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SUPPORTING INFORMATION

The existence of metastable intermediate lysozyme conformation highlights the role of alcohols in altering protein stability

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Transition between two states monitored by the first moment of emission spectra.

The transition between two conformational states of a protein is usually monitored as a function of temperature by using different spectroscopic techniques, such as circular dichroism or fluorescence. The classical assumption is that the total spectroscopic signal S(E,T), at emission energy E and temperature T, results from the the linear combination of the signals due to states 1 and 2, weighted by the fractions (f_1, f_2) of the population of each state:

$$S(E,T) = f_1 S_1(E, T) + f_2 S_2(E, T) \qquad [A1]$$

where:

$$f_1 = Z^{-1} ; \qquad f_2 = Z^{-1} \exp(-\Delta G_{12}/ k_B T) ; \qquad Z = 1 + \exp(-\Delta G_{12}/ k_B T) \qquad [A2]$$

$$\Delta G_{12} = \Delta H_{12} + \Delta C_{12} (T - T_{12}) - T \Delta H_{12} / T_{12} - T \Delta C_{12} \ln(T /T_{12}) \qquad [A3]$$

In the latter expressions, as specified in the main text, ΔG_{12} is the free energy of the transition from state 1 to state 2, T_{12} is the midpoint temperature, H_{12} is the van't Hoff enthalpy at T_{12} , and C_{12} is the difference in the heat capacity between the two states.

In the case of fluorescence experiments, each signal is due to the normalized emission spectrum $P_i(E, T)$ and to the quantum yield Y_i (for i=1,2): $S_i(E,T) = Y_i P_i(E,T)$.

The first moment of the total signal M_T is:

$$M_T = \int E\, S(E,T)\, dE \,/\, \int S(E,T)\, dE = [\, M_1 Y_1 f_1 + M_2 Y_2 f_2 \,] / [\, Y_1 f_1 + Y_2 f_2 \,]$$

$$= [\, M_1 f_1 + q M_2 f_2 \,] / [\, f_1 + q f_2 \,] \qquad [A4]$$

where $q = Y_2 / Y_1$ is the ratio between the quantum yields of the two states, and M_i (for i=1,2) are the first moments of the emission spectra of the two states: $M_i = \int E\, P_i(E,T)\, dE$

The latter expression would be exactly equivalent to expression 1 of the main text only if the quantum yields of the two states were equal, which is not likely to occur. In order to restore the equivalence, we can rearrange this expression by defining an equivalent free energy ΔG_{12}^*:

$$f_1^* = Z^{*-1} ; \qquad f_2^* = Z^{*-1} \exp(-\Delta G_{12}^* / k_B T) ; \; Z^* = 1 + \exp(-\Delta G_{12}^* / k_B T) \qquad [A5]$$

$$\Delta G_{12}^* = \Delta G_{12} - k_B T \ln(q) \qquad [A6]$$

One obtains the following expression which is equivalent to the equation 1 of the main text and resemble the typical linear expression used for any spectroscopic signal:

$$M_T = M_1 f_1^* + M_2 f_2^* \qquad [A7]$$

Now this equivalence becomes an identity for the purpose of numerical fitting, if we can take the approximation $\Delta G_{12}^* \sim \Delta G_{12}$. This approximation holds if the entropic terms are much larger than $k_B \ln(q)$, that is:

$$\log q \ll |\Delta H_{12}| / (k_B T_{12} \ln 10) \quad \text{or} \quad \log q \ll |\Delta C_{12}| / (k_B \ln 10) \qquad [A8]$$

Now, for a transition occurring at observable temperatures, we may consider the extreme case of T_{12} = 100 °C and $\Delta C_{12} = 0$, thus obtaining:

$$\log q \ll 0.14\, \Delta h \qquad [A9]$$

where Δh is the numerical value of ΔH_{12} in kJ mol^{-1}.

In an unfolding (or partial unfolding) experiment, the typical values of van't Hoff enthalpy are of

a few hundreds of kJ mol^{-1}. Therefore, the correction term would be significant only if the quantum yields of the two state were different by several orders of magnitude, which is very unlikely and typically not observed. In the case of a very moderate conformational transition (like in the transition that we have studied between the intermediate and the unfolded state), we may argue to have much lower values such as a few tens of kJ mol^{-1}. In such a case one would typically expect a much closer resemblance of the quantum yields of the two states. However, the correction term is still not significant.

In summary, we have pointed out (and demonstrated) that the first moment of the emission spectra can be safely written as a linear combination of the first moments of the different species involved, in the case of our experiments and quite generally for most of the typical protein conformational transitions.

Experimental methods: Atomic force microscopy (AFM).

For the aggregation experiments, the samples of lysozyme (20 mg/ml) were incubated at 65 °C for 1, 3 or 20 days, and then filtered by a sterile gauze to remove the huge white lysozyme aggregates. Part of the incubated and gauze filtered samples were used for AFM imaging. A few microliters of the solution were dropped onto a freshly cleaved mica substrate. After a few minutes, the sample was washed dropwise with Millipore SuperQ water, and then dried with a gentle stream of dry nitrogen. Images of the incubated lysozyme samples were recorded with a Multimode Nanoscope V Atomic Force Microscope (Veeco Instruments, Santa Barbara, CA, USA), operating in tapping mode. We used rigid cantilevers (model RFESP) with resonance frequencies of about 85 kHz, and equipped with single crystal silicon tips with a nominal radius of curvature of 5 to 10 nm. Typical scan size was 500 x 500 nm^2 (512 x 512 points), and line scan rate was 0.5 Hz.

AFM experiments.

Lysozyme has been found to form amyloid fibrils in the presence of TFE upon incubation at high temperatures (Krebs et al. 2000). We performed AFM experiments in order to check if the enhanced aggregation propensity at 10-15% TFE is consistent with the formation of amyloid fibrils. In most cases, the AFM images display an amorphous clustering of aggregates with a size of the orders of tens of nanometers. In one case, we observed an elongated amyloid-like structure 1 μm long and 20 nm thick. Since at 65 °C the intermediate state is more populated at a TFE concentration of 10 or 15 %, these results suggest that this metastable intermediate state is also prone to favor lysozyme aggregation (and maybe fibrillation).

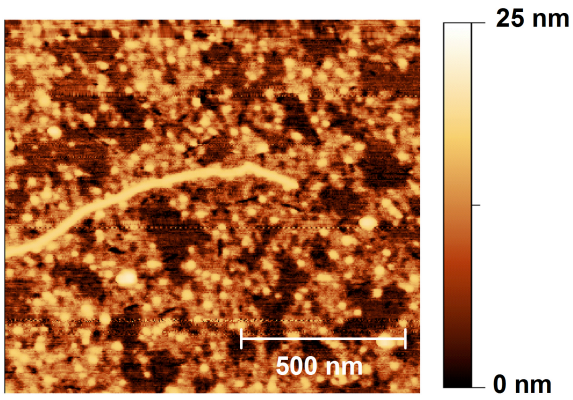

FIGURE A1. AFM image of a lysozyme sample

with 15% TFE upon incubation at 65 °C for 20 days.

REFERENCES

Krebs, M. R. H.; Wilkins, D. K.; Chung, E. W.; Pitkeathly, M. C.; Chamberlain, A. K.; Zurdo, J.; Robinson, C. V.; Dobson, C. M. J. Mol. Biol. 2000, 300, 541–549.