An important and often overlooked aspect of Timasheff's mechanism is the role of solid phase chemical potentials. Adding protective excipients to a protein solution increases the chemical potential of both the protein's native and denatured state. There is a limit to how high the chemical potential can be raised, however. This is because the *solid phase* chemical potentials of the protein are largely unaffected by the addition of excipients to the liquid solution. Thus, once the chemical potential of the native state is increased to a value greater than that of the solid state, precipitation of the protein will be favored. Protein precipitated will be at high concentration; this may pose problems due to aggregation upon drying and/or storage. Thus, there is a clear trade-off between conformational stability of the protein, which is increased with increasing cryoprotectant concentration, and stability against aggregation, which is decreased with increasing cryoprotectant concentration.

Finally, it is important to consider mechanistically how to explain the much greater potency of PEGs as cryoprotectants relative to other compounds such as sucrose. The data for one case, which are shown in Figure 9 and Table 1, illustrate this point. Figure 9 compares cryoprotection of LDH by PEG 8000, PEG 400, and sucrose (MW 342). LDH is completely protected during freeze-thawing

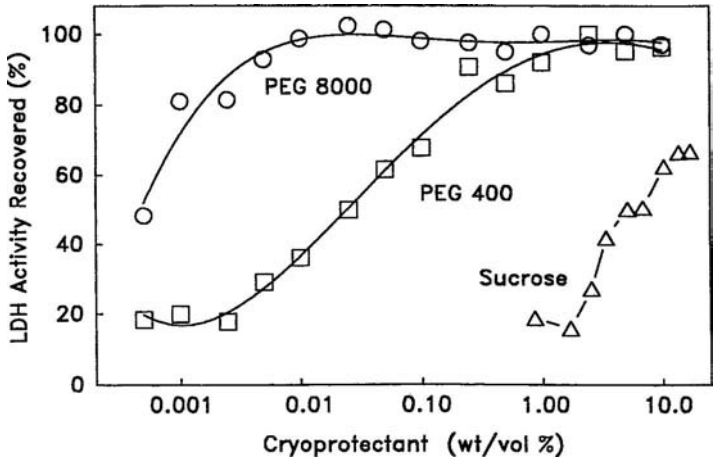

FIGURE 9 Effects of polyethylene glycols (PEG) and sucrose on lactate dehydrogenase stability during freeze-thawing. *Source*: Data taken from Refs. 59 and 70.

TABLE 1 Parameters for Solute Interactions with Chymotrypsinogen

Solute	Concentration	$(\delta m_3/\delta m_2)_{\mu 1,\ \mu 3}$	$(\delta \mu_3/\delta m_3)_{m2}$[a]	$(\delta \mu_2/\delta m_3)_m$[b]
Sucrose[c]	1.27 m	−10.35	0.56	5.7
PEG 400[d] (0.27 m)	10% wt/vol	−6.87	2.42	16.6
PEG 6000[d] (0.0017m)	1% wt/vol	−0.62	480.00	297.6

[a]kcal/mol of solute/mol of solute in 1000 kg H_2O.
[b]kcal/mol of protein/mol of solute.
[c]Data taken from Ref. 83.
[d]Data taken and calculated from Ref. 84.