QUESITI COLLOQUIO - 2024S31

Busta n. 1:

1. Descrivere i principi analitici e le modalità operative di una tipica analisi lipidomica.

Busta n. 2:

1. Descrivere le caratteristiche strumentali più rilevanti per un'analisi metabolomica untarget tramite spettrometria di massa.

> 8% are not uncommon. Caution must be exercised in interpreting increased ACCR values,[57] because elevations have been observed also in *burns, ketoacidosis, renal insufficiency, myeloma, light-chain proteinuria, and march hemoglobinuria,* and following *extracorporeal circulation, large intravenous doses of corticosteroids, duodenal perforations,* and *extraperitoneal surgical procedures.* It is possible that the increased clearance of amylase in these conditions is due to the release of S- or P-type amylase from other tissues. In *macroamylasemia,* ACCR is usually < 2%.

Methods for the Determination of Amylase Activity[4,73,89]

Specimen

The serum enzyme is quite stable; activity loss is negligible at room temperature in the course of a week or at refrigerator temperatures over a two-month period. In urine, an acid pH may make the enzyme less stable; therefore, pH should be adjusted to ~7.0 before storage. With the exception of heparin, all common anticoagulants inhibit amylase activity because they chelate Ca(II); citrate, EDTA, and oxalate inhibit it by as much as 15%. As a consequence, amylase assays should be performed only on serum or heparinized plasma.

Starch-Based Methods[73]

It has been estimated that some 200 methods for the assay of amylase activity have been described, based on nine different principles and various substrates. However, until a decade ago, three main methods (each with several variations) predominated throughout the world, namely, the *saccharogenic,* the *amyloclastic,* and the *chromolytic.* These techniques will be outlined, but first it is necessary to mention the difficulties associated with the amylase substrate. Different starches vary considerably in their proportion of amylose and amylopectin, and the average chain length of the starch molecules will depend on the manner of preparation of the starch and the method used to prepare a solution. Starch does not disperse in water to form a true molecular solution but forms instead a colloidal sol containing hydrated starch micelles of various sizes. The degree of dispersion varies with temperature; at lower temperatures amylase chains aggregate into large micelles. Potato, corn, and Lintner's "soluble" starch have been most commonly used, although pure amylose, amylopectin, and glycogen were preferred by some. Starch sols deteriorate rather rapidly as a result of mold contamination; benzoic acid, sodium azide, or *p*-hydroxypropylbenzoate may be added as a preservative. Sterile (autoclaved) sols keep well for several months. Obviously, starch substrates must be prepared, treated, and stored in a consistent manner.

Saccharogenic Assays

In saccharogenic assays the course of the enzyme reaction is followed by measuring the quantity of reducing materials (sugars, dextrins) formed. Any of the common procedures for measuring reducing substances, such as the Folin-Wu or Somogyi-Nelson methods, may be used. Methods based on the reduction of picrate, ferricyanide, and 3,5-dinitrosalicylic acid and on the anthrone-sugar reaction have also been advocated. The results obtained with these methods are reported in terms of milligrams of total reducing substances formed, expressed as "apparent glucose." The chief reducing sugar present is maltose, which, mass for mass, has about 40% of the reducing capacity of glucose. Quantities of reducing sugars produced are determined in protein-free filtrates of the reaction mixtures. With some starch preparations, it is difficult to obtain clear, nonopalescent filtrates. Opalescence does not affect the assay, provided that the turbidity is due to starch and not due to incompletely precipitated proteins; turbidity due to starch clears up at a later stage of the procedure.

Several automated forms of saccharogenic procedures have been described. In one such method glucose oxidase and catalase are used to destroy endogenous glucose; starch is then added, and after incubation the reducing sugars are determined using the cupric-neocuproine reaction. This type of assay is the most reliable of the older assays but it is very time consuming, the preparation of reproducible starch solutions is difficult (see above), and sample blanks are frequently high. These factors probably account for the 1978 finding that < 3% of all amylase assays done in the United States are performed using the saccharogenic method. The reader is referred to a detailed description of the Somogyi saccharogenic method as modified by Henry and Chiamori.[62] In the last decade, reaction rate saccharogenic assays have been devised; these will be described later in this section along with more recent assay formulations.