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A COMPARISON OF THE α-HELIX FORMING PROPENSITIES AND HYDROGEN BONDING PROPERTIES OF SERINE PHOSPHATE AND α-AMINO-γ-PHOSPHONOBUTYRIC ACID

Sebastian Liehr[a] and H. Keith Chenault*[,b]

Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19711, U.S.A.
Current addresses: [a]Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104, U.S.A.
[b]DuPont Central Research and Development, Experimental Station, P.O. Box 80328, Wilmington, DE 19880-0328, U.S.A.

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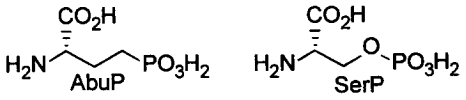
Abstract: The ability of serine phosphate (SerP) or α-amino-γ-phosphonobutyric acid (AbuP) and arginine to form a salt bridge between their side chains appears to be much greater when they are spaced *i*/*i*+4 than when they are spaced *i*/*i*+3. The side chain-side chain interaction between SerP/Arg and AbuP/Arg, positioned *i*/*i*+4, contribute 0.45 and 0.62 kcal mol^{-1}, respectively, toward stabilizing the α-helical conformation of a peptide.

Phosphonic acids and their derivatives have been prepared and studied as analogs of naturally occurring phosphate esters.[1] These phosphonate analogs exhibit a variety of biological activities and have the advantage that they do not hydrolyze under the chemical or enzymatic conditions that hydrolyze ordinary phosphate esters. Phosphorylated amino acids1c,[2] are of particular interest, since the phosphorylation of proteins regulates processes such as glycogen metabolism, glycolysis, hormonal responses, and muscle contraction.[3] In each of these processes, regulation depends on changes in protein structure that may involve the formation or breaking of interactions between serine phosphate and a hydrogen-bond donor such as arginine.[3c] In general, hydrogen bonds between serine phosphate and arginine are important in stabilizing structural motifs of globular proteins.[3c]

Peptides containing serine phosphate[4] (SerP) and α-amino-γ-phosphonobutyric acid (AbuP) have been synthesized previously.[5] However, while the biological activities of some of these peptides have been tested, the effects of side chain–side chain interactions between SerP or AbuP and arginine on conformational stability have not been measured. Likewise, the helix forming propensities of SerP and AbuP have not been compared.



Herein, we report the use of short, alanine-rich peptides containing SerP or AbuP at position *i* and argenine at position *i*+3 or *i*+4 both to determine the intrinsic α-helix forming propensities of SerP and AbuP and to measure the strength of hydrogen-bonding interactions between SerP or AbuP and argenine. The peptides[6] used in this work (Table 1) were based on that of Marqusee and Baldwin,[7] which has the advantages of being short, water soluble, and 50% α-helical at 25 °C. Since the equilibrium between α-helix and random

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coil is 1.0 at room temperature, small energetic perturbations in the stability of the α-helix are easily detected. For the synthesis of peptides **1** and **2**, enantiomerically pure *N*-Fmoc-L-AbuP, protected as the dimethyl-phosphonate, was prepared in seven steps from γ-*tert*-butyl *N*-Boc-L-aspartate.[5]

Table 1. Peptide Sequences

peptide	sequence
1	Ac-YEAAAK(SerP)AARAEAAAKA-NH_2
2	Ac-YEAAAK(SerP)AAAREAAAKA-NH_2
3	Ac-YEAAAK(AbuP)AARAEAAAKA-NH_2
4	Ac-YEAAAK(AbuP)AAAREAAAKA-NH_2

Because studies with the Marqusee–Baldwin peptide have shown that the side chains of glutamate and lysine spaced *i*/*i*+3 interact only slightly,[7] side chain–side chain interactions between SerP or AbuP and Arg spaced *i*/*i*+3 were considered to be negligible.[8] Thus, the helicities of peptides **1** and **3** are determined largely by the helical propensities of their constituent amino acids. Because the helical propensities of all other amino acids used are known,[9] those of SerP and AbuP were treated as variables when fitting α-helicities predicted by modified Lifson–Roig theory to the actual measured values. With peptides **2** and **4**, SerP or AbuP and arginine are spaced *i*/*i*+4. The helix propagation parameters (*w*) determined for SerP and AbuP from peptides **1** and **3**, respectively, were used as fixed parameters, and the magnitudes of the side chain-side chain interactions were varied in order to fit calculated α-helicities to those that had been measured.

The α-helicities of peptides **1–4** were determined from circular dichroism (CD) measurements.[10] At low temperatures, each peptide showed a certain degree of α-helicity, as indicated by CD minima at 222 and 208 nm and a CD maximum at 192 nm. At higher temperatures, the CD signals characteristic of the α-helix disappeared, and a CD minimum at 200 nm, characteristic of the random coil state appeared.

At 1 °C, peptides **1–4** were 44.6, 68.3, 46.1, and 78.8% α-helical, respectively. As expected, peptides with SerP or AbuP and arginine spaced *i*/*i*+3 were less helical than those with the charged side chains spaced *i*/*i*+4. Apparently charge–charge or hydrogen-bonding interactions between the side chains do help to stabilize the α-helical conformation. To determine the various energetic contributions favoring α-helix formation, an extended Lifson–Roig model that includes side chain–side chain and charge–dipole interactions was used to describe the multi-state transition from random coil to α-helix.[7] This extended model allows calculation of the intrinsic helical propensities (helix propagation parameters, *w*) of individual amino acids as well as the energetic contributions of interactions involving side chains (*p*) in order to fit calculated α-helicities to those that have been measured.

Table 2. Helix propagation and side chain interaction parameters

amino acid	w $(\Delta G)^a$	p^b $(\Delta G)^a$
SerP	0.46 (0.43)	2.3 (–0.45)
AbuP	0.41 (0.48)	3.1 (–0.62)

[a]For coil to helix, in kcal mol^{-1}. [b]For SerP7/Arg11 or AbuP7/Arg11.

Using peptides **1** and **3** and assuming no interaction between SerP or AbuP and arginine spaced *i*/*i*+3, the helix propagation parameters (*w*) of SerP and AbuP were calculated to be 0.46 and 0.41, respectively. Thus,

SerP and AbuP have very similar intrinsic helical propensities, and peptides **1** and **3** exhibit similar helical stabilities. We probably overestimate the *w*-values of SerP and AbuP slightly because of the assumption that *i*/*i*+3 interactions are negligible. However, these *w*-values compare well with those of glutamate (0.43) and serine (0.36), the closest structural analogs of SerP and AbuP that have measured values of *w*.

Using values of *w* for SerP and AbuP calculated from peptides **1** and **3** as well as the α-helicities of peptides **2** and **4**, the magnitude of interactions between the side chains of SerP or AbuP and arginine, spaced *i*/*i*+4, were determined. For SerP/arginine, $p = 2.3$, corresponding to an α-helix-stabilizing energy of –0.45 kcal mol-1. For AbuP/arginine, $p = 3.1$, corresponding to a stabilization energy of –0.62 kcal mol^{-1}. Because the values of *w* used to calculate them are probably overestimated, the helix stabilizing effects of these side chain-side chain interactions are probably underestimated. Their magnitude is similar to that of the interaction between glutamate and lysine spaced *i*/*i*+4 (–0.50 kcal mol^{-1})[11] and suggests that the interactions between side chains involve only a single hydrogen bond, not two, as might have been expected.

AbuP was found to be a good mimic of SerP. In an α-helical environment AbuP and SerP exhibit similar helix forming propensities (*w*) of 0.41 and 0.45, respectively. Interestingly, the *w*-value for serine phosphate is slightly higher than that of serine, suggesting that phosphorylation increases the capability of a peptide or protein segment to form an α-helix. Because the phosphonate group is more electron-rich than the corresponding phosphate ester, the side chain of AbuP is a better hydrogen-bond acceptor than that of SerP. The AbuP/arginine interaction stabilizes α-helix formation by 0.17 kcal mol^{-1} more than that of SerP/arginine. This difference is fairly small and should not limit the use of AbuP as a mimic of SerP. Since AbuP cannot be hydrolyzed by enzymes, its use may aid the study of SerP in nature.

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6. Peptides were synthesized and HPLC purified by PeptidoGenic & Co. Peptides were synthesized on a 0.1 mmol scale, using 4 mole equivalents of Fmoc protected amino acid and HBTU/HOBt as coupling reagents at each coupling step. Peptides were acetylated, cleaved from the resin, and then deprotected using methods developed by Perich.5a Purity was determined to be 98.1, 95.6, 100, and 99.3% for peptides **1–4**. All peptides were characterized by ESI-MS, UV, ^{31}P NMR, and CD.

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8. Indeed, models indicate that, whereas side chains spaced *i*/*i*+4 are oriented with a dihedral angle of approximately 30°, those spaced *i*/*i*+3 have a dihedral angle of approximately –70°.

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10. Percent helicities of peptides were calculated from their mean residue ellipticities at 222 nm ($[\theta]_{222}$), using –29,717 deg cm^2 dmol^{-1} as the calculated value of $[\theta]_{222}$ for the fully α-helical peptide[8d] and 640 deg cm^2 dmol^{-1} as the value of $[\theta]^{222}$ for the fully random coil peptide. For comparison, values of $[\theta]_{222}$ for the "completely" α-helical peptides **1–4** estimated by titration with 2,2,2-trifluoroethanol were –27,950, –27,930, –28,710, and –28,680 deg cm^2 dmol^{-1}, respectively. Thus, the calculated and measured values of ellipticity for fully α-helical peptides were virtually identical. We used the value of $[\theta]_{222}$ calculated for a fully helical peptide since this was the value used in derivation of the Lifson–Roig parameters used in our study.

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