by PEG 8000 at concentrations of $\geq$0.01% (wt/vol). In contrast, full protection in the presence of PEG 400 is not realized until the concentration is at least 2.5% (wt/vol). On a weight percentage basis, PEG 8000 is 250-fold more potent as a cryoprotectant. On a molar basis, the higher molecular weight PEG is 5000-fold more potent. Sucrose is much less effective than even PEG 400. Even at sucrose concentrations as high as 10% (wt/vol) the protein is not fully protected.

In the past, we have ascribed these differences in protein stabilization to Timasheff's thermodynamic mechanism. The only protein for which the needed thermodynamic parameters have been measured in the presence of all three cryoprotectants is chymotrypsinogen (83,84) (Table 1). Although these data are not directly applicable to LDH, the general trends shown should be relevant to any protein. The increase in chymotrypsinogen chemical potential, $(\delta\mu_2/\delta m_3)_{m_2}$, in the presence of either of two different molecular weights of PEG (e.g., $M_r = 400$ or 6000) is greater than that noted in the presence of the sucrose, even though the PEGs are excluded to a lesser degree, on a per mole of solute basis. Comparing the two PEG molecules indicates that the larger the PEG the less it is excluded on a mole basis, but the more it increases protein chemical potential.

The basis for these observations can be explained by examining equation (20). The other major component in determining the effect of solute on protein chemical potential is the self-interaction parameter for the solute, $(\delta\mu_3/\delta m_3)_{m_2}$. The value for this parameter is several-fold greater for PEG 400 and almost three orders of magnitude greater for PEG 6000 than that for sucrose. The self-interaction parameter is given by

$$\left(\frac{\delta\mu_3}{\delta m_3}\right)_{m_2} = \left[\left(\frac{RT}{m_3}\right) + RT\left(\frac{\delta \ln \gamma_3}{\delta m_3}\right)_{m_2}\right] \tag{2}$$

where γ_3 is the activity coefficient of the solute and R is the universal gas constant (reviewed in Refs. 4, 78, and 79). The molal concentrations needed for preferential exclusion of PEG are very small and the activity coefficient of PEG is quite large, relative to values for sucrose. Therefore, the self-interaction parameter for PEG is very large compared to that for sucrose. In addition, as the size of PEG increases there is a great increase in such nonideality (Table 1).

This argument does indeed support the contention that on a per-mole basis PEG is much more effective than sucrose at increasing protein chemical potential. And for cases where relatively high concentrations of PEG (e.g., >1% wt/vol) are needed to confer cryoprotection, the Timasheff mechanism may be applicable. However, it seems unlikely that a PEG concentration of 0.01% (wt/vol) would have a significant effect on the thermodynamics of the system. This is because the actual parameter of interest is the transfer free energy of the native versus denatured protein from water into cryoprotectant solution. The difference between the values for the two states determines the magnitude of the effect on the free energy of denaturation. The transfer free energy is obtained by integrating equation (1) from zero to the molal concentration of cryoprotectant of interest. With PEG 8000 at 0.01% wt/vol concentration, the molality is so low that the calculated transfer free energy would be extremely small. Thus, the effect on the free energy of denaturation would be trivial.

So, how can we account for the potent protection afforded by PEGs? The concentration range for cryoprotection is actually very similar to that seen with nonionic surfactants such as Tweens. PEGs have been shown to be surface