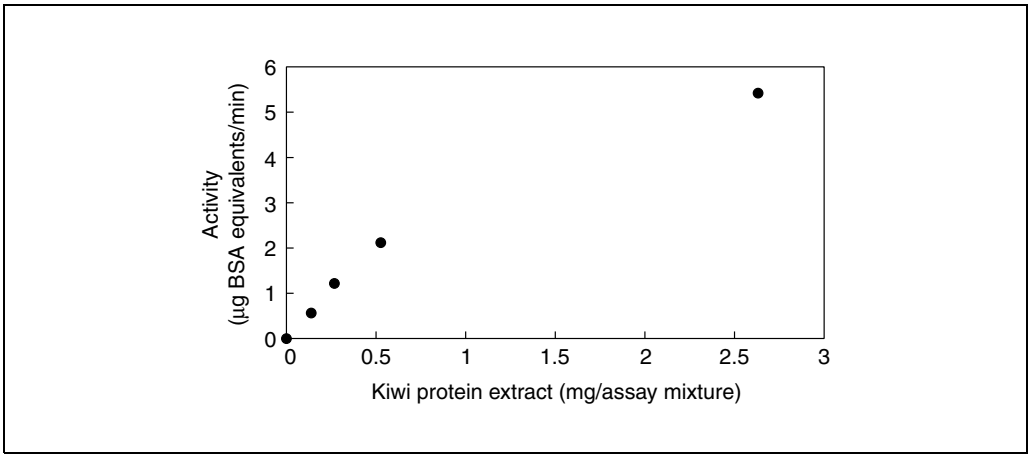

Figure C2.2.4 Enzyme extract activity profiles for raw kiwi fruit. Results were obtained using Basic Protocol 1 with Hammerstein casein substrate (ICN Biomedicals).

buffer (0.1 M NaH_2PO_4, pH 7.0) was used for both enzyme and substrate preparation. Figure C2.2.4 illustrates the relationship between measured peptidase activity and volume of kiwi fruit extract included in the reaction mixture. The relationship is essentially linear at the lower enzyme concentrations (lower amounts of extract added to the reaction mixture). The linear relationship begins to break down at higher enzyme concentrations. The data provide a first estimate of the range of kiwi fruit peptidase activities that are appropriate for analysis using Basic Protocol 1. Clearly, the analyst would want to work with enzyme levels that fall within the linear range of the assay.

The amount of active enzyme associated with a given enzyme preparation is typically reported as units of activity per milliliter, units of activity per milligram, or units of activity per milligram protein. The latter expression (units/mg protein) is referred to as the preparation's specific activity (Segel, 1975). Measured specific activities for the peptidase preparation employed in this experiment are given in Table C2.2.1. Notice that values calculated from data obtained in the linear range of the assay are in good agreement, as would be expected. In contrast, the value associated with the highest enzyme concentration tested is significantly below those obtained at the lower enzyme concentrations—again illustrating the importance of working in the linear portion of the assay.

Time Considerations

A typical assay with the casein or hemoglobin substrates (Basic Protocol 1 or Alternate Protocol) takes ~2 hr following reagent and enzyme preparation. Assays employing the azo-substrates (Basic Protocol 2) are considerably shorter, ~1 hr, since the color development step is not necessary.

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Table C2.2.1 Specific Activity Values for a Single Kiwi Fruit Peptidase Extract Measured at Different Enzyme/Protein Concentrations Using Basic Protocol 1

Enzyme extract (µl/reaction mixture)	Activity[a] (µg BSA eq/min)	Protein[b] (mg/reaction mixture)	Specific activity (µg BSA eq/min/mg protein)
0	0.0	0	0.0
5	0.5	0.32	4.2
10	1.2	0.263	4.6
20	2.1	0.527	4.0
100	5.4	2.63	2.0

[a]Measured as in Basic Protocol 1 with casein substrate.

[b]Measured by the BCA protein assay of Smith et al. (1985).

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This text is devoted solely to peptidase enzymes, although the information can be extrapolated to other systems. The text covers a wide range of topics, including assay methods, mechanisms of action, inhibition, and technological applications of peptidases, in a relatively straightforward manner.

Eisenthal, R. and Danson, M.J. (eds.) 1992. Enzyme Assays: A Practical Approach. Oxford University Press, Oxford.

This text covers the design and execution of enzyme assays. Chapters 1, 9, and 11 ("Principles of enzyme assay and kinetic studies," "Techniques of enzyme extraction," and "Buffers and the determination of protein concentration," respectively) are particularly relevant to this unit.

Whitaker, J.R. 1994. Principles of Enzymology for the Food Sciences, 2nd ed. Marcel Dekker, New York.

A classic text, directed at the food sciences, covering the fundamental principles of enzymology. Chapters covering enzyme purification, pH effects, temperature effects, enzyme inhibitors, and the proteolytic enzymes are particularly relevant to this unit.

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This text is a good source of information on the chemical mechanisms underlying the different modes of peptidase catalysis. Three important enzymes are covered: subtilisin, a serine endopeptidase; papain, a cysteine endopeptidase; and chymosin, an aspartic endopeptidase.

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