Application of Old and New Values of α-Helix Propensities to the Helix−Coil Transition of Poly(L-glutamic acid)

Alfred Holtzer

Department of Chemistry, Washington University St. Louis, Missouri 63130

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It is generally accepted that the equilibrium states in the helix−coil transition in peptides are well-described by theories of the Zimm−Bragg type.[1,2] Realization of this theory for heteropeptides requires values of the parameters for helix initiation (σ) and propagation (s) for each type of amino acid. A complete set of such values for all the types commonly found in proteins has been available for some time.[3,4] Recently, several groups have questioned the legitimacy of these older values, and a new set has been put forward.[5] We compare here the success of each set, employed "as is", in describing extant data on the helix−coil transition in poly(L-glutamic acid).[6] Surprisingly, we find that the older values describe the experimental data quantitatively in spite of the chemical differences between glutamic and host peptide side chains. Not surprisingly, the newer ones, which refer to a host peptide context with severely limited side-chain interactions, are in serious disagreement with the same data.

The older values were obtained by including each target amino acid in a water-soluble host polypeptide of a non-natural amino acid, specifically, poly[N^5-(3-hydroxypropyl)-L-glutamine] (PHPG) or its 4-hydroxybutyl analog (PHBG).[3] This older complete set includes the temperature dependence of s for each amino acid.[3,4] This PHP/BG set of values has been criticized because it does not agree with experiments on various short, alanine-based peptides (SABP host).[5] These newer experiments stem from various laboratories, which are in rough agreement with one another on the relative values. A complete set of absolute values has now appeared, but only for a single temperature, 0.0 °C.[5]

Doubtless, many tests will be made of the legitimacy of these parameters. One rather limited test can be made immediately, because the data already exist. Some years ago, the standard free energy, enthalpy, and entropy for the helix−coil transition in poly(L-glutamic acid) were measured by potentiometric titration.[6] This technique provides the standard thermodynamic parameters for the conversion of the fully protonated polymer from complete helix to complete random coil.[7] We emphasize that these results are not only independent of any specific statistical mechanical model or theory, such as Zimm−Bragg theory,[7] but also predate the relevant PHP/BG and SABP helix propensity values by many years.[3,5,6]

The reaction in question, then, is

$$(E^0)_n^{\text{helix}} \rightleftarrows (E^0)_n^{\text{coil}} \qquad (1)$$

wherein E^0 stands for a protonated glutamic acid residue, n is the degree of polymerization, and the superscript indicates the conformation of the polymer. Long-extant measurements show that the standard thermodynamic properties for this transition are as summarized in Table 1. They were obtained using three polymers of degree of polymerization 620, 680, and 740, respectively, the average being 680.[6]

Table 1. Experimental Thermodynamic Parameters for Helix → Coil in Poly(L-glutamic acid)

$[\Delta H^\infty/n]^a$	$[\Delta S^\infty/n]^b$	$[\Delta G^\infty(0\ °C)/n]^a$
975 ± 50	2.67 ± 0.1	246 ± 72

[a] In cal/residue-mol. [b] In cal/(K·residue-mol).

We next compare the experimental free energy at 0 °C with those given by Zimm−Bragg theory, the latter being realized using the PHP/BG or the SABP set of parameters. The theory defines the standard chemical potential of the completely random homopeptide as 0 and that of the completely helical homopeptide as $-RT \ln(\sigma s^n)$.[1,2] Thus, the standard free energy change for reaction 1 is, per residue,

$$\Delta G^\infty/n = [(RT \ln \sigma)/n] + RT \ln s \qquad (2)$$

Insertion of the numerical values for the degree of polymerization of the homopeptide and for σ and s into eq 2 allows calculation of the standard free energy of reaction 1. The result can then be compared with the experimental value given above.

First, we implement eq 2 for the protonated glutamic residues using the PHBG set of values.[8] In this set, $\sigma = 0.0100$. This parameter is of lesser importance, because, as eq 2 reveals, the term in which it appears is divided by the degree of polymerization, which in this case is very large (680). The PHBG results give the propagation parameter as a function of temperature[8] and are well fit by

$$RT \ln s = RTB_0 + RB_1 \qquad (3)$$

with $B_0 = -1.6047$ and $B_1 = 557.82$.[9] Inserting eq 3 into eq 2 and also using the numerical values, we find $\Delta G^\infty(0\ °C)/n =$ 233 cal/(residue-mol). This value is in rather close agreement, well within error, with the experimental value obtained independently and given in Table 1, differing by only 5%. Thus, the free energy given by Zimm−Bragg theory, using PHBG realization, agrees with the experiments on poly(L-glutamic acid).

We next implement eq 2 for the protonated glutamic residues using the SABP set of parameters.[5] These values are $\sigma =$ 0.00191 and $s(0\ °C) = 0.63$, insertion of which into eq 2 yields $\Delta G^\infty(0\ °C)/n = -256$ cal/(residue-mol). This result not only differs from the experimental value by over 200% but also is qualitatively incorrect. If the glutamic residues in poly(L-glutamic acid) had the helix propensities they display in the SABP host, the polymer would be randomly coiled, not helical at all.

Since most readers are probably more familiar with the s values than with the corresponding free energies, it may clarify the issues to proceed a bit differently. Let us accept the experimental value for the free energy and insert it into eq 2 along with the value of σ (the latter being only a small contributor, anyway) and then calculate the value of s. Using the PHBG value of σ in this fashion, we find $s(0\ °C) = 1.58$, a value in excellent agreement with that obtained from the PHBG data summarized in eq 3, the latter giving 1.55. On the other hand, if we use the experimental free energy with the SABP value of σ (the latter being again a small perturbation) in eq 2, we obtain $s = 1.59$, a value indistinguishable from the PHBG-determined one, but a far cry from the SABP-determined value of 0.63.

(1) Zimm. B.; Bragg, J. *J. Chem. Phys.* **1959**, *31*, 526−535.
(2) Poland, D.; Scheraga, H. *Theory of Helix−Coil Transitions in Biopolymers*; Academic Press: New York, 1970.
(3) Scheraga, H. *Pure Appl. Chem.* **1978**, *50*, 315−324.
(4) Skolnick, J.; Holtzer, A. *Macromolecules* **1982**, *15*, 812−821.
(5) Chakrabartty, A.; Kortemme, T.; Baldwin, R. L. *Protein Sci.* **1994**, *3*, 843−852 and references therein.
(6) Olander, D. S.; Holtzer, A. *J. Am. Chem. Soc.* **1968**, *90*, 4549−4560.
(7) Nagasawa, M.; Holtzer, A. *J. Am. Chem. Soc.* **1964**, *86*, 538−543 (in particular, Appendix I).
(8) Maxfield, F.; Alter, J.; Taylor, G.; Scheraga, H. *Macromolecules* **1975**, *8*, 479−491.
(9) Holtzer, M. E.; Holtzer, A.; Skolnick, J. *Macromolecules* **1983**, *16*, 462−465.

0002-7863/94/1516-10837$04.50/0

It thus appears that the older, PHBG-based values are to be preferred in the case of fully protonated poly(L-glutamic acid). However, this is not to say that either source of propensities is, in general, "correct" or "incorrect" for glutamics. It may very well be that strong helix-favoring forces exist in poly(L-glutamic acid) that do not exist in alanine-based peptides. These forces, for example, could be side chain to side chain hydrogen bonds,[6] which can form in the homopolymer (wherein a glutamic has only other glutamics as neighbors) but could not exist in an alanine-rich host. The agreement with the PHBG values would then be seen as fortuitous. One could argue that the host polymer in the PHBG experiments supplies interactions with the glutamic side chains that happen to mimic in free energy the hydrogen bonds (or whatever) that exist in the homopolymer.

To test the plausibility of this hypothesis, we next examine not only the free energy but also its constituent enthalpy and entropy. The standard enthalpy given by the Zimm–Bragg theory can be obtained in the usual way by partial differentiation of the free energy with respect to T. Applying this procedure to eq 2, while recognizing that σ is independent of T and using eq 3, yields

$$\Delta H^{\circ}/n = -RT^2\partial(\ln s)/\partial T = RB_1 \qquad (4)$$

Insertion of numerical values into eq 4 gives $\Delta H^{\circ} = 1108$ cal/residue-mol, showing that the Zimm–Bragg theory, realized via the PHBG values for helix propensities, provides not only a standard free energy but also an enthalpy (and therefore an entropy as well) in rather good agreement with the experimental values for poly(L-glutamic acid) in Table 1. This indicates that the attempt to rationalize the agreement with the free energy as fortuitous now requires belief in a double coincidence.

Is it possible that side-chain hydrogen bonds (or whatever else raises propensities to levels above the newer values) in the PHBG host polymer exactly mimic those in poly(L-glutamic acid), thus causing all the thermodynamic properties of glutamic to be the same in either context? This seems unlikely, in view of the difference in chemical nature of the side chains. Moreover, the one extant measurement of the enthalpy in the case of the SABP hosts agrees with that for poly(L-glutamic acid).[10] One would therefore have to believe, at the same time, that these extra hydrogen bonds (or whatever), while strongly augmenting the stabilizing free energy in the homopolymer over what it is in the SABP peptides, do nothing whatever to the enthalpy. These difficulties raise the possibility that the alanine-rich host peptides may also possess some special, disqualifying feature. In any case, these discrepancies will have to be reconciled before workers can employ any set of values with confidence.

It seems possible that both sets of propensities may be useful. The SABP set perhaps provides values for an environment wherein side-chain interactions are minimized.[5,11] This set may be appropriate as a basis for a much more elaborate theory in which such interactions are added in a manner particular to the sequence of interest. Such a comprehensive theory, however, may be some time in coming.

Meanwhile, the PHP/BG set, although evidently inappropriate for alanine-based peptides, may be more useful than appears at first sight. Evidently, it succeeds in mimicking the polar side-chain interactions in poly(L-glutamic acid), thus augmenting the backbone helix propensity of a glutamic guest. Very likely, the same would be true of other polar side chains, such as glutamine *et al.* Moreover, very recent work indicates that PHP/BG side chains also augment the helix propensities of nonpolar amino acids.[11] Although alanine is a quite common amino acid in proteins, a given amino acid side chain in an arbitrary, varied sequence is far more likely to be near in space to a polar or nonpolar side chain larger than alanine than to alanine or glycine. For example, in a random peptide of the composition of tropomyosin (a highly helical protein), the odds are more than 6 to 1. Thus, *because* it provides versatile host–guest side-chain interactions, the PHP/BG set may mimic the mean environment found in many peptide sequences sufficiently well to yield useful estimates even for "off-the-rack" calculations, unaided by more detailed theories. It therefore is worth investigating how well this older set will do in treating peptides more like those found in proteins.

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(10) Scholtz, J. M.; Marqusee, S.; Baldwin, R. L.; York, E. J.; Stewart, J. M.; Santoro, M.; Bolen, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 2854–2858.

(11) Padmanabhan, S.; York, E. J.; Gera, L.; Stewart, J. M.; Baldwin, R. L. *Biochemistry* **1994**, *33*, 8604–8609.