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Chymotrypsin Inhibitor

Key Words: Casein; chymotrypsin inhibitor; casein; chymotrypsin determination; chymotrypsin unit; protein degradability; amino acid; peptide availability.

1. Introduction

Similar to the trypsin inhibitor, the chymotrypsin inhibitor also decreases protein degradability in the intestine, resulting in lower availability of amino acids and peptides for production purposes. This adversely affects growth and other productive responses. This inhibitor is also heat labile.

1.1. Present in

Erythrina caffra, Glycine max, Phaseolus lunatus, Arachis hypogaea, Macrotyloma axillare, Vigna unguiculata, Solanum tuberosum, black gram, chick pea, pigeon pea, *Phaseolus vulgaris, Psophocarpus tetragonolobus, Vicia faba, Vigna subterranea, Vigna umbellata*.

1.2. Principle of Assay

The method described here is based on the spectrophotometric determination of the breakdown products of casein at 280 nm produced by a given concentration of chymotrypsin, in the presence and absence of the inhibitor. It is based on the method of Kakade et al. ***(1)***.

2. Materials

1. *Borate buffer, 0.1M, pH 7.6. Stock solution A:* 0.2M solution of boric acid (12.4 g in 1 L of distilled water). *Stock solution B:* 0.05M solution of borax (19.05 g in 1 L of distilled water; 0.2M in terms of sodium borate). 50 mL of solution A +2 mL

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of solution B and dilute to a total of 200 mL with distilled water. Check pH, which should be 7.6.

2. *Casein solution*. Suspend 1 g of casein in 80 mL of 0.1M the borate buffer, pH 7.6, and completely dissolved by heating on a steam bath for 15 min. Cool this solution, adjust the pH to 7.6 and make the volume up to 100 mL by the borate buffer.
3. *Chymotrypsin stock (40 μg/mL)*. Dissolve 4 mg chymotrypsin in 100 mL of 0.001M HCl containing 0.08M $CaCl_2.2H_2O$.
4. *Trichloroacetic acid (TCA) reagent*. Take 18 g of TCA and 18.0 g of anhydrous sodium acetate, add 20 mL of glacial acetic acid, and make the volume up to 1000 mL with distilled water.
5. *HCl (0.001M) containing 0.08M $CaCl_2.2H_2O$*. Dilute 0.09 mL concentrated HCl [37% weight/volume (w/v)] to approximately 900 mL distilled water. To it add 11.76 g $CaCl_2.2H_2O$ and make the volume up to 1 L with distilled water.
6. *NaOH (1M)*. Dissolve 4 g NaOH in 100 mL distilled water.

3. Methods

3.1. Preparation of Extract

Take 1 g of defatted (by using petroleum ether) and ground sample (ground preferably using a ball mill) and suspend in 10 mL of distilled water (*see* **Note 1**). Adjust its pH to 7.6 using 1M sodium hydroxide solution. After shaking for 1 h on a magnetic stirrer, centrifuge (3000 g, 10 min) the suspension.

3.2. Preparation of Calibration Curve

1. Pipette the stock solution of chymotrypsin (0.2 to 1.0 mL) into a triplicate set of tubes (one set for each level of enzyme) and make the volume up to 1.0 mL with 0.001M HCl containing 0.08M Ca^{2+}.
2. Add 1 mL of 0.1M borate buffer (pH 7.6) to each tube, and transfer the tubes to a water bath at 37°C. To one of the triplicate tubes add 6 mL of the TCA reagent (this tube serves as a blank for the other two). Then to the other two tubes in each set add 2 mL of the casein solution prewarmed to 37°C.
3. Allow the tubes to remain at 37°C for exactly 10 min, and then stop the reaction by adding 6 mL of the TCA to the tubes.
4. After allowing it to stand at room temperature for at least 30 min, filter the suspension, and measure the absorbance of the filtrate at 275 nm against the appropriate blank.
5. One chymotrypsin unit (CU) is arbitrarily defined as an increase of 0.01 absorbance unit at 275 nm in 10 min per 10 mL of the reaction mixture under the conditions described here.

3.3. Determination of Inhibitor

1. Take the sample extract (0.25, 0.5, 0.75 mL) into a triplicate set of test tubes (one set for each level of the extract), bring the volume to 1.0 mL with the borate buffer,

and add 1 mL of the stock chymotrypsin. Transfer these tubes to a water bath adjusted at 37°C.

2. To one of the triplicate tubes add 6 mL of the TCA reagent (this tube serves as a blank for the other two). Then to each tube add 2 mL of the casein solution (prewarmed to 37°C). After exactly 10 min, stop the reaction by adding 6 mL of the TCA to the other two tubes.
3. After allowing it to stand at room temperature for at least 30 min, filter the suspension and measure the absorbance of the filtrate at 275 nm against the appropriate blank.
4. The chymotrypsin inhibitor activity is defined as the number of chymotrypsin units inhibited (CUI), and the results can be expressed as CUI per milligram of protein or per gram of the sample. For expressing CUI per milligram of protein, the protein content of the extract can be determined using the method of Lowry et al. ***(2)***. True chymotrypsin inhibitor activity may be obtained by taking different volumes of the sample extract and then extrapolating to zero volume of the inhibitor (sample) solution.

4. Note

1. The 0.1M borate buffer (pH 7.6) can also be used for the extraction of chymotrypsin inhibitor from the samples.

References

1. Kakade, M. L., Swenson, D. H., and Liener, I. E. (1970) Note on the determination of chymotrypsin and chymotrypsin inhibitor activity using casein. *Anal. Biochem.* **33**, 255–258.
2. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.