Phosphorylation of Annexin A1 by TRPM7 Kinase: A Switch Regulating the Induction of an α-Helix

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§Supporting Information

ABSTRACT: TRPM7 is an unusual bifunctional protein consisting of an α-kinase domain fused to a TRP ion channel. Previously, we have identified annexin A1 as a substrate for TRPM7 kinase and found that TRPM7 phosphorylates annexin A1 at Ser5 within the N-terminal α-helix. Annexin A1 is a Ca^{2+}-dependent membrane binding protein, which has been implicated in membrane trafficking and reorganization. The N-terminal tail of annexin A1 can interact with either membranes or S100A11 protein, and it adopts the conformation of an amphipathic α-helix upon these interactions. Moreover, the existing evidence indicates that the formation of an α-helix is essential for these interactions. Here we show that phosphorylation at Ser5 prevents the N-terminal peptide of annexin A1 from adopting an α-helical conformation in the presence of membrane-mimetic micelles as well as phospholipid vesicles. We also show that phosphorylation at Ser5 dramatically weakens the binding of the peptide to S100A11. Our data suggest that phosphorylation at Ser5 regulates the interaction of annexin A1 with membranes as well as S100A11 protein.

Phosphorylation of amino acids within proteins is an important mechanism for signal transduction in the cell; however, the effects of phosphorylation on protein structure are not well understood. It has been demonstrated that phosphorylation of threonine or serine can affect the helix-forming propensity of proteins.1,2 Since protein interactions often involve α-helices, phosphorylations modulating formation of α-helices might be a mechanism for regulating protein interactions.

Recently, we have discovered a novel family of protein kinases, α-kinases.3,4 These kinases can phosphorylate their substrates within α-helices, unlike conventional protein kinases, which phosphorylate substrates within β-turns, loops, and irregular structures.5-8 TRPM7 is an unusual bifunctional molecule in which an α-kinase domain is fused to a TRP ion channel. TRPM7 channel can conduct both Mg^{2+} and Ca^{2+} and is believed to play an important role in Mg^{2+} and Ca^{2+} homeostasis, regulating cell growth and proliferation, cell adhesion, as well as cell death during anoxia.7 The role of the kinase domain in TRPM7 function is not fully understood and may involve autophosphorylation of TRPM7 as well as phosphorylation of other target proteins. Previously, we have identified annexin A1 as a target of TRPM7.8 We have found that annexin A1 is phosphorylated by TRPM7 at Ser5 within the N-terminal tail.8 The existing data indicate that, when not phosphorylated, the N-terminal tail of annexin A1 adopts an amphipathic α-helix conformation upon interacting with membranes9 or the S100A11 protein.10

Annexin A1, a Ca^{2+}-dependent membrane-binding protein, which is involved in the regulation of membrane trafficking and reorganization, is a mediator of the anti-inflammatory action of glucocorticoids and is implicated in the regulation of proliferation, differentiation, and apoptosis.11,12 Annexin A1, a protein of ∼38 kDa, consists of a Ca^{2+}-binding core domain, with a slightly curved disk shape, and an N-terminal tail domain of ∼40 amino acids. Annexin A1 requires calcium for binding to negatively charged phospholipid membranes through the convex side of its core domain.11 Existing evidence suggests that the N-terminal tail domain can regulate the membrane binding properties of annexin A1 and can function as a secondary Ca^{2+}-dependent membrane-binding site.11,13,14 The N-terminal tail domain can also interact with S100A11 in a Ca^{2+}-dependent manner.10,15,16 S100A11 is a homodimeric EF-hand Ca^{2+}-binding protein that is involved in a variety of intracellular activities, including coordination of membrane association upon interaction with annexin A1.12 The important characteristic of annexin A1 is its ability to connect two adjacent membranes. According to the current model, annexin A1 can connect membranes by two distinct mechanisms11,13,14 in the presence of Ca^{2+}, annexin A1 binds to a membrane through the core domain and releases its N-terminal

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tail and (i) the N-terminal tail can bind a second membrane or (ii) two annexin A1 molecules bound to two separate membranes can be bridged via their N-terminal tails by an S100A11 dimer.

The crystal structure of annexin A1 in the absence of Ca$^{2+}$ showed that the first 17 amino acid residues form an α-helix, which is buried inside the core domain. The first 12 N-terminal amino acid residues form an amphipathic α-helix containing Met3, Val4, Phe7, Leu8, and Trp12 on one side of the helix and adopt an flexible linker formed by residues 18–26 and a flexible linker formed by residues 27–41. In the presence of Ca$^{2+}$, however, the N-terminal tail is expelled from the core domain and is disordered in X-ray structure. The crystal structure of the S100A11 protein in complex with the N-terminal peptide of annexin A1 revealed that the peptide also forms an amphipathic α-helix upon interaction with S100A11. It has also been demonstrated that the N-terminal peptide of annexin A1, while in a random-coil conformation in aqueous solution, forms an amphipathic α-helix in membrane-mimetic environments as well as upon interaction with phospholipid membranes. Thus, existing data indicate that the N-terminal tail of annexin A1, while unstructured in aqueous solution, adopts the conformation of an amphipathic α-helix upon interaction with membranes, S100A11 protein, or the core domain of annexin A1 itself, suggesting that the α-helical conformation of the N-terminal tail is induced by these interactions.

Here we investigate the consequences of phosphorylating Ser5 on the ability of the N-terminal peptide of annexin A1 to adopt an α-helical conformation in the presence of membrane mimetics and also analyze the effect of this phosphorylation on the ability of the peptide to bind to S100A11.

**EXPERIMENTAL PROCEDURES**

**Materials.** Chemicals, unless mentioned otherwise, were obtained from Sigma. Dimyristoylphosphatidylcholine (DMPC), dimyristoylphosphatidylserine (DMPS), and dodecylphosphocholine (DPC) were obtained from Avanti Polar Lipids. Dodecyl β-o-glucoside was obtained from Fisher Scientific. Peptides were synthesized at Sigma-Genosys at >95% purity. The sequences and the purity of peptides were verified by mass spectrometry and high-performance liquid chromatography. The peptides were N-acetylated, and the sequences of the peptides were as follows: Ac1–18, Ac-AMVSFLKQAWFIENEEQ; Ac1–18P, Ac-AMV[pSer]EFLKQAWFIENEEQ.

Since in the cell annexin A1 exists as a protein modified by the cleavage of the N-terminal methionine with subsequent N-acetylation, N-acetylated peptides of annexin A1 corresponding to residues 2–19 of annexin A1 were used. The concentrations of peptide stock solutions were verified by UV spectra and calculated with an extinction coefficient of 5500 M$^{-1}$ cm$^{-1}$.

Recombinant porcine S100A11 protein was expressed and purified as described previously. The purity of the protein was verified by SDS gel electrophoresis to be >95%. The concentration of the protein was determined by the bicinchoninic acid protein assay (Pierce). The protein solution was adjusted to 1 mM DTT and 2 mM NaCN and stored at 4 °C.

**Circular Dichroism (CD) Spectroscopy.** CD measurements were taken at 25 °C on an Aviv model 400 spectropolarimeter equipped with a thermoelectrically controlled cell holder. CD spectra were recorded at 0.5 nm intervals with an averaging time of 5 s in the wavelength range of 190–260 nm. Cylindrical fused quartz cells with a path length of 0.1 cm were used. For measurements in the presence of SDS, 200 μM peptide stocks in buffer solution [50 mM Tris-HCl (pH 7.4), 150 mM NaCl, and 0.2 mM EGTA] were used. Peptide (20 μM) in a 300 μL sample volume was used for measurements in buffer solution [5 mM Tris-HCl (pH 7.4), 15 mM NaCl, and 0.02 mM EGTA]. Increasing concentrations of SDS were obtained by sequential addition of the stock solution (the corresponding peptide at 20 μM in 347 mM SDS) to the cuvettes. The buffer signal was measured at each SDS concentration via addition of 347 mM SDS to the cuvette containing 5 mM Tris-HCl (pH 7.4), 15 mM NaCl, and 0.02 mM EGTA. The CD signals of SDS were subtracted to yield the presented CD spectra. In the experiments with 150 mM NaCl, the salt concentration was adjusted accordingly.

For measurements in the presence of TFE, 200 μM peptide stocks in buffer solution [50 mM Tris-HCl (pH 7.4), 150 mM NaCl, and 0.2 mM EGTA] were mixed with water and the corresponding amount of TFE to yield 20 μM peptide in a 300 μL sample. The TFE signal was measured at each concentration of TFE by mixing the corresponding amount of TFE, water, and 30 μL of buffer solution [50 mM Tris-HCl (pH 7.4), 150 mM NaCl, and 0.2 mM EGTA] to produce a 300 μL sample. The CD signals of TFE were subtracted to yield the presented CD spectra.

For measurements in the presence of dodecylphosphocholine (DPC), dodecyl β-o-glucoside (DG), octyl β-o-glucoside (OG), or dodecyltrimethylammonium bromide (DTAB), 200 μM stock solutions of peptides in 50 mM Tris-HCl (pH 7.4) were used. Peptide (20 μM) in a 300 μL sample volume was used for measurements in buffer solution [5 mM Tris-HCl (pH 7.4) and 20 mM sodium phosphate buffer (pH 7.4)] and the indicated amounts of detergents. The signals of detergents alone in the buffer were subtracted to yield the presented CD spectra.

For CD measurements in the presence of phospholipids, DMPC/DMPS small unilamellar vesicles (SUVs) were prepared as described previously. DMPC/DMPS (3:1 molar ratio) SUVs were prepared at a concentration of 10 mg/mL in 10 mM sodium phosphate buffer (pH 6.2); 250 μM stock solutions of peptides in 20 mM Hepes (pH 7.4) were used. The stock solutions of the peptides were diluted with 10 mM sodium phosphate buffer (pH 6.2) and mixed with DMPC/DMPS SUVs to yield final concentrations of 25 μM for peptide and 4 mM for SUVs in a 300 μL sample. The SUVs alone produced a strong signal in the CD spectrum. The CD signal of SUVs was subtracted to yield the presented CD spectra.

**Steady-State Fluorescence Spectroscopy.** The emission spectra were recorded with a PTI (Lawrenceville, NJ) fluorometer with 2 nm excitation and 4 nm emission slit widths. Quartz cells with 0.4 and 1 cm path lengths in the excitation and emission directions, respectively, were used. Emission spectra were recorded between 300 and 500 nm with excitation at 295 nm for the intrinsic tryptophan fluorescence. Two hundred μM peptide stocks in buffer solution [50 mM Tris-HCl (pH 7.4), 150 mM NaCl, and 0.2 mM EGTA] were used. The fluorescence emission spectra were recorded in 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.2 mM EGTA, and 0.7 mM CaCl$_2$ or, as a control, without Ca$^{2+}$. For titration experiments, aliquots of the mixture of 250 μM S100A11 and the respective peptide at 10 μM were sequentially added to a 10 μM solution of Ac1–18 or Ac1–18P. To obtain the spectra of S100A11 alone, aliquots of 250 μM S100A11 were sequentially added to the buffer solution. The absorbance of the solutions at 295 nm did not exceed 0.1. The experiment was run in three separate cells in parallel using four-cell holder. The
spectra recorded for each sample were corrected by subtraction of the signal provided by the buffer in the corresponding cell. Then the spectra at each concentration of S100A11 were corrected by subtraction of the spectra of S100A11 alone. The data were processed using KaleidaGraph version 4.0 (Synergy Software). The dissociation constants were determined by fitting the S100A11-induced changes in the fluorescence of the peptide at 335 nm using the following equation (eq 1):

\[
I = I_0 + \frac{I_\infty - I_0}{2} \left( \frac{[S]_{\text{tot}} + [P]_{\text{tot}} + K_d}{[S]_{\text{tot}} + [P]_{\text{tot}} + K_d} \right)^2 - 4 \frac{[S]_{\text{tot}} [P]_{\text{tot}}}{([S]_{\text{tot}} + [P]_{\text{tot}} + K_d)^2}
\]

where \(I_0\) and \(I\) are the fluorescence emission intensities of the peptides in the absence and presence of S100A11, respectively, \(I_\infty\) is the fluorescence emission intensity of the peptide in the presence of an infinite S100A11 concentration, and \([S]_{\text{tot}}\) and \([P]_{\text{tot}}\) are the total concentrations of S100A11 and peptide, respectively. The equation describes a model with one peptide-binding site per S100A11 monomer.

**RESULTS**

In this work, we employed the N-terminal peptide of annexin A1 containing 18 N-terminal residues (Ac1–18), which has been used previously in binding studies with S100A11 protein. To examine the effect of phosphorylation by TRPM7, we used a similar peptide phosphorylated at Ser5, named Ac1–18P.

To investigate the effect of phosphorylation on the ability of the N-terminal peptide of annexin A1 to form an \(\alpha\)-helix in the membrane environment, we examined the structures of Ac1–18 and Ac1–18P peptides in the presence of sodium dodecyl sulfate (SDS) micelles, which mimic the environment of anionic phospholipid membranes. We have found that phosphorylation of Ser5 prevents induction of an \(\alpha\)-helical conformation in the N-terminal peptide of annexin A1 in the presence of SDS.

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**Figure 1.** Effect of Ser5 phosphorylation on the structure of the Ac1-18 peptide in the presence of SDS or TFE. (A) CD spectra of 20 \(\mu\)M Ac1-18 (left) and Ac1-18P (right) in the presence of the indicated concentrations of SDS and 15 mM NaCl. (B) CD spectra of 20 \(\mu\)M Ac1-18 (left) and Ac1-18P (right) in the presence of the indicated concentrations of TFE and 15 mM NaCl.
micelles. According to the CD spectroscopy analysis, both phosphorylated and unphosphorylated peptides have mostly random-coil conformation in aqueous buffer (Figure 1A). At increasing concentrations of SDS, we observed a dramatic increase in the α-helical content of Ac1–18 as the SDS concentration reaches the critical micelle concentration (CMC) for SDS at 15 mM NaCl18,19 (Figure 1A, left panel). In the buffer alone or at a SDS concentration below the CMC, the shape of the CD spectrum indicates mostly random-coil conformation of Ac1–18. In the presence of SDS at concentrations above the CMC, however, the positions of the maximum and minimum on the CD spectra indicate an α-helical conformation for Ac1–18. In contrast, phosphorylated peptide Ac1–18P remained mostly random coil at concentrations of SDS high above the CMC (Figure 1A, right panel). In Figure 1A of the Supporting Information, these data are also presented as the dependence of the mean residue ellipticity at 222 nm on the concentration of SDS.

In a buffer containing 150 mM NaCl (as compared to 15 mM), we observed similar ellipticity changes occurring now at a lower concentration of SDS, in agreement with the known lower CMC for SDS at a salt concentration of 150 mM18,19 (Figure 1B of the Supporting Information). These results support the assertion that the formation of micelles and not simply the concentration of SDS is the critical factor for induction of an α-helical conformation in the peptide.

We have also examined the ability of the peptides to adopt an α-helical conformation in the presence of trifluoroethanol (TFE), which has the ability to stabilize an α-helical conformation of peptides. In aqueous TFE solutions, both Ac1–18 and Ac1–18P are similarly able to form α-helices in a TFE concentration-dependent manner (Figure 1B), indicating that phosphorylation does not affect the α-helical propensity of the peptide in a hydrophobic TFE environment.

We also investigated whether the ability of the peptides to form an α-helix in the presence of micelles depends on the ionic nature of the headgroup of the detergent. Using CD spectroscopy, we examined the structures of Ac1–18 and Ac1–18P in the presence of dodecylphosphocholine (DPC), dodecyl β-D-glucoside (DG), or dodecyltrimethylammonium bromide (DTAB) micelles, which have the same 12-carbon aliphatic tail as SDS but possess a zwitterionic, nonionic, or cationic headgroup, respectively, in place of the anionic headgroup of SDS. In the presence of 4 mM DPC (CMC = 1.1), we observed a dramatic increase in the α-helical content of Ac1–18 similar to that in the presence of SDS micelles (Figure 2A). However, the helical content of Ac1–18P in the presence of DPC was significantly decreased in comparison with that of Ac1–18 (Figure 2A). Therefore, phosphorylation at Ser5 interferes with the induction of an α-helical conformation in the peptide in the presence of zwitterionic DPC micelles, though to a lesser degree than in the presence of anionic SDS micelles. The ability of Ac1–18 to form an α-helix in the presence of DPC is consistent with previous data showing that unlike the primary binding through the annexin A1 core, which has a strict requirement for anionic phospholipids, the secondary binding through the N-terminal tail can occur with both anionic and zwitterionic phospholipids.

In the presence of 0.25 mM DG (CMC = 0.19 mM), both peptides have a mostly random-coil conformation (Figure 2B). Similarly, in the presence of 30 mM octyl β-D-glucoside (CMC = 25 mM), another detergent with a nonionic headgroup, we did not observe significant changes in the structure of the peptides (data not shown). In the presence of 15 mM DTAB (CMC = 14.6 mM), we could obtain CD spectra only above 215 nm, because of the high absorbance and/or scatter of DTAB micelles below 215 nm. The values of mean residue elipticities at 222 nm for both Ac1–18 and Ac1–18P increased dramatically upon addition of DTAB (Figure 2C), similar to the mean residue ellipticity at 222 nm of Ac1–18 in the presence of SDS or DPC. These results indicate that phosphorylation at Ser5 does not prevent the induction of an α-helical conformation in the peptide in the presence of cationic DTAB micelles. Overall, our data suggest that the presence of the ionic headgroup in the detergent is important for the ability of the peptide to form an α-helix and that phosphorylation of the peptide inhibits the induction of an α-helical conformation in the presence of anionic or zwitterionic micelles.

Next we investigated the effect of phosphorylation at Ser5 on the ability of the Ac1–18 peptide to form an α-helix in the presence of phospholipid vesicles. It has been demonstrated previously that the N-terminal peptide corresponding to residues 2–26 of annexin A1 adopts an α-helical conformation in the presence of phospholipid vesicles (DMPC/DMPS small
S100A11 in the presence of 0.5 mM Ca\(^{2+}\) intensity in the absence of S100A11 (peptide to S100A11 protein. Changes in the intrinsic tryptophan fluorescence of 10 emission intensity at 335 nm (experimental data to eq 1. We normalized the obtained fluorescence of which can be induced by excitation at 295 nm. Since S100A11 lacks tryptophan, the recorded emission spectrum reflects solely the signal from tryptophan of Ac1−18. The shift of the maximum of the tryptophan emission spectrum to a shorter wavelength (blue shift) with a concomitant increase in fluorescence intensity is indicative of binding of the peptide to S100A11, because upon binding, Trp12 of the peptide partitions into a hydrophobic environment of the S100A11-binding pocket.10,15 To investigate how phosphorylation at Ser5 affects binding of the Ac1−18 peptide to S100A11, we recorded the emission spectra of Ac1−18 or Ac1−18P upon sequentially increasing concentrations of S100A11 in the presence of 0.5 mM Ca\(^{2+}\) (Figure 2 of the Supporting Information). In the absence of S100A11, the fluorescence maximum for both peptides is located at \(\sim 350\) nm, corresponding to emission of fully exposed tryptophan. The addition of increasing concentrations of S100A11 induced a blue shift in the emission spectra of Ac1−18 and Ac1−18P in a concentration-dependent manner and a concomitant increase in the fluorescence intensity. The emission spectra of the peptides alone were not affected by the addition of Ca\(^{2+}\), and the addition of S100A11 to Ac1−18 or Ac1−18P in the absence of Ca\(^{2+}\) did not produce a blue shift in the emission spectra (data not shown).

![Figure 3. Effect of Ser5 phosphorylation on the structure of the Ac1−18 peptide in the presence of DMPC/DMPS vesicles. CD spectra of 25 \(\mu M\) Ac1−18 (A) or Ac1−18P (B) in the presence (circles) or absence (triangles) of 4 mM DMPC/DMPS (3:1 molar ratio) small unilamellar vesicles (SUV).](image)

Our results show that phosphorylation of the N-terminal annexin A1 peptide interferes with the peptide’s ability to form an \(\alpha\)-helix upon interaction with anionic or zwitterionic membrane-mimetic micelles and phospholipid vesicles. Our results also show that phosphorylation of the peptide dramatically weakens its binding to S100A11. However, phosphorylation of Ser5 does not significantly affect the helicity of the peptide in the presence of TFE. Since the phosphorylated peptide is able to adopt an \(\alpha\)-helical conformation in the uniformly hydrophobic environment of TFE, A1 contains a single tryptophan, the fluorescence of which can be induced by excitation at 295 nm. Since S100A11 lacks tryptophan, the recorded emission spectrum reflects solely the signal from tryptophan of Ac1−18. The shift of the maximum of the tryptophan emission spectrum to a shorter wavelength (blue shift) with a concomitant increase in fluorescence intensity is indicative of binding of the peptide to S100A11, because upon binding, Trp12 of the peptide partitions into a hydrophobic environment of the S100A11-binding pocket.10,15 To investigate how phosphorylation at Ser5 affects binding of the Ac1−18 peptide to S100A11, we recorded the emission spectra of Ac1−18 or Ac1−18P upon sequentially increasing concentrations of S100A11 in the presence of 0.5 mM Ca\(^{2+}\) (Figure 2 of the Supporting Information). In the absence of S100A11, the fluorescence maximum for both peptides is located at \(\sim 350\) nm, corresponding to emission of fully exposed tryptophan. The addition of increasing concentrations of S100A11 induced a blue shift in the emission spectra of Ac1−18 and Ac1−18P in a concentration-dependent manner and a concomitant increase in the fluorescence intensity. The emission spectra of the peptides alone were not affected by the addition of Ca\(^{2+}\), and the addition of S100A11 to Ac1−18 or Ac1−18P in the absence of Ca\(^{2+}\) did not produce a blue shift in the emission spectra (data not shown).

To determine dissociation constants (\(K_d\)) for the binding of Ac1−18 or Ac1−18P to S100A11, S100A11-induced changes in fluorescence at 335 nm were plotted versus S100A11 concentration (Figure 4), and the data were fitted to eq 1. We found that Ac1−18 binds to S100A11 with a \(K_d\) value of 2.1 ± 0.2 \(\mu M\), which is similar to a previous estimate.23 The \(K_d\) value for binding of Ac1−18P to S100A11 was 6.8 ± 1 \(\mu M\), indicating that phosphorylation of the N-terminal peptide of annexin A1 at Ser5 significantly decreases its affinity for S100A11 association.

### DISCUSSION

Our results show that phosphorylation of the N-terminal annexin A1 peptide interferes with the peptide’s ability to form an \(\alpha\)-helix upon interaction with anionic or zwitterionic membrane-mimetic micelles and phospholipid vesicles. Our results also show that phosphorylation of the peptide dramatically weakens its binding to S100A11. However, phosphorylation of Ser5 does not significantly affect the helicity of the peptide in the presence of TFE. Since the phosphorylated peptide is able to adopt an \(\alpha\)-helical conformation in the uniformly hydrophobic environment of TFE,
the effects observed in our work may reflect the decrease in the α-helix forming ability of the phosphorylated peptide specifically upon interaction with membrane mimetics or S100A11. Because of the amphiphilic nature of the Ac1–18 peptide, the structure of the peptide could be stabilized upon interaction with membrane mimetics or S100A11 by hydrophobic interactions on one side and electrostatic interactions on the other side of an amphiphatic helix.

The existing data suggest that membrane binding of the N-terminus of annexin A1 is driven by hydrophobic as well as electrostatic interactions. Via analysis of the membrane-bound state of the N-terminal peptide of annexin A1, it has been found that the peptide adopts a peripheral mode of binding and is oriented parallel to the membrane surface. Therefore, the effect observed in our work could be due to the electrostatic repulsion of phosphorylated Ser5 by the negatively charged membrane-mimetic or phospholipid headgroups, making the induction of an amphiphatic α-helix energetically unfavorable in these membrane-mimetic environments. This assumption is consistent with our results, which show that phosphorylation of the peptide has a dramatic effect on its ability to form an α-helix in the presence of anionic micelles, a weaker effect in the presence of zwitterionic micelles, and no effect in the presence of cationic micelles. The ability to form an amphiphatic α-helix, observed for many membrane-interacting peptides and proteins, is crucial for the interaction with membranes. Therefore, the inability of the phosphorylated peptide to form an α-helix in the presence of micelles and phospholipid vesicles suggests that phosphorylation weakens substantially, if not prevents, its binding.

The crystal structure of the S100A11 protein in a complex with Ac1–18 revealed that the peptide also forms an amphipatic α-helix. When calcium binds, S100A11 exposes a hydrophobic surface, which can then interact with the hydrophobic side of the N-terminal α-helix of annexin A1. The helical conformation of the N-terminal peptide of annexin A1 is probably induced by the environment of the binding pocket of S100A11 protein. In the complex of the N-terminal peptide of annexin A1 with S100A11, the hydrophobic residues of the peptide are buried within the complex, while the hydrophilic residues of the peptide form hydrogen bonds with the N-terminal helix of S100A11, where Glu9 of S100A11 forms a hydrogen bond with Ser5 of the peptide. The weakened binding of the phosphorylated peptide to S100A11 might reflect the decrease in the α-helix forming ability of the phosphorylated peptide in the environment of the S100A11-binding pocket. Alternatively, it is possible that phosphorylation results in unfavorable steric contacts of phospho-Ser5 and/or electrostatic repulsion of phospho-Ser5 in the proximity of Glu9.

In summary, our data show that phosphorylation of Ser5 prevents the N-terminal peptide of annexin A1 from adopting an α-helical conformation in the presence of membrane mimetics and phospholipid vesicles as well as dramatically weakens binding of the peptide to S100A11 protein. Our results suggest that phosphorylation at Ser5 modulates the interactions of the N-terminal tail of annexin A1 with membranes as well as S100A11 protein that can have important physiological implications for the binding activities of annexin A1 in the cell.

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