Actin oligomers at the initial stage of polymerization induced by increasing temperature at low ionic strength: Study with small-angle X-ray scattering

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Using small-angle X-ray scattering (SAXS), we have studied the initial stage (nucleation and oligomerization) of actin polymerization induced by raising temperature in a stepwise manner from 1°C to 30°C at low ionic strength (4.0 mg ml–1 actin in G-buffer). The SAXS experiments were started from the mono-disperse G-actin state, which was confirmed by comparing the scattering pattern in q- and real space with X-ray crystallographic data. We observed that the forward scattering intensity I(q→0), used as an indicator for the extent of polymerization, began to increase at ~14°C for Mg-actin and ~20°C for Ca-actin, and this critical temperature did not depend on the nucleotide species, i.e., ATP or ADP. At the temperatures higher than ~20°C for Mg-actin and ~25°C for Ca-actin, the coherent reflection peak, which is attributed to the helical structure of F-actin, appeared. The pair-distance distribution functions, p(r), corresponding to the frequency of vector lengths (r) within the molecule, were obtained by the indirect Fourier transformation (IFT) of the scattering curves, I(q). Next, the size distributions of oligomers at each temperature were analyzed by fitting the experimentally obtained p(r) with the theoretical p(r) for the helical and linear oligomers (2–13mers) calculated based on the X-ray crystallographic data. We found that p(r) at the initial stage of polymerization was well accounted for by the superposition of monomer, linear/helical dimers, and helical trimer, being independent of the type of divalent cations and nucleotides. These results suggest that the polymerization of actin in G-buffer induced by an increase in temperature proceeds via the elongation of the helical trimer, which supports, in a structurally resolved manner, a widely believed hypothesis that the polymerization nucleus is a helical trimer.

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