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Collagenase Assay

Method

Assay methods utilizing labelled collagen have been reported by Gisslow and McBride (1975), Robertson et al. (1972) and Sakamoto et al. (1972). Since true collagenase attacks the helical region of the molecule, change in optical rotary dispersion reflects collagen degradation (Keil et al. 1975). Worthington collagenase products CLS and CLSPA are assayed as described below:

Method: A modification of the procedure of Mandl et al. (1953). Collagenase is incubated for 5 hours with native collagen. The extent of collagen breakdown is determined using the Moore and Stein (1948) colorimetric ninhydrin method. Amino acids liberated are expressed as micromoles leucine per milligram collagenase. One unit equals one micromole of L-leucine equivalents from collagen in 5 hours at 37° C and pH 7.5 under the specified conditions.

Reagents

0.05 M TES [tris(hydroxymethyl)-methyl-2-aminoethane sulfonate] buffer with 0.36 mM calcium chloride, pH 7.5

4% Ninhydrin in methyl cellosolve with 7.1 mM stannous chloride

0.2 M Sodium citrate, pH 5.0

Ninhydrin-citric acid mixture: Prepare by mixing 50 ml of the 4% ninhydrin in methyl cellosolve containing 7.1 mM stannous chloride with 50 ml of 0.2 M citrate pH 5.0. Allow mixture to stir for 5 minutes.

50% n-Propanol

Substrate: Worthington bovine achilles tendon collagen (Code: CL) and vitamin free casein

50% (w/v) Trichloroacetic acid

Enzyme

Dissolve enzyme at a concentration of 1 mg/ml in 0.05 M TES with 0.36 mM calcium chloride, pH 7.5. Dilutions run are 1/10 and 1/20 in the above buffer.

Procedure

Weigh 25 mg of bovine collagen into each of four test tubes. Include at least two tubes to serve as blanks which will contain no enzyme. Add 5.0 ml of 0.05 M TES buffer to the tubes and incubate at 37° C for 15 minutes. Start the reaction by adding 0.1 ml of enzyme dilution to appropriate tubes.

After 5 hours, stop the collagenase reaction by transferring 0.2 ml of solution (leaving behind the collagen) to test tubes containing 1.0 ml of ninhydrin-citric acid mixture. Include an enzyme blank (collagen incubated with 0.1 ml TES buffer in place of enzyme). Heat for 20 minutes in a boiling water bath. After cooling, dilute with 5 ml of 50% n-propanol. Let stand for 15 minutes and read absorbance at 600 nm. From an L-leucine standard curve determine micromoles amino acid equivalent to leucine liberated.

Non-specific protease activity (i.e. caseinase activity) is determined using the above assay and substituting 25 milligrams vitamin free casein for collagen. The reaction is stopped after 5 hours by the addition of 0.5 ml of 50% trichloroacetic acid. After centrifugation, 0.2 ml of the supernatant is transferred to 1.0 ml of ninhydrin and treated as above. Caseinase activity is calculated as collagenase activity.