Cocktail Ingredients: An Explanation

Extraction

The purpose of extraction is to "freeze" the enzyme in the particular state in which it exists. Extraction breaks down barriers (e.g., walls and membranes) that would prevent access of the enzyme to the substrates that you will provide. There are myriad potentials for artifacts that must be avoided. The extraction cocktail is formulated with these artifacts in mind:

(1) proteolysis—The extract is made and maintained at 0°C, at which proteolytic enzymes are usually less active. (Many products are available for inclusion in raw-extract cocktails to diminish proteolysis. These protease inhibitors are more or less specific for the various classes of proteases.)

(2) denaturation—*p*H is strictly maintained by the buffer tris (base)/tris-Cl, and the protein concentration is maintained above the (arbitrary) threshold of 1 mg mL^{-1}. (For particular enzymes, inclusion of other substances to maintain the native conformation is required. E.g., glycerol may be included at high concentration to cause the protein to aggregate into active oligomers.)

(3) deleterious cations—Some divalent cations, e.g. Cu^{2+}, may cause difficulty during extraction or subsequent assay. It usual practice to include a chelator, such as EDTA, to bind these ions, which, of course, have effect only as the free species.

(4) divalent cations—Many plant enzymes are "protected" during extraction by inclusion of Mg^{2+} or Mn^{2+}.

(5) centrifugation—Particulates (e.g., cell wall, starch) that would interfere with the assay must be removed.

(6) other—Many other protocols are found to be effective for extract preparation. E.g., if one suspects that the phosphorylation state is important, inclusion of a phosphatase inhibitor would be in order.

Assay

The assay-cocktail pre-mix contains tris, again, as a *p*H buffer; BSA, to stabilize the dilute protein in the cocktail and to serve as an alternative substrate for cellular proteases that were released during tissue disruption; and Mg^{2+}, which is required by many enzymes for full activity. In this assay, Mg^{2+} associates with ATP to form the kinase substrate, ATP□Mg.

The substrate for the enzyme of interest, PGal DH, is 1,3-diPGA. This substance, as the free acid, is unstable, and the commercial form (which must be treated by a fairly long and drawn out process) is expensive. For these reasons, we will synthesize it in the assay tube according to the following reaction:

$$\text{3-PGA} + \text{ATP}\square\text{Mg} \xrightarrow[\text{PGAK}]{} \text{1,3-PGA} + \text{ADP}\square\text{Mg}$$

Thus, although we refer to "substrate-dependence" as "PGA-dependence," the real substrate is 1,3-diPGA and "PGA-dependence" is fitting only because PGA, ATP, Mg^{2+}, and the analytical enzyme PGAK synthesize the real substrate.

In the direction assayed, PGal DH has two substrates, namely, 1,3-diPGA, as discussed, and NADPH. NADPH also serves as the assay indicator.