Intermolecular Interactions in α Helical Oligo- and Poly(L-glutamic acid) at Acidic pH

Erik J. Spek, Youxiang Gong, and Neville R. Kallenbach*

Department of Chemistry, New York University
New York, New York 10003

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Studies of amino acid substitutions in helical peptides show that some side chains stabilize α helix structure, while the majority do not.[1] The influence of a side chain on helical structure is measured by scales of its "helix propensity", which corresponds to the free energy for stabilizing a (nucleated) α helix at the expense of other conformations for that side chain.[2] Recent propensity scales show good agreement concerning the ability of a given side chain to stabilize or destabilize the helical structure in short peptides[1] or at surface sites of helices in proteins.[3] However, the scale derived from substitutions in a hydroxy-alkylated glutamine copolymer background fails to predict the extent of helical structure in short helical peptides containing Ala or other natural side chains.[4] Short alanine-rich peptides tend to be helical.[1c,d] This discrepancy has been investigated by introduction of one or more hydroxy-alkylated glutamine side chains into short peptides; the results reveal the presence of strong side chain−side chain interactions in these molecules.[5]

The availability of several propensity scales has stimulated discussion concerning the applicability of the scales derived from different models.[6] Holtzer points out that the scale of helix propensity derived from substitution experiments on short, alanine-rich peptides predicts that poly(Glu) should not be helical at any pH.[6] By contrast, the scale of values determined from synthetic copolymers with hydroxy-alkylated glutamine side chains[4] predicts that poly(Glu) is helical at acidic pH.[6] Experimentally poly(Glu) is helical at acidic pH, and its thermodynamic properties have been determined by potentiometric titration: the value cited is $\Delta G^\circ = -0.250 \pm 0.07$ kcal/(mol of residue), based on data from high molecular weight polymers at about 4 °C and an ionic strength of 0.01 M.[7] This value is at variance with that determined from alanine-based peptides.[1d]

We demonstrate here that the helical structure in poly(Glu) or shorter oligo(Glu) molecules is intermolecular. This means that the free energy value determined in poly(Glu) includes a major contribution from side chain-side chain interactions to the stability of the acid helix, as Holtzer infers, but not simply that present in hydroxy-alkylated glutamine containing copolymers.[4] An older literature reference reported that the helix−coil transition in poly(Glu) is concentration dependent,[8] while poly(Glu) at acidic pH precipitates on standing.[9]

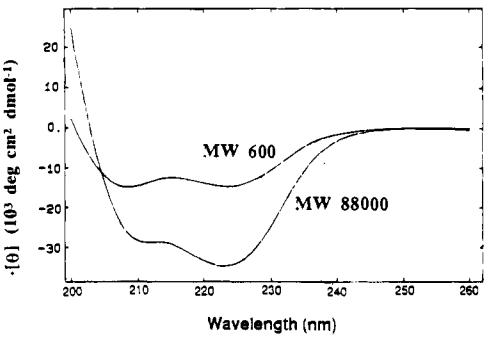

Figure 1. Circular dichroism spectra of the oligo(Glu) MW = 600 and poly(Glu) MW = 88 000, in 50 mM phosphate adjusted with HCl to pH = 3.5 at 4 °C. Spectra were recorded on an Aviv 60 DS spectropolarimeter equipped with an HP Model 89100A temperature controller, using a cuvette with a 1 mm path length, averaging over three scans with a step size of 0.5 nm. Sample concentrations were ca. 0.8 mM per residue, determined by weight.

To clarify the interactions which stabilize helical glutamic acid, we have investigated the helix−coil transition in oligo(Glu) chains with different mean lengths. Sequences of poly(Glu) occur in the acidic "tails" of transcription factors and thus are of practical interest in their own right.[10] We have studied two sets of chains in this work: a short oligomer with mean dp 5 residues, and a high molecular weight polymer with mean dp 600 residues. The degrees of polymerization were determined by light-scattering measurements, provided with the samples.[11] Figure 1 shows the CD signal of these samples at pH = 3.5. The CD signal implies the presence of a helical conformation in both chains; the spectrum of oligo(Glu) indicates a mix of helix and coil conformations, while that of poly(Glu) is more fully helical. The 208 nm band is more sensitive to the chain length of α helices than the 222 nm band; for example, the former is weak in helices with fewer than 10 residues.[12] A unimolecular model for helical poly(Glu) should show strong chain length dependent helical properties and a very weak value of $[\theta]_{208}$ and would not predict helix formation of the oligo(Glu) fraction of five residues. While the spectra of the two fractions are not identical, the appearance of an α helix signal in oligo(Glu) suggests that the helical structure in oligo(Glu) is not intramolecular.

In Figure 2 $[\theta]_{222}$ of oligo(Glu) and poly(Glu) is recorded as a function of the pH. At higher pH values there is no helical structure, which can be attributed to the low helix propensity of glutamate, together with the repulsion between the charged glutamate side chains. Reducing the pH results in protonation of the side chains, allowing the formation of aggregates that account for helix formation and the ellipticity signal.

At still lower pH the ellipticity disappears; the aggregates precipitate with increasing neutralization of the side chains. Olander and Holtzer[7b] assumed in fact that aggregation of poly(Glu) is confined to a distinct "aggregation region", in which the polymer precipitates. The drop in apparent ellipticity of poly(Glu) at lower pH and concentrations has been referred to as "anomalous behavior".[9]

Further information on the aggregation and on the size of the aggregate comes from gel sizing experiments. A sample

* Department of Chemistry, New York University, New York, NY 10003. Telephone: (212) 998-8757. FAX: (212) 260-7905. E-mail: kallnbch@is.nyu.edu.

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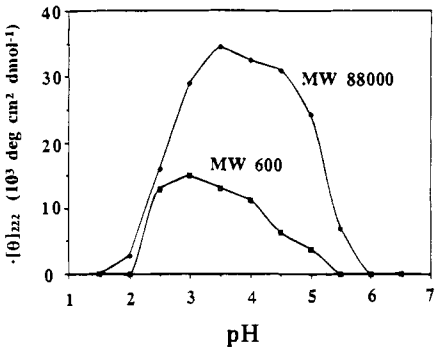

Figure 2. Ellipticity at $[\theta]_{222}$ of the oligo(Glu) MW = 600 and poly-(Glu) MW = 88 000 as a function of pH. The peptides were in 50 mM phosphate. The pH was adjusted with HCl. Other experimental characteristics are as described in Figure 1.

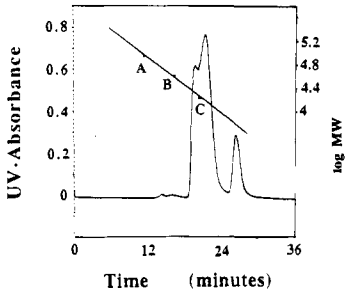

Figure 3. FPLC chromatogram of oligo(Glu) MW = 600 at pH = 4. A 2.5 mg sample was run on a Pharmacia Superose 12 column using a Biologic 750-0049 apparatus. The peptide was detected by UV absorbance at 215 nm. Eluent was 50 mM phosphate buffer adjusted to pH = 4 with HCl; the flow rate was 1 mL/min. A reference line with marker proteins at neutral pH is shown: (A) conalbumin MW = 77 000; (B) chicken ovalbumin MW = 45 000; (C) myoglobin MW = 17 000.

of the short oligo(Glu) chain (five residues) run on a size exclusion column produces a broad profile at pH 4 as shown in Figure 3, with a peak corresponding in volume to a globular protein (at neutral pH) of approximately 10 000 MW relative to the marker proteins shown.

Additional evidence for association is provided by NMR experiments (Figure 4). The 1D ^{1}H-NMR spectrum of oligo-(Glu) (five residues) contains a variety of peaks in the amide region at low temperature (panel A). Chemical shift averaging in the random coil conformation should result in a maximum of five peaks, most likely only one, depending on the lifetime of different states present. On the other hand, an aggregate of small oligo(Glu) strands leads to many amide peaks, since it is unlikely that there is only one environment for all residues in the associated state. Raising the temperature results in reduction

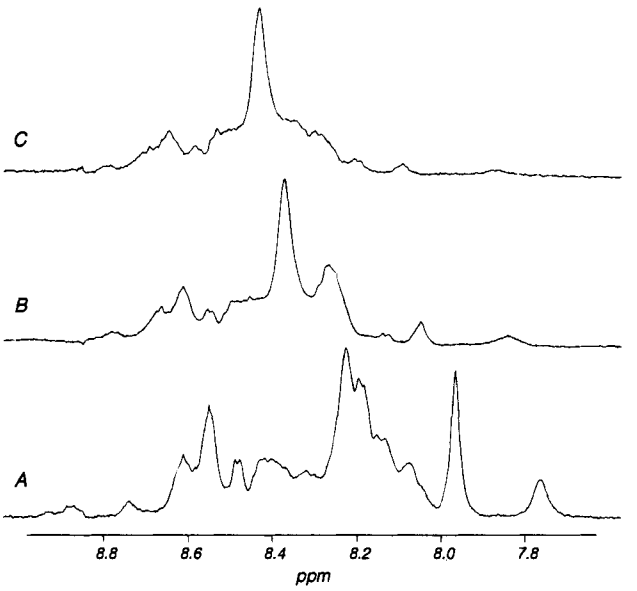

Figure 4. Expanded amide region of a 1D ^{1}H-NMR spectrum of oligo-(Glu) MW = 600 at various temperatures. The spectrum was recorded on a Varian UNITY 500 NMR at residue concentration 8 mM, pH = 4. The solvent was suppressed by presaturation: (A) 20 °C; (B) 40 °C; (C) 50 °C.

of the number of peaks (Figure 4B,C). The extent of association presumably diminishes, favoring the random coil conformation in short chains. This is consistent with the observation of a single strong peak at ppm 8.4 at high temperature (panel C). Samples with concentrations necessary for NMR spectroscopy tend to precipitate within a few hours, limiting further investigations into the nature of the structure present.

In this paper we have shown that intermolecular interactions play a dominant role in poly(Glu) interactions at acidic pH. The intrinsic helix propensity of Glu cannot then be evaluated from data on such a system,[7] as it can in single Glu substitutions.[1a,d] What is the nature of the helical state?

We can only speculate at present. A plausible explanation is that neutralization of glutamate residues allows formation of multistranded amphipathic structures, such as coiled coils. The possibility that intramolecular COOH−COOH interactions also occur cannot be excluded by our data.[13]

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