Determination of the size of folding nuclei of protofibrils from the concentration dependence of the rate and lag-time of their formation

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In this work a kinetic model of the process of formation of amyloid protofibrils is suggested which allows calculation of the size of the nuclei using only kinetic data. In addition to the stage of primary nucleation, which is believed to be present in many protein aggregation processes, the given model includes both linear growth of protofibrils (proceeding only at the cost of attaching of monomers to the ends) and exponential growth of protofibrils at the cost of growth from the surface, branching, and fragmentation with the secondary nuclei. Theoretically, only the exponential growth is compatible with the existence of a pronounced lag-period (which can take much more time then the growth of aggregates themselves). According to our theory, one can distinguish some mechanism of growth on the basis of kinetic data. Thus, the small (<0.2) value of L_{rel} (the lag-time to the growth time ratio) together with independence of L_{rel} on the total concentration $M_{\sum}$ of protein in solution determines applicability of the linear regime of growth, and in case of inapplicability of the latter consideration of the exponential mechanism of growth is required [1]. For insulin and LysPro insulin we have $L_{rel} \approx$ 2-3, which means that it is definitely an exponential growth scenario. One can roughly define three types of possible mechanisms of exponential growth: growth from the surface, bifurcation, and fragmentation. Despite the difference of the processes, in kinetic experiment fragmentation and bifurcation show a very similar behavior if the size of the secondary nucleus is equal to zero. According to our theory, there is no chance to distinguish between these two scenarios of exponential growth without direct experiments demonstrating what exact scenario takes place in the given case. However, the behavior of kinetic dependencies in the case of growth from the surface scenario differs from that in the fragmentation/bifurcation scenario [2].

One of the conclusions that can be made from our theory is that the dependence of $\ln T_2$ (T_2 being the transition time) on $\ln[M_\Sigma]$ in the case of exponential growth must be linear and moreover, in the case of growth from the surface its tangent coefficient $k=\frac{d(\ln T_2)}{d(\ln[M_\Sigma])}$ must be -1, while in the case of the bifurcation scenario (in our case the branching process was determined to be the primary one, according to the results on Electron Microscopy data) it must be $-\frac{n_2+1}{2}$ where n_2 is the size of the secondary nucleus.

The experiments support our theory resulting in linear dependence of $\ln T_2$ on $\ln[M_\Sigma]$. Calculations of the tangent coefficient give -0.52±0.13 for insulin and -0.40±0.51 for LysPro insulin which means that, according to our theory, $n_2 = -1 - 2\frac{d(\ln T_2)}{d(\ln[M_\Sigma])}$ is very close to zero for the "bifurcation" scenario.

In accord with the developed theory and the experimental data we obtained that the size of the primary nucleus is equal to one monomer and the size of the secondary nucleus is zero in both insulin and LysPro insulin.

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2. O.M. Selivanova et al. (2014) How to Determine the Size of Folding Nuclei of Protofibrils from the Concentration Dependence of the Rate and Lag-Time of Aggregation. II. Experimental Application for Insulin and Lys-Pro Insulin: Aggregation Morphology, Kinetics and Sizes of Nuclei. *J. Phys. Chem.*, 118:1198-1206.