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NMR study of the cataract-linked P23T mutant of human γD-crystallin shows minor changes in hydrophobic patches that reflect its retrograde solubility ☆

Ajay Pande, Jianchao Zhang, Priya R. Banerjee, Shadakshara S. Puttamadappa, Alexander Shekhtman, Jayanti Pande *

Department of Chemistry, University at Albany, State University of New York, 1400 Washington Avenue, Albany, NY 12222, USA

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ABSTRACT

The Pro23 to Thr (P23T) mutation in human γD-crystallin (HGD) shows several cataract phenotypes. We found earlier [A. Pande, O. Annunziata, N. Asherie, O. Ogun, G.B. Benedek, J. Pande, Decrease in protein solubility and cataract formation caused by the Pro23 to Thr mutation in human gamma D-crystallin, Biochemistry 44 (2005) 2491–2500] that the mutation dramatically lowers the solubility of P23T but the overall protein fold is maintained. Recently we observed that solutions of P23T showed liquid–liquid phase transition behavior similar to that of HGD but the liquid–protein crystal phase transition was altered, suggesting an asymmetric distribution of "sticky" patches on the protein surface [J.J. McManus, A. Lomakin, O. Ogun, A. Pande, M. Basan, J. Pande, G.B. Benedek, Altered phase diagram due to a single point mutation in human gammaD-crystallin, Proc. Natl. Acad. Sci. USA 104 (2007) 16856–16861]. Here we present high-resolution NMR studies of HGD and P23T in which we have made nearly complete backbone assignments. The data provide a structural basis for explaining the retrograde solubility of P23T by (a) identifying possible "sticky" patches on the surface of P23T and (b) highlighting their asymmetric distribution.

A number of studies of the mutants of human γD-crystallin provide compelling evidence that the aggregation and lowered solubility of these mutants may be directly involved in cataract formation [1–3]. In many cases, the mutant protein largely maintains its secondary and tertiary structure. One example of this is the P23T mutation, reported to be geographically widespread and universally linked to cataract formation. In a previous study we provided the molecular basis of opacity due to this mutation and showed that the overall protein fold of P23T is maintained not only in solution, but also in the aggregated state [1]. However, the retrograde solubility pattern (i.e. inverse dependence of solubility on temperature) shown by P23T suggested that there may be "sticky" patches on the protein surface which facilitate aggregation due to hydrophobic, protein–protein interactions. Other investigators [4] have used synchrotron-based circular dichroism (CD) spectroscopy and shown that minor structural changes occur in P23T. Such minor differences between a cataract-associated mutant and HGD have been observed previously by us and others in the high-resolution X-ray crystal structure of the Arg58 to His (R58H) and the Arg36 to Ser (R36S) mutants [2,5], although here again, these mutations resulted in a dramatic lowering of protein solubility.

In order to ascertain what the minor structural changes in P23T could be which lead to its lower and retrograde solubility relative to HGD, we examined the solution structure of P23T and HGD using high-resolution NMR spectroscopy. This technique is well suited to examining proteins such as P23T, since our attempts and those of others [4] to crystallize this mutant have not been successful thus far.

Materials and methods

Cloning, expression, and purification of HGD and P23T have already been described [1]. For the NMR experiments, *Escherichia coli* cells were grown in LB broth until induction, and transferred to either (i) the M9 minimal medium containing 1 g/L [U–^{15}N]NH_4Cl and 2 g/L D-glucose (for [U-,^{15}N]-labeled protein), or (ii) to the M9 minimal medium containing 1 g/L [U–^{15}N]NH_4Cl and 2 g/L [U–$^{13}C_6$] D-glucose [for U-,^{15}N-,^{13}C-labeled protein]. The protein was obtained in the soluble form and purified as already described for the unlabeled protein [1]. Protein samples in the concentration range 0.3–0.5 mM were dissolved in the NMR buffer (10 mM KPO_4 (pH 7.0), 100 mM NaCl, 0.02% NaN_3, 90% H_2O and 10% D_2O).

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* Corresponding author.
E-mail address: jpande@albany.edu (J. Pande).

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NMR spectra were collected at 25 °C in a Bruker Avance spectrometer, operating at a ^{1}H frequency of 500 MHz, and equipped with a *z*-axis gradient TCI cryoprobe. Standard double and triple resonance NMR experiments, 2D ^{1}H–^{15}N HSQC and 3D CBCA (CO)NH, HNCACO, HNCO, and HNCACB, were used for the backbone chemical shifts assignments [6]. All spectra were processed using TOPSPIN 2.1 (Bruker, Inc.) and the NMR chemical shift assignments were made using CARA [7]. Chemical shift differences (shown in Fig. 3), are weighted averages for each $^{1}H/^{15}N$ pair, and calculated as $[(\Delta\delta^2_{NH} + \Delta\delta^2_{N}/25)/2]^{1/2}$, as in [8], where $\Delta\delta_{NH}$ and $\Delta\delta_{N}$ are differences in the amide ^{1}H and ^{15}N chemical shifts for HGD and P23T, respectively.

Amino acid residues are numbered according to the sequence of HGD (www.expasy.ch: seq. ID CRGD_human). However, residue 86 in the sequence is at 87 in the X-ray structure of HGD. Therefore, in Figs. 3 and 4 we have adopted the numbering according to the X-ray structure.

Results and discussion

Fig. 1 shows the ^{15}N HSQC spectrum of HGD. It provides a fingerprint of the 3D structure, and each backbone amide except proline is expected to contribute a single cross-peak. Besides proline the following 11 residues could not be assigned in HGD: G1, N24, Y62, G85, Y98, R99, I121, L146, M147, D156, and W157, and the following 13 residues could not be identified in P23T: G1, T23, N24, Y62, R76, Y98, R99, I121, R140, L146, M147, D156, and W157. N-terminal amines are usually unassigned during standard NMR procedures [6]. Except for G1, all other residues are located in the solvent exposed loop structures of HGD and are possibly broadened due to chemical exchange of amides with water. Most of the unassigned residues are common to the two proteins. Thus, overall, about 90% of the backbone amides have been assigned. We note that several assigned resonances appear to be of low intensity, e.g. residues D21, H22, L71, S84, and Q154. This is probably due to the unfavorable exchange rates of these residues.

Fig. 2 shows the overlay of the spectra for HGD (black) and P23T (red). The large dispersion in both ^{15}N and ^{1}H dimensions indicates that both HGD and P23T are well folded, and the striking congruence of the spectra suggests that the two 3D structures are nearly

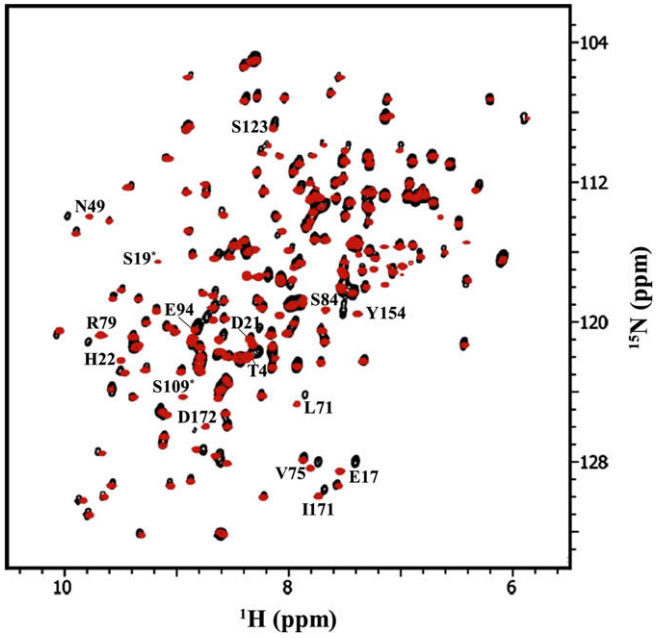

Fig. 2. 2D ^{15}N–^{1}H HSQC spectrum of 500 μM [U-,^{15}N] HGD (black contours), and 500 μM [U-,^{15}N] P23T (red contours). Residues with large average amide chemical shift differences between the two proteins are marked (compare with Figs. 3 and 4). The contours marked with an asterisk (*) are those for which HGD contours are not visible at this contour level.

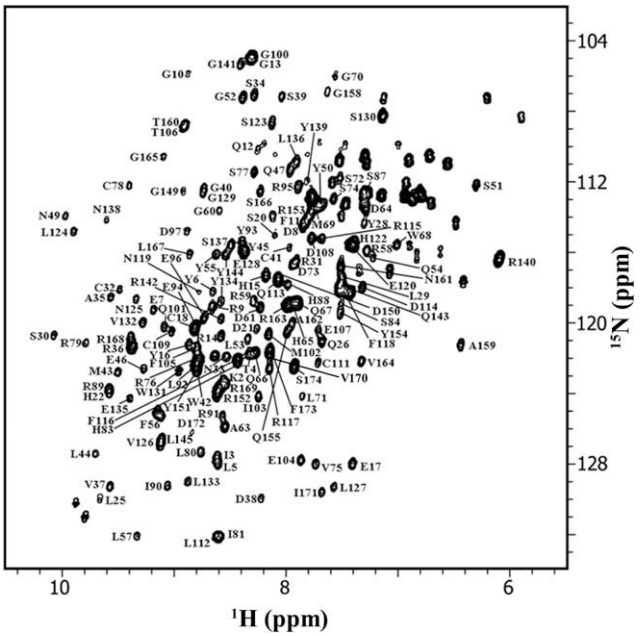

Fig. 1. 2D ^{15}N–^{1}H HSQC spectrum of 500 μM [U-,^{15}N] human γD-crystallin (HGD), with assignments indicated by residue number and identity. The protein sample was dissolved in the NMR buffer (10 mM KPO_4 (pH 7.0), 100 mM NaCl, 0.02% NaN_3, 90% H_2O, and 10% D_2O). NMR data was acquired on 500 MHz Bruker Avance NMR spectrometer at 25 °C.

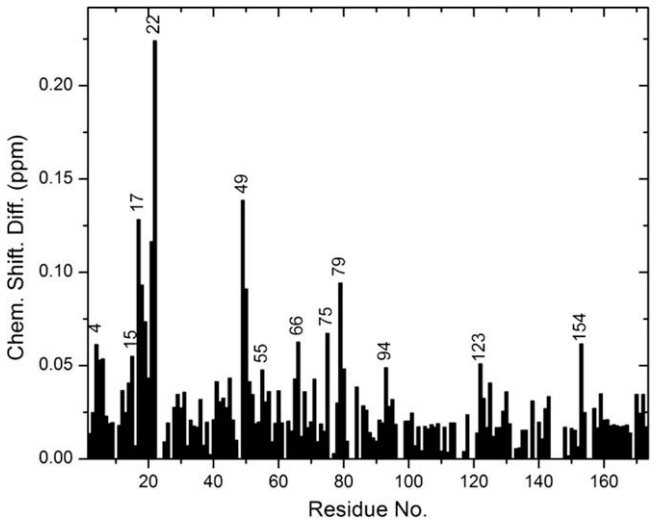

Fig. 3. Average amide chemical shift difference for all the assigned residues in HGD and P23T. The weighted averages of the ^{1}H and ^{15}N chemical shifts differences are calculated as $[(\Delta\delta^2_{NH} + \Delta\delta^2_{N}/25)/2]^{1/2}$ (see text for details). The residue numbering for the C-terminal end (i.e. 87–174) was shifted up by one residue to match the numbering in the X-ray structure [5]. Only high (greater than 0.1 ppm) and moderate (between 0.1 ppm and 0.05 ppm) chemical shift differences are indicated by the individual residue numbers. For the sake of clarity, all consecutive residues have not been marked.