during freezing and/or during drying. As a measure of the degradation during freezing, "freeze-thaw" stability studies are carried out, and to estimate (roughly) the degradation during drying, stability during freeze-drying is compared to stability during freeze-thaw. The basic assumption is that degradation during thawing is comparable to degradation during reconstitution, and therefore the difference in activity between a freeze-dried-reconstituted sample and a freeze-thawed sample is a measure of the loss in activity during drying. This assumption is likely a reasonable approximation for a fast thawing process, at least if air bubbles released during thawing are not a major factor. One observation of particular significance is that some excipients stabilize during both freezing *and* drying (denoted "lyoprotectants"), while others stabilize only during freezing (denoted "cryoprotectants") (26,30,31). Data for phosphofructokinase (PFK), active as a tetramer, illustrate this observation quite well (Fig. 4). With no "stabilizer" added to the formulation, PFK is completely deactivated during freeze-thaw. With all stabilizers at the relatively high level of 0.5 M, some stabilize reasonably well during freeze-thaw but offer no protection during freeze-drying (proline and trimethylamine *N*-oxide). The disaccharides (trehalose, sucrose, and maltose) stabilize both during freezing and drying, and therefore, at a level of 0.5 M, are effective lyoprotectants. Low levels of disaccharides are not good cryoprotectants (31) and therefore cannot be good lyoprotectants. Of course, if a given formulation offers no protection during freezing, any potential protection during drying will be invisible with the usual experimental design. Polyethylene glycol (PEG) is found to be an exceptionally efficient cryoprotectant for PFK but offers no protection during drying (31). Trehalose and glucose at low levels ($\approx$ 0.1 M) offer essentially no protection during freeze-drying because, at low levels, they are not cryoprotectants. However, when PEG is used in combination with a low level of trehalose (or glucose), nearly complete stabilization during freeze-drying is

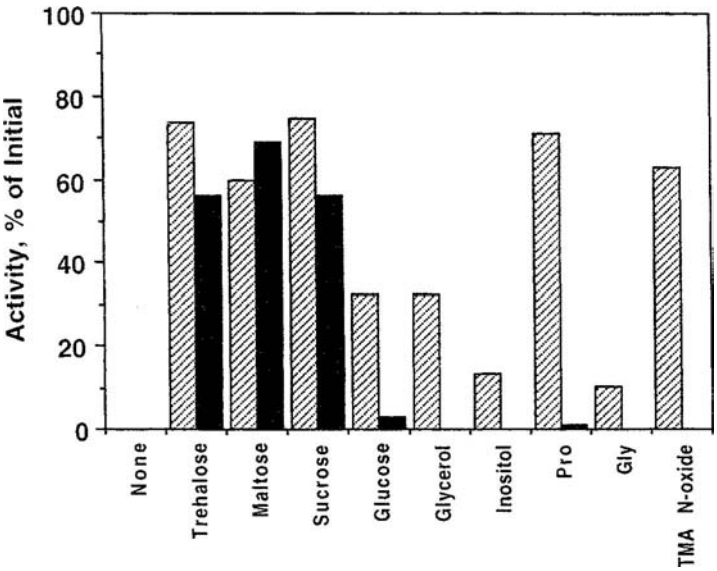

FIGURE 4 Comparison of freeze-thaw stability with freeze-dry stability: phosphofructokinase with additives at 0.5 M. Key: shaded bar = freeze-thaw, solid bar = freeze-dry. *Source*: Data from Ref. 26.