LETTER

Is pressure-induced signal loss in NMR spectra for the Leu99Ala cavity mutant of T_4 lysozyme due to unfolding?

In PNAS, Nucci et al. (1) present an NMR study suggesting the pressure-induced denaturation of individual domains of the T_4 lysozyme mutant Leu99Ala (T_4L L99A). The denaturation equilibrium was considered to be responsible for the pressure-induced loss of ^{15}N-^{1}H heteronuclear single quantum coherence (HSQC) cross-peaks, which were preferentially observed for residues in the C-terminal domain. Independently, our laboratories have studied the L99A mutant by high-pressure NMR spectroscopy (2).

In the study of Nucci et al., the signal intensity was taken to be proportional to the population of the folded state and used to derive the equilibrium for unfolding at individual amide positions. The heterogeneous response that was observed was interpreted as local unfolding at the level of secondary structure. Signal loss was more severe for the C-domain than for the N-domain and taken to mean that the stability of the C-domain was lower than that of the N-domain. At the same time, amides in helices 4 and 7 in the C-terminal domain did not sense this destabilization.

Consistent with the study of Nucci et al., we observed pressure-induced signal loss in ^{15}N-^{1}H HSQC for L99A. At the same time, methyl groups facing the internal cavity of the C-domain showed a selective and stronger signal decrease in ^{13}C-^{1}H HSQC spectra, whereas new signals indicative of unfolded protein were not observed in 1D ^{1}H-NMR and 2D HSQC spectra (2). Given that L99A experiences pervasive line broadening due to exchange between two structured states at atmospheric pressure (3), we considered that the loss of cross-peak intensities might be caused by conformational exchange between the ground state (G) and the high-energy state (HES), rather than unfolding. Indeed, we found a close correspondence between the methyl groups showing large chemical shift changes to the exited state and signal loss under high pressure (2) and further showed by spectral simulation that an increase in the high-energy state (3) population with pressure could explain the observed line broadening.

Why did Nucci et al. not deem an increase in the excited state population as an adequate explanation? The notion is discarded, as detailed in the legend to their figure S2: movement of the Leu133 ^{15}N-^{1}H HSQC peak with pressure is in a direction opposite to that expected if the excited state population would increase. However, the large pressure-induced chemical shift changes typically observed in protein ^{15}N-^{1}H HSQC spectra (see, for example, figures S2–S4 in ref. 1) may overwhelm population-dependent changes and obstruct the use of NMR peak position as a reliable proxy for the G ⇔ HES conformational equilibrium.

Two experiments may help to resolve the interpretation differences: First, hydrogen/deuterium exchange may be used as an alternative method to determine local thermodynamic stability, as has previously been done for the two domains in WT. Second, transverse relaxation dispersion experiments allow the conformational equilibrium to be followed in detail along the pressure coordinate. New pressure-resistant cells now offer sufficient sensitivity for this approach. Application of these experiments to L99A is therefore eagerly waited on.

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1 Nucci NV, Fuglestad B, Athanasoula EA, Wand AJ (2014) Role of cavities and hydration in the pressure unfolding of T_4 lysozyme. *Proc Natl Acad Sci USA* 111(38):13846–13851.

2 Maeno A, et al. (2015) Cavity as a source of conformational fluctuation and high-energy state: High-pressure NMR study of a cavity-enlarged mutant of T4Lysozyme. *Biophys J* 108(1):133–145.

3 Bouvignies G, et al. (2011) Solution structure of a minor and transiently formed state of a T4 lysozyme mutant. *Nature* 477(7362):111–114.

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The authors declare no conflict of interest.

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