Perid Assay of Yeast Invertase

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The enzyme couple between glucose oxidase (EC 1.11.1.7) and peroxidase (EC 1.1.3.4) for the oxidation of perid was first used to assay for glucose by Werner et al. (1970). Sookias (M. J. Sookias, unpublished work) assayed yeast invertase (EC 3.2.1.26) with perid [purchased from the Boehringer Corp. (London) Ltd., London W5 2TZ, U.K.] to determine the quantity of glucose produced by the action of the enzyme on sucrose. He found that the perid reagent (containing the enzyme couple together with the chromagen, perid, in 0.1 M-phosphate buffer, pH 7.0) contained sucrase activity. Woodward & Wiseman (J. Woodward & A. Wiseman, unpublished work) confirmed this, but found that even the use of appropriate substrate, enzyme, and reagent blanks was unsatisfactory, because batches of perid reagent were found to have different sucrase activities, and some gave substrate blank readings that were extremely high.

Commercial preparations of glucose oxidase can contain a variety of possible contaminant carbohydrases. For example, Blecher & Glassman (1962), found that commercial preparations of glucose oxidase had various amounts of sucrase activity. Dahlqvist (1960) found that glucose oxidase preparations had maltase activity and isomaltase activity. Rerup & Lundquist (1967) claimed that this specific method of glucose determination, with glucose oxidase, yielded quantitative reactions with glycogen, starch and maltose. Scharlach et al. (1962), used the glucose oxidase system for the generation of \( \text{H}_2\text{O}_2 \) as an oxidative reagent in experiments with thyroid particles, prepared in 0.25 M-sucrose! They found that the enhancement of organic iodine formation that occurs from the addition of glucose and crude glucose oxidase also occurred in the absence of glucose.

Unless inhibition of the contaminating carbohydrases of glucose oxidase is accomplished, then the assay for glucose is inaccurate in systems where the substrates of these carbohydrases are present.

The use of Tris buffer at alkaline pH has been used to overcome this problem. Larner & Gillespie (1956), noticed the inhibition of maltase and 1,6-oligoglucosidase by several amines including Tris at alkaline pH. Friedman (1960) noted that Tris was inhibitory to maltase contaminants in commercial glucose oxidase, but he made no analytical use of this. White & Subers (1961) used Tris buffer, pH 7.6, to inhibit maltase activity in a glucose oxidase-peroxidase coupled reagent and hence provided a suitable reagent for routine maltase assay. Jørgensen & Andersen (1973) used Tris to inhibit
sucrase activity in a glucose oxidase-peroxidase-coupled assay reagent for determination of yeast invertase activity. None of these used perid.

The perid reagent powder contains the enzyme couple, chromagen and buffer which needs to be diluted with water before use. Pharr & Dickinson (1973) have reported that a convenient dilution procedure was found to decrease β-glucosidase contamination in an assay reagent for glucose determination. Sookias (M. J. Sookias, unpublished work) had noted that if one part of the perid reagent is diluted by a further eight parts of water, glucose could still be measured quantitatively, although with far less sensitivity. Indeed this further dilution of perid also decreased the contaminating sucrase activity. Woodward & Wiseman (J. Woodward & A. Wiseman, unpublished work), however, found that one part of perid reagent diluted by two parts of water was insensitive to glucose concentrations greater than 30 μM.

We have diluted the perid powder with 0.1 M-Tris-HCl buffer, pH 7.2, and used this perid reagent as a routine to determine the quantity of glucose formed by the action of invertase on sucrose. To determine whether Tris affected the sensitivity of perid, we compared the standard curve for glucose perid in Tris with those obtained with three batches of perid reagent (perid 1, 2, and 3), made up to the recommended concentration with water.

The glucose standard curve (Fig. 1) was produced in the range 5.0 × 10⁻² to 26.5 × 10⁻² μmol of glucose in 5.0 ml of perid reagent (10–53 μM). Colour development was carried out at room temperature (18°C) for 25 min, and extinction was measured in the EEL portable colorimeter (OB3 filter, 600–650 nm range). The four standard curves were not significantly different, thus Tris does not affect the sensitivity of perid to glucose.

We also investigated the effect of different sucrose concentrations on the sucrase activity of perid–Tris, perid 1, 2 and 3. For this determination a range of sucrose quantities were pipetted into 5.0 ml of perid reagent and the assay was performed as described above. Fig. 2 shows that the perid reagent had various amounts of sucrase activity, as seen with batches of perid 1, 2 and 3. The use of Tris clearly inhibits the sucrase. Therefore dissolving the perid powder in 0.1 M-Tris–HCl buffer, pH 7.2, provides a suitable assay
reagent for glucose even when sucrose is present, and has provided a good method for the routine assay of yeast invertase, for our studies of its conformational stability.

The reaction mixture found to give a satisfactory assay for invertase activity linear with invertase dilution in the range 0.8–16 units/ml, consists of 0.4 ml of a 50% sucrose solution, 1.5 ml of 0.1 M-acetate buffer, pH 4.7, and 0.1 ml of diluted invertase. The mixture is incubated for 10 min at 25°C after which a 0.1 ml portion is pipetted into 5.0 ml of perid-Tris, pH 7.2 [the reaction is stopped by dilution (M. J. Sookias, unpublished work)]. The colour is developed as described above.

Perid in fact has several advantages over other methods that are used in measuring reducing sugar. It is a relatively harmless reagent, the method is one hundred times more sensitive than the 3,5-dinitrosalicylic acid reagent we have used for reducing sugar determination and it is four times more sensitive than the glucose oxidase-peroxidase coupled reagent with O-dianisidine as the chromagen.


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