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pH jump induced α-helix folding

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Abstract. pH can be used to impact the folding equilibrium of peptides and proteins. This fact is utilized, similarly to temperature jumps, in pH jump experiments employing laser time-resolved spectroscopy to study the function and structural dynamics of these molecules. Here the application of pH jumps in folding experiments was investigated. Experiments with poly-L-glutamic acid alpha-helix formation shown the critical aspects of pH jump experiments and yielded direct information about the folding kinetics monitored with the amide I IR band.

1 Introduction

Time-resolved studies of protein folding and functionality are an essential to understand the biological role of those macro molecules and the physical interactions responsible for their complex structure and activity. Taking advantage of time-resolved IR laser spectroscopy in the amide I range allows collection of non-equilibrium kinetic data on phenomena like protein folding [1,2]. For recording transient spectra of the rearranging molecule effective methods of initiating the process are needed. The discussed case of peptide and protein folding equilibrium, like all other thermodynamic equilibria, is temperature dependent. Additionally it is influenced, even more directly, by the properties of the biomolecule such as local charge and hydrophobic/hydrophilic interactions [1,3]. These, even for a given compound, can often be altered and controlled by pH. This is possible since one third of the 21 naturally occurring amino acids carry protonatable carboxyl or amine groups in their side chains. Side chain protonation affects the amino acid's properties by changing its charge, solvation and, in general, ability to interact with nearby molecules. As a result, side chain protonation has a dramatic impact on the protein or peptide secondary and tertiary structure. Among many examples of how protonation impacts structure and functionality, the influenza A M2 membrane proton channel [4] and folding of GCN4 leucine zippers [5] can be mentioned.

When dynamic control over temperature or pH is obtained they can be used as folding triggers. In fact, laser induced temperature jumps are an established method to observe the unfolding of proteins caused by the temperature increase [2, 6]. In this project, the effort is directed to explore the experimental potential of pH jumps. It is expected that this method opens access to, new more direct and truly biomimetic folding experiments. Ultimately the folding equilibrium can be switched completely, starting from completely unfolded samples.

Here, formation of an α-helix was studied in pH jump experiments on poly-L-glutamic acid (poly-L-glu). The experiments had a two-fold aim. On one hand, they were conducted on a relatively simple system to explore the challenges of pH jump triggering. On the other hand, their goal was to

confront the observed folding kinetics with the rich literature knowledge about poly-L-glutamic acid helix formation [1,2,6].

2 pH jumps, rapid protonation and folding

Poly-L-glutamic acid has a high α-helix propensity. Nevertheless, in neutral and alkaline solutions it remains unfolded due to the electrostatic repulsive interactions of ionized carboxyl groups. Upon protonation (pKa value of 4.2) the side chains are neutralized and poly-L-glu forms an α-helix. The correlation between charge neutralization and folding was studied by comparing titration and folding curves obtained with circular dichroism (CD) measured at 222 nm (fig 1A). From the relative shift of the two sigmoids it is clear that the peptide needs about 50% protonation before the folding begins. Understanding the two curves allows to estimate the buffer capacity of the sample and predict the resulting shift on the folding curve. It illustrates the practical necessity to control the amount of released protons (commonly the limiting factor in pH jump experiments) and initiating the jump at pH near to the transition (with the peptide partially protonated). Moreover minimizing the sample's buffer capacity even at the cost of lowering the peptide's concentration can make the jump effective in terms of folding.

A well studied proton cage, ortho-nitrobenzaldehyde (oNBA), was used in these experiments [7]. Its solubility and proton release yield limit the theoretical proton delivery to 4 mM. In experiments 1-2 mM protons were observed [8, 9]. They are delivered upon dissociation of the oNBA photoproduct at a timescale of 21 ns observed in water solutions [7]. These characteristics are sufficient for applying in most folding experiments. In the case of poly-L-glu_{50} studied in 1mg/ml concentration (7 mM residual concentration), the amount of delivered protons is nearly enough to change the folding equilibrium by 50% of the transition seen in the CD folding curve in fig 1A.
The proton transfer between the proton cage and the peptide was observed based on carboxyl marker bands of the oNBA photo product (carboxylate: 1605 cm^{-1} and carboxyl: 1710 cm^{-1}) and glutamate side chain (carboxylate: 1570 cm^{-1} and carboxyl: 1715 cm^{-1}). The bands were identified based on pH dependent FTIR data (spectra not shown). Kinetic fits to the time evolution of those bands return an estimate of the poly-L-glu protonation timescale between 25 and 55 ns.

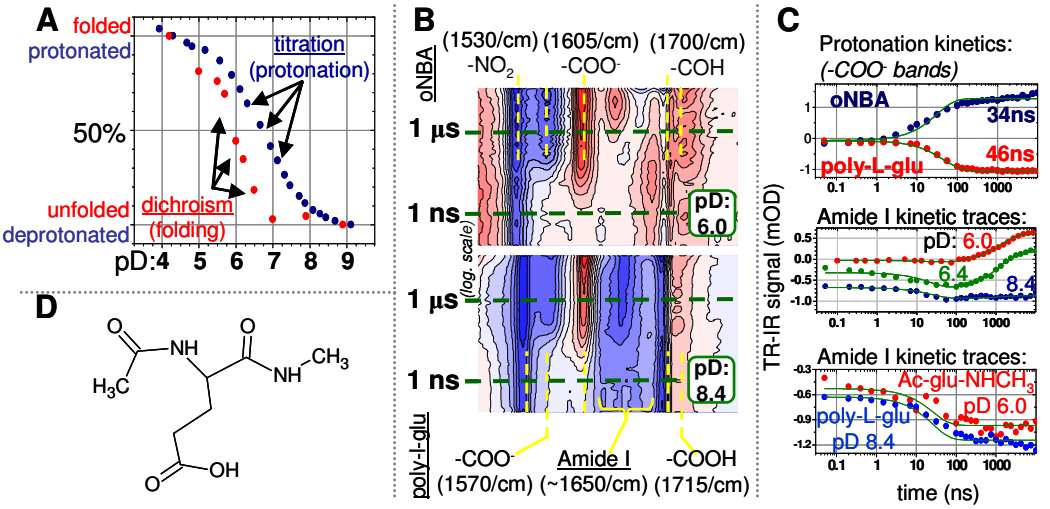

Fig. 1. A) poly-L-glu_{50} titration and folding curve (helicity estimated with a CD measurement of Θ_{222}); B) time-resolved IR spectra measured in a pH jump experiment in a oNBA and poly-L-glu solution; C) Kinetic traces of bands characteristic for the proton transfer and α-helix formation together with fitting results discussed in text; D) model molecule reproducing a single residue of poly-L-glu with the corresponding amide I band.

3 Kinetics of the helix formation

Information about the folding process of poly-L-glu$_{50}$ homopeptide was extracted from the time evolution of the amide I band. The dynamics recorded in that spectral region differed qualitatively depending on the initial pH of the sample. For basic samples (pD 8.4), initiated at full deprotonation, a fast, nanosecond, bleach was observed. For samples initiated close to or within the folding transition (pD 6.4, 6.0) the initial bleach of the amide I was accompanied by further bleaching of the blue side and a positive signal appeared at the red side of the band (fig 1B). The time constants fitted to these processes were 25 ns and 1.3 μs (fig 1C).

A good match of the fast kinetic component and the timescale, together with its presence in all samples (also the basic ones, in which only a marginal shift to the folding equilibrium was obtained) suggested this signal is a direct response to the side chain protonation rather then a result of a structural change. It can be interpreted as a Stark Effect on the amid I band. This was confirmed by experiments with Ac-glu-NHCH$_3$ (fig. 1D). This model molecule well simulated the amide I band of poly-L-glu whereas having only one amino acid was incapable of folding. Matching behaviour of the amid I band of the two compounds in the nanosecond timescale confirmed the electrostatic nature of the observed spectral changes (fig 1C).

The slower process (1.3 μs) represented the folding. The fitted timescale was found matching literature values reported in a T jump experiment in which folding was observed with the CD signal [6].

4 Conclusions

Ply-L-glutamic acid folding was observed on a 1.3 μs timescale. Initial fast (25 ns) dynamics in the amide I band were associated with the protonation event which resulted in a Stark Shift of the amide I band. The structural meaning of this kinetic step was excluded in a model experiment with molecules incapable of folding. Here, pH jumps were investigated in the context of protein folding but their application to initiate other pH controlled structural changes of proteins, such as membrane channel gating, is possible. In case of protein folding they open a new range of possibilities coming from the fact of direct observation of non-equilibrium peptide and protein folding systems out of the equilibrium conditions. It is feasible to use pH-jumps for initiating transitions over the entire folding curve if the magnitude of the pH jump and the buffer capacity of the sample are matched. This could not be demonstrated with the oNBA and ply-L-glu samples due to limited proton delivery and high buffer capacity of the homopolypeptide consisting of only carboxylate terminated side chains. Nevertheless the discussed issue largely eliminated when examining peptides of a lower content of protonatable amino acids such as Leucine zippers. pH jumps are a still demanding experimental technique, but they provide a biomimetic method of triggering protonation dependent biological processes in the near neutral pH range (pH 4-10).

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