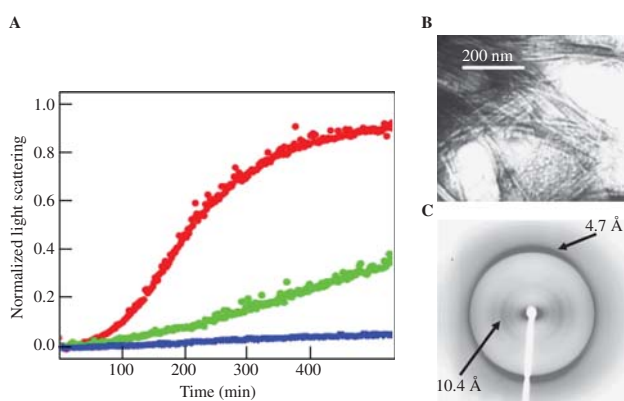


**FIGURE 39.2** Effects of the mutations on the stability and global cooperativity of lysozyme. (A) Thermal unfolding monitored by far-UV CD at 228 nm (upper panel) and by ANS fluorescence (lower panel) of the I56T (●), the D67H (●), the T70N (●), and the wild-type (●) lysozymes. The lysozyme concentration was 0.2 mg/mL in 0.1 M acetate buffer pH 5.0. (B) Electrospray mass spectra of an equimolar mixture of [ $^{14}\text{N}$ ]I56T variant and  $^{15}\text{N}$ -labeled wild-type lysozyme (upper panel) and of  $^{15}\text{N}$ -labeled I56T and  $^{14}\text{N}$ -labeled T70N variant (lower panel). Mass spectra were recorded following exposure to hydrogen exchange conditions at pH 8.0 and 37°C for periods of time ranging from 0.4 to 3600 s. The four proteins were exposed to  $\text{D}_2\text{O}$  initially to replace all the labile hydrogens with deuterium atoms; the exchange process therefore involved subsequent replacement of these deuterium atoms with hydrogen atoms from the solvent  $\text{H}_2\text{O}$ . The peaks observed in spectra of control samples recorded after complete H/D exchange are shown in black. The peaks colored red (I56T and WT) and green (T70N) arise from the gradual loss of deuterium during the course of exchange that occurs via an EX2 mechanism due to local fluctuations [12,28,31]. The peaks colored yellow were observed in the spectra of the I56T variant but not in that of the T70N and the wild-type lysozyme. They result from a transient and cooperative unfolding event that gives rise to exchange by an EX1 mechanism. Note that in these experiments, the D67H variant has been found to behave like the I56T variant [31]. (C) Ribbon diagram of the lysozyme, showing in green regions of the protein involved in the transient cooperative unfolding event observed for the I56T and D67H variants as determined by real-time H/D exchange experiments analyzed by MNR [28, 31]. The 3<sub>10</sub>- and  $\alpha$ -helices are labeled A to D, and the four disulfide bonds are shown in blue.



**FIGURE 39.3** (A) Time course of the aggregation of the I56T (upper curve), the T70N (middle curve), and the wild-type (lower curve) lysozyme, as monitored by light scattering. Lysozyme solutions (0.1 mg/mL) were incubated at 48°C in 0.1 M citrate buffer pH 5.5 containing 3 M urea. Note that in these experiments, the D67H variant behaves like the I56T protein. (Adapted from [12].) (B) Representative image of fibrils formed from the D67H variant as produced by transmission electron microscopy. (C) X-ray diffraction pattern of the same type of fibril showing a prominent meridional reflection at 4.7 Å and an equatorial reflection at 10.4 Å, features typical of the cross- $\beta$  structure of amyloid fibril. (Adapted from [29].)