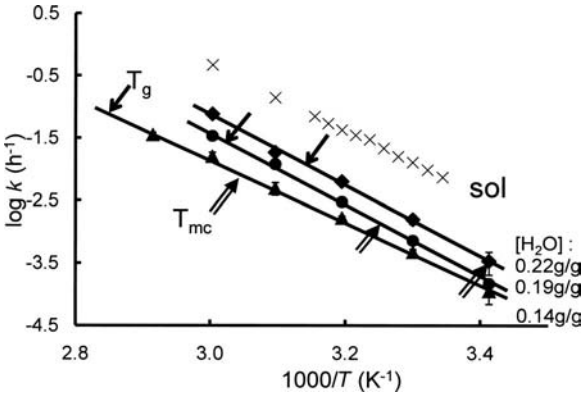


**FIGURE 14** Temperature dependence of cephalothin hydrolysis in freeze-dried formulation containing dextran at 23% (▲), 60% (●), and 75% RH (◆).

temperature dependence of the apparent first-order rate constant is linear at all humidities in a manner similar to that of hydrolysis in aqueous solution, regardless of their $T_{mc}$ indicated by arrows in the figure. The temperature dependence is also unaffected by the $T_g$ of the formulations that are approximately 20°C higher than the $T_{mc}$. Since the translational mobility of drug and water molecules in freeze-dried formulations is affected by $T_g$ and/or $T_{mc}$, the hydrolysis rate should be affected by $T_g$ and/or $T_{mc}$ if the translational diffusion of the drug and/or water molecules is rate limiting. The absence of a break in temperature dependence around $T_g$ and $T_{mc}$ suggests that the translational diffusion is not rate limiting. Since the translational diffusion of water can be considered to be much faster than that of the larger cephalothin molecule, the diffusion barrier of water molecules may be smaller than the chemical activational barrier. This interpretation is supported by the finding that the activation energy for the hydrolysis of cephalothin in the freeze-dried formulations containing dextran (between 23 and 26 kcal/mol) is close to the apparent activation energy obtained for hydrolysis in solution (24 kcal/mol). Because of the small diffusion barrier of water in freeze-dried formulations compared to the activational barrier, the hydrolysis rate of cephalothin is not affected by $T_g$ and/or $T_{mc}$, even if the translational mobility of water molecules changes around $T_g$ and/or $T_{mc}$.

### Correlations Between Storage Stability and Fast Dynamics as Determined by NMR Relaxation Times

Protein aggregation, one of the most common degradation pathways of freeze-dried protein formulations, also appears to be closely related to the structural relaxation as reflected by NMR relaxation times. Figure 15 shows the temperature dependence of the time required for 10% protein aggregation ($t_{90}$) in freeze-dried β-galactosidase formulation containing methylcellulose (43). At 60% RH, the slope changes around the $T_g$ measured by NMR ($T_{mc}$). No change in temperature dependence of $t_{90}$ is observed at 12% RH, at which the $T_{mc}$ is higher than the highest temperature for the measurement.

Apparent correlation between protein aggregation rate and structural relaxation is also observed for β-galactosidase freeze-dried with sugars. As shown in Figure 16, the slope of $t_{90} - T_g/T$ plots changes at around $T_g$, suggesting that aggregation rate is related to structural relaxation (33). However,