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Supplemental Information

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SUPPORTING MATERIAL

The current paper is devoted to aggregation of sw ApoMb and its mutated forms under non-denaturing conditions. It is shown that the major factor that determines both aggregation propensity and characteristics of the formed aggregates is stability of the protein native state. Here we show additional experimental data and describe standard methods for investigation of protein folding and mathematical data processing, as well as peculiarities of these methods used in our study.

To confirm that the heat-denatured state of sw ApoMb is an intermediate state we compared the melting curves obtained by far UV CD and calorimetry (Fig. S1). Since the calorimetric technique is restricted to the N→I transition for this protein, the coincidence of the melting temperature obtained by both methods indicates that the melting of sw ApoMb is a transition from its native state to intermediate state.

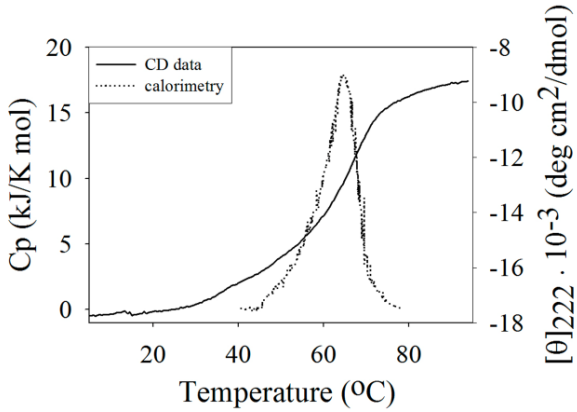

FIGURE S1 Melting curves of WT sw ApoMb monitored by far UV CD and differential scanning microcalorimetry.

To confirm that the estimation of sw ApoMb and its mutant forms structure stability in accordance with the melting temperatures and on the basis of populations of the N and I states at pH 5.5 and 40°C give a similar results we plotted the dependence of T_m on f_I and f_N. Calculated values of r and P are represented in fig. S2 and report that the T_m values correlate with f_I and f_N.

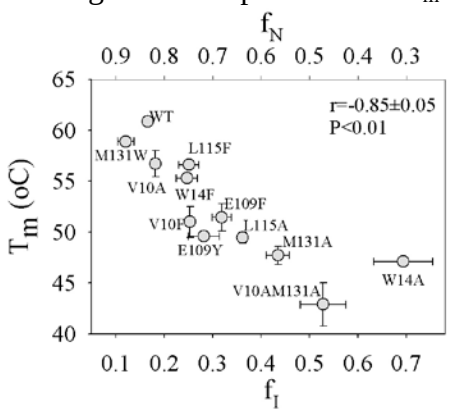

FIGURE S2 Dependence of the melting temperature (T_m) at pH 5.5 of WT sw ApoMb and its mutant forms on fractions of the intermediate f_I and the native states f_N at 40°C and pH 5.5.

For additional confirmation that aggregates formed by WT sw ApoMb and its mutant forms exhibit amyloid structure properties, the method of ThT fluorescence was used. The increase in ThT fluorescence after protein incubation at 40°C indicates that the formed aggregates contain the cross β-structure (Fig. S3).

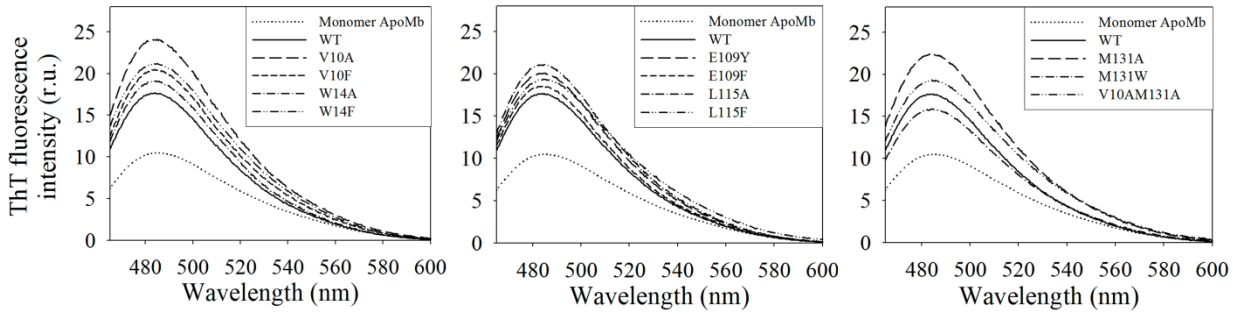

FIGURE S3 ThT fluorescence spectra of WT sw ApoMb and its mutant forms after incubation at 40°C, the spectrum of WT ApoMb before incubation presented for comparison (Monomer ApoMb).

The aggregation propensity of WT sw ApoMb and its mutant forms was determined using the staining intensity of the monomeric gel band in the electrophoregram of native electrophoresis. To be able to quantify the amount of formed aggregates according to the electrophoresis data, it was necessary to plot the concentration dependence of the gel band staining intensity and determine the shape of this dependence. Electrophoregram of monomeric sw ApoMb applied onto the slots in sequential concentrations decrease from 1.0 to 0.2 mg/ml is represented in fig. S4 *a*, all samples applied onto the slots were equal in volume. As follows from Fig. S4 *b*, the staining intensity of the monomeric sw ApoMb band shows linear dependence on the protein concentration; this helps much in quantifying its aggregation propensity.

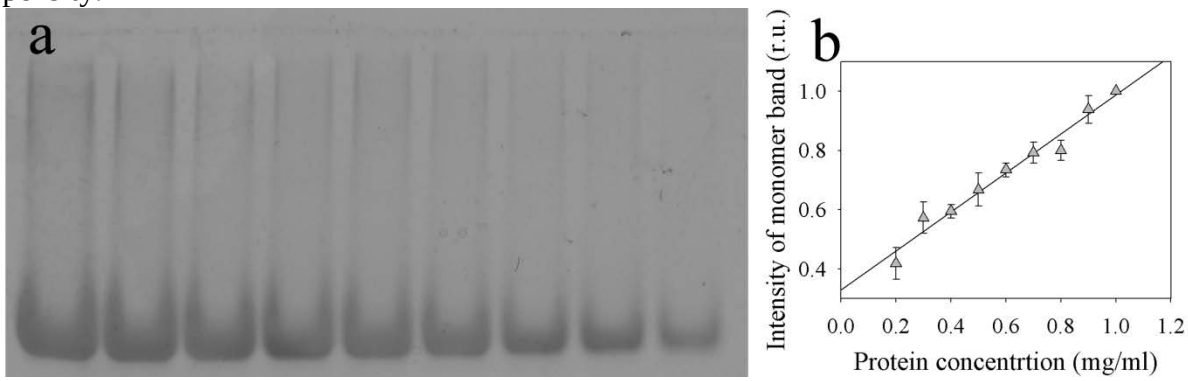

FIGURE S4 Monomer concentration dependence of the gel band staining intensity: *(a)* electrophoregram of monomeric sw ApoMb applied onto the slots in sequential concentrations decrease from 1.0 to 0.2 mg/ml; *(b)* calculated gel band staining intensity as a function of concentration of monomeric protein applied onto the slots. The monomer band staining intensities are normalized to that of sample with the concentration 1 mg/ml.

CD

Sample preparation for acidic equilibrium unfolding of WT sw ApoMb and its mutants was performed as follows: sw ApoMb was dissolved in sodium acetate buffer (NaAc), pH 6.2 (to preserve its native state), and divided into aliquots where the required pH was reached by addition of the appropriate amount of HCl.

Molar ellipticity [θ] was calculated according to the formula:

$$[\theta]_\lambda = [\theta]_\lambda \times MRW/l \times c, \qquad \text{(Eq. S1)}$$

where θ_λ is the ellipticity value measured at a wavelength of λ, mdeg; MRW is the average residue molecular weight calculated from the amino acid sequence; l is the optical path length, mm; c is the protein concentration, mg/ml. All experiments were carried out at 11°C in a buffer

system with 0.01 M NaAc. For every mutant protein, two independent experimental unfolding curves were measured.

Calculation of the native- and intermediate state fractions

Populations of the intermediate- and native states at 40°C and pH 5.5 were estimated using both the data on heat melting curves and acidic pH-induced equilibrium unfolding transitions at 11°C. The former allowed calculating conformational state populations at 11°C and pH 5.5, and then the fractions of the two states were estimated using the data on protein melting at 40°C. As it follows from acidic pH-induced equilibrium unfolding transitions, at pH 5.5, sw ApoMb molecules are in the native-like or intermediate states, but never of the sw ApoMb mutants undergo the I-U transition. Therefore, for our calculations we used the formulae for one-stage transitions (1):

$$f_{I(11℃,\mathrm{pH5.5})} = (\theta_{\mathrm{N}} - \theta_{\mathrm{pH5.5}})/(\theta_N - \theta_I), \quad \text{(Eq. S2)}$$

$$f_{N(11℃,\mathrm{pH5.5})} = 1 - f_{I(11℃,\mathrm{pH5.5})}, \quad \text{(Eq. S3)}$$

where $f_{N(11℃,\mathrm{pH5.5})}$ and $f_{I(11℃,\mathrm{pH5.5})}$ are fractions of the native- and intermediate states observed at 11°C and pH 5.5, respectively; θ_N and θ_I are the values of molar ellipticity for the native- and intermediate states, respectively, obtained from acidic pH-induced equilibrium unfolding transitions, deg cm^2/dmol; $\theta_{\mathrm{pH5.5}}$ is the value of molar ellipticity at pH 5.5, deg cm^2/dmol.

Then, basing on the results on protein heat melting at pH 5.5, we calculated fractions of the native- and intermediate states at 40°C. Since a melted conformation of sw ApoMb is its intermediate state, in these calculations sw ApoMb melting is taken to be a one-stage N-I transition. On the heat melting curve there are two base lines: one of these is observed at high temperature and consistent with the heat-denatured state, while the other is for the range from 5°C to 20°C and is consistent with the values of $f_{N(11℃,\mathrm{pH5.5})}$ and $f_{I(11℃,\mathrm{pH5.5})}$ calculated from the pH-induced unfolding transitions. Therefore, populations of the conformational states occurring at 40°C and pH 5.5 were calculated on the basis of heat melting curves using the following formulae:

$$f_{I(40℃,\mathrm{pH5.5})} = f_{I(11℃)} + [(1 - f_{I(11℃)}) \times ((\theta_{11℃} - \theta_{40℃})/(\theta_{11℃} - \theta_T))], \quad \text{(Eq. S4)}$$

$$f_{N(40℃,\mathrm{pH5.5})} = 1 - f_{I(40℃,\mathrm{pH5.5})}, \quad \text{(Eq. S5)}$$

where $f_{N(40℃,\mathrm{pH5.5})}$ and $f_{I(40℃,\mathrm{pH5.5})}$ are fractions of the native- and intermediate states at 40°C and pH 5.5, respectively; $f_{I(11℃)}$ is the intermediate state fraction at 11°C; θ_T is the molar ellipticity value of the heat-denatured state, deg cm^2/dmol; $\theta_{11℃}$ is the molar ellipticity value on the base line in the 5-20°C range, deg cm^2/dmol; $\theta_{40℃}$ is the molar ellipticity value at 40°C, deg cm^2/dmol.

Calculation of experimental errors in parameter measurements

The mean square errors of the data presented at the Table 1 were calculated as follows:

$$S = (\sum(\bar{x} - x_i)^2/(n-1))^{1/2}, \quad \text{(Eq. S6)}$$

where S is the mean square error;

$\bar{x}$ is the average value of the measured parameter: $x = \sum_{i=1}^{n} x_i /n$;

n is the number of measurements taken.

Supporting References

1. Pace, C. N. 1986. Determination and analysis of urea and guanidine hydrochloride denaturation curves. Methods Enzymol. 131:266-280.

Acknowledgements

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