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Synthesis and initial characterization of FGFR3 transmembrane domain: consequences of sequence modifications

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Abstract

Receptor Tyrosine Kinases (RTKs) conduct biochemical signals via lateral dimerization in the plasma membrane, and defects in their dimerization lead to unregulated signaling and disease. RTK transmembrane (TM) domains are proposed to play an important role in the process, underscored by the finding that single amino acids mutations in the TM domains can induce pathological phenotypes. Therefore, many important questions pertaining to the mode of signal transduction and the mechanism of pathology induction could be answered by studying the chemical–physical basis behind RTK TM domain dimerization and the interactions of RTK TM domains with lipids in model bilayer systems. As a first step towards this goal, here we report the synthesis of the TM domain of fibroblast growth factor receptor 3 (FGFR3), an RTK that is crucial for skeletal development. We have used solid phase peptide synthesis to produce two peptides: one corresponding to the membrane embedded segment and the naturally occurring flanking residues at the N- and C-termini (TM_{wt}), and a second one in which the flanking residues have been substituted with diLysines at the termini (TM_{KK}). We have demonstrated that the hydrophobic FGFR3 TM domain can be synthesized for biophysical studies with high yield. The protocol presented in the paper can be applied to the synthesis of other RTK TM domains. As expected, the Lys flanks decrease the hydrophobicity of the TM domain, such that TM_{KK} elutes much earlier than TM_{wt} during reverse phase HPLC purification. The Lysines have no effect on peptide solubility in SDS and on peptide secondary structure, but they abolish peptide dimerization on SDS gels. These results suggest that caution should be exercised when modifying RTK TM domains to render them more manageable for biophysical studies.

Keywords: Receptor tyrosine kinase; FGFR3; Solid phase peptide synthesis; Terminal lysine

1. Introduction

RTKs are type I transmembrane proteins with three distinct domains: an N-terminal extracellular ligand-binding domain, usually several hundred amino acids long and containing characteristic arrays of structural motifs; a TM domain; and a catalytic domain related to soluble tyrosine kinases [1,2]. Signal transduction is initiated when a ligand (a growth factor) binds to the receptor. This is believed to occur via a ligand-induced conformational change in the extracellular domain that results in the dimerization of the receptor [3–7]. The contact between the two cytoplasmic domains results in the intermolecular autophosphorylation of the receptor subunits, triggering signaling cascades.

Some recent findings suggest that RTK TM domains may be critical for the dimerization process. The work of Tanner and Kyte [8] showed that, at least for EGFR, the TM domains are the driving force behind dimerization,

Abbreviations: TM, transmembrane; RTK, receptor tyrosine kinase; FGFR3, fibroblast growth factor receptor 3; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; HFIP, hexafluoroisopropanol

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while the extracellular domains act to prevent dimerization in the absence of the ligand. This view is supported by recent crystal structures of RTK extracellular domains [9,10], and by the finding that the TM domains of ErbB1-4 dimerize in the absence of extracellular domains and ligands [11]. Furthermore, Bell et al. [12] have demonstrated that the TM domains in the dimer position the catalytic domains in such a way that they can autophosphorylate each other. The importance of RTK TM domains in signal transduction is further underscored by the fact that single amino acid substitutions in the TM domains can lead to constitutive receptor activation and therefore to pathologies such as cancers and growth disorders [13,14].

Many important questions pertaining to the mode of signal transduction and the mechanism of pathology induction could thus be answered by studying the chemical–physical basis behind RTK TM domain dimerization and the interactions of RTK TM domains with lipids in model bilayer systems. An important issue, therefore, is the production of pure TM domains with high yield. One option for protein production is expression in bacteria. Often, however, a specifically labeled residue needs to be introduced at a chosen site, without perturbing the same amino acid elsewhere in the sequence. This specific labeling approach, in conjunction with solid state NMR, has already provided valuable information about the mechanism of Neu constitutive activation [15,16]. If specific labeling is required, chemical synthesis is the method of choice for peptide production.

The TM domain of FGFR3, an RTK critical for skeletal development, has multiple mutations known to cause developmental abnormalities and cancer: Gly380→Arg, Ala391→Glu, Gly370→Cys370, Ser371→Cys371, and Tyr373→Cys373 [17–19]. These mutations are restricted to the TM domain, such that biophysical studies of the TM domain can provide insight into the mechanism of pathogenesis. As a first step towards this goal, we have synthesized the FGFR3 TM domain with high yield. This experience will be likely useful for the synthesis of other RTK TM domains.

Often, Lys residues are added to the N- and C-termini of the peptides to render them manageable for biophysics studies [20–23]. We therefore synthesized a version of FGFR3 TM domain in which the flanking polar residues were substituted with diLysines. We then studied the effect of the terminal lysines on the overall hydrophobicity of the peptide, on its secondary structure, and on its dimerization propensity in SDS. We found that the diLysine flanking segments can affect FGFR3 TM domain dimerization in SDS micelles. However, a previous study of Melnyk et al. [24] has demonstrated that flanking lysines do not disrupt the oligomeric state of glycophorin A and the influenza A virus M2 ion channel in SDS. Therefore, the effect of the flanking Lysines on the dimerization of hydrophobic peptides can vary with the peptide sequence.

2. Materials and methods

2.1. Synthesis

The peptides were synthesized via solid phase synthesis on a model 431 ABI peptide synthesizer (Applied Biosystems), using 9-fluorenylmethoxycarbonyl (Fmoc) chemistries. Fmoc-amino acids were purchased from Anaspec Inc. (San Jose, CA). The following protected amino acids were used: Arg(Pbf) and Lys(*t*-Boc), Cys(trityl), Asp(*t*-butyl), Glu(*t*-butyl), Ser(*t*-butyl), Thr(*t*-butyl), and Tyr(*t*-butyl). Capping reagent was prepared as follows: 19 mL acetic anhydride was mixed with 9 mL DIEA and 6 mL of a 1 molar solution of HOBt in NMP, and NMP was added to a final volume of 400 mL. The resin used was CLEAR-amide resin, 100–200 mesh (Peptides International, Louisville, KY). To enhance synthetic yields by limiting on-resin peptide–peptide interactions, a reduced resin loading protocol was employed. Less than one equivalent of the first amino acid was added to the resin and coupled for the usual time. The resin was then washed to limit further coupling. All unreacted sites were acetylated with acetic anhydride with HOBt. The rationale behind this step, which reduces the resin loading by up to 50%, was that only the highly exposed sites on the resin will react with the first amino acid, and only they should be used in the synthesis. All additional amino acids were double or triple coupled. The three Phe residues in the middle of the sequence, and the YVAGI segment at the N-terminus of the hydrophobic segment required triple coupling. After each coupling step, the remaining unreacted sites were acetylated.

2.2. Cleavage/deprotection and purification

For the cleavage/deprotection, the dry resin was suspended in 500 μL of ethanedithiol (EDT, scavenger) (Aldrich Chemical Co., Milwaukee, WI). After incubation for 3 min with the scavenger alone, 9.5 mL of neat TFA was added and allowed to react for 90 min. After cleavage, the peptide was precipitated with 35 mL diethyl ether and washed four times with ether. The peptide was then redissolved in 75% acetonitrile in water. This solution was extracted with ether (liquid/liquid) two additional times and then dried in vacuo. The dried peptide was redissolved in a HFIP/water mixture and then purified using reverse phase HPLC on a Vydac 214TP54 C4 column, using a water/acetonitrile gradient (Solvent A: Water containing 0.1% TFA; Solvent B: 90% acetonitrile containing 0.1% TFA). The preferred method was as follows: the column was equilibrated with 30% B, the sample was injected and held for 5 min at 30% B, followed by a 25 min linear gradient from 30% to 100% B, 15 min at 100% B, a 5 min gradient from 100% B to 30% B, and finally 5 min at 30% B. Peptide fraction peaks were monitored at 220 nm and collected manually.

Molecular weight was confirmed using MALDI-TOF mass spectrometry.

2.3. Circular dichroism

CD spectra of FGFR3 TM domain in HFIP/water, MeOH, SDS, and lipid vesicles were collected using a Jasco 710 spectropolarimeter. For the experiments in detergents and liposomes, we used 10 mM phosphate buffer, pH 7. The concentrations of the peptides in the samples, required for calculating molar ellipticities, were determined from absorbance measurements in a Cary 50 (Varian) UV/VIS spectrophotometer.

2.4. Oriented CD

Oriented CD measurements were performed as previously reported [25–28]. Peptides and lipids were co-dissolved in HFIP/chloroform. Dropwise, the solution was deposited on a quartz slide and the solvent was removed under a stream of nitrogen to form a mutilamellar sample containing the peptides. The quartz slide was mounted on a custom designed chamber. To hydrate, a drop of water was placed in the chamber and the sample was equilibrated overnight. The chamber was placed in the Jasco 710 spectropolarimeter such that the multilayers were perpendicular to the beam. The sample was rotated around the beam axis in increments of 45°. Eight discreet spectra were thus collected and averaged.

2.5. SDS-PAGE

The peptide samples were subjected to SDS-PAGE using 10–20% tricine precast gels (Novex, San Diego, CA). The TM domains were dissolved in SDS-containing sample buffer, reduced with NuPAGE reducing agent, boiled for 5 min, and loaded onto the gels. The peptides were visualized with Coomassie blue.

3. Results and discussion

3.1. Synthesis of FGFR3 TM domains

The chemical production of long hydrophobic TM domains has become feasible with the development of new solid phase synthesis methods and instrumentation [16,29–34]. Here we report the synthesis and initial characterization of the TM domain of FGFR3. We have synthesized two peptides: one corresponding to the membrane embedded segment of FGFR3 and the naturally occurring flanking residues at the N- and C-termini (TM_{wt}), and a second one in which the flanking residues have been changed with diLysines (TM_{KK}) (see Table 1). The yield of the synthesis was >70% for TM_{wt} and >90% for TM_{KK}. Fig. 1 shows the HPLC traces of the crude peptides, run over a Vydac 214TP54 C4 column using a water/acetonitrile gradient. The major peaks correspond to the correct peptide products. The measured molecular weights of TM_{wt} and TM_{KK} matched the calculated ones, 3520.2 and 2931.6.

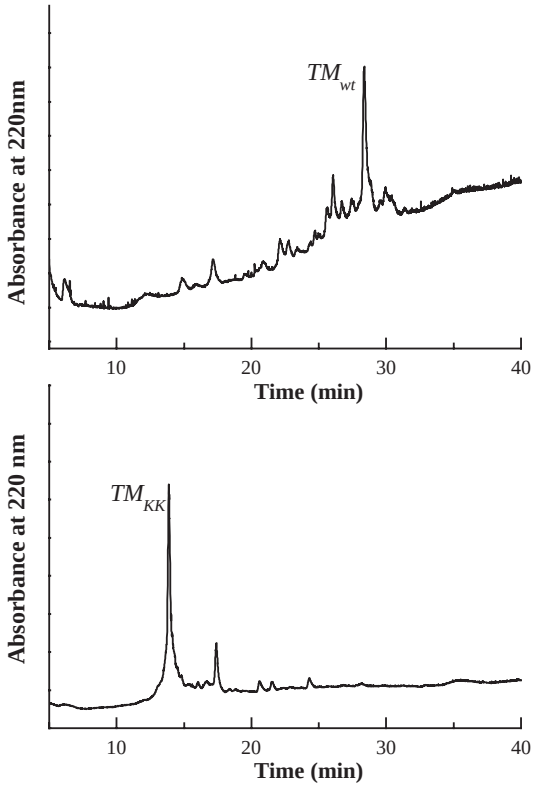

Fig. 1. HPLC traces of crude TM_{wt} and TM_{KK}, obtained using ProStar HPLC (Varian) and Vydac 214TP54 C4 column. The main peaks correspond to the correct synthesis products, as identified by mass spectrometry. TM_{KK} elutes much earlier than TM_{wt}, indicating that the flanking Lysines reduce the over-all hydrophobicity of the peptide at pH 2.

The HPLC traces in Fig. 1 show that the peptides elute at different times: TM_{wt} elutes at about 85% acetonitrile, while TM_{KK} elutes at about 50% acetonitrile under the acidic conditions employed. The difference in

Table 1
Amino acid sequences of the synthesized peptides: (1) FGFR3 TM_{wt}; (2) FGFR3 TM_{KK}

Sequence	MW	ΔG pH 2/7 (kcal/mol)	ΔG_{AVE} pH 2/7 (kcal/mol)	ΔG_{core} pH 2/7 (kcal/mol)
NH_2-DEAGS-core-TLCRLR-$CONH_2$	3520.2	−2.36/7.89	−0.07/0.23	−9.42/−9.42
NH_2-KK-core-TKK-$CONH_2$	2931.6	7.78/7.78	0.28/0.28	−9.42/−9.42

The hydrophobic core is *VYAGILSYGVGFFLFILVVAAV*. The table reports values for the over-all free energy of transfer from water to octanol [43], ΔG, for TM_{wt} and TM_{KK}, at pH 2 and 7 [44]. ΔG is a measure of the over-all hydrophobicity of the two peptides; ΔG_{AVE} is the "per residue" value, obtained by dividing ΔG by the number of amino acids in the sequence.

elution time demonstrates that the Lys flanks substantially reduce the hydrophobicity of the peptide at low pH, as expected from calculations of the over-all hydrophobicity at pH 2 (Table 1).

3.2. Secondary structure of FGFR3 TM domain

A major problem with long hydrophobic peptides is that they may misfold or aggregate with a change in secondary structure. Therefore, CD was used to monitor the secondary structure of the synthesized TM domains. As discussed below, we found that the secondary structure is different in different solvents.

Both TM_{wt} and TM_{KK} are highly soluble in HFIP (solubility >10 mg/mL), and both peptides are helical in the presence of HFIP. Fig. 2 shows the CD spectra of TM_{wt} and TM_{KK} in HFIP/water (1,2). In these experiments, the peptides were first dissolved in HFIP, and water was added afterwards. Fig. 2 shows a typical α-helix spectrum, with minima at 208 and 222 nm. The helicities of TM_{wt} and TM_{KK} are very similar, suggesting that the flanking Lys–Lys segments do not affect the secondary structure.

HFIP can be easily removed under a stream of nitrogen, and the peptide can be then dissolved in other solvents, such as detergent solutions. TM_{wt} and TM_{KK}, dissolved in 4% SDS in a sodium phosphate buffer, pH 7.0, after HFIP removal, are α-helical (solid line in Fig. 5). The solubility of TM_{wt} and TM_{KK} in SDS is similar, about 2 mg/mL in 4% SDS. This finding, which may seem unexpected at first, reflects the similar over-all hydrophobicities of TM_{wt} and TM_{KK} at pH 7 (see Table 1).

In order to produce liposomes containing the peptides, the peptides were mixed with lipids in the presence of HFIP, the organic solvent was removed, and the dry protein/lipid mixtures were hydrated in a sodium phosphate buffer, pH 7.0. The CD of TM_{wt} and TM_{KK} spectra in POPC liposomes, presented in Fig. 3, show that the incorporated peptides are α-helical.

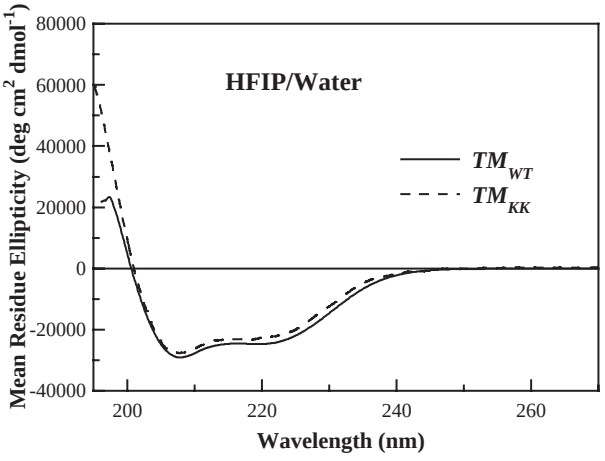

Fig. 2. Circular dichroism spectra of FGFR3 TM_{wt} (solid line) and TM_{KK} (dashed line) in HFIP:H_2O (1:2). Peptides were first dissolved in HFIP, followed by the addition of H_2O. Both peptides are helical in this environment; the flanking Lysines do not affect the helicity of the peptide.

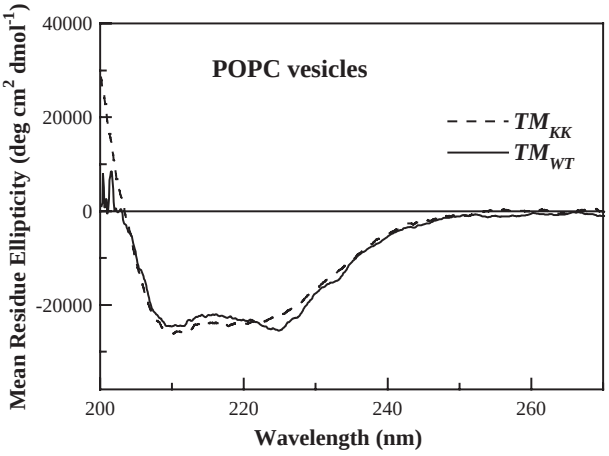

Fig. 3. Circular dichroism spectra of FGFR3 TM_{wt} (solid line) and TM_{KK} (dashed line) in liposomes. POPC and peptides were mixed in HFIP/chloroform. The solvents were evaporated, and the samples were redissolved in a sodium phosphate buffer, 100 mM NaCl, pH 7. Samples were equilibrated after three freeze–thaw cycles. Both TM_{wt} and TM_{KK} are α-helical.

The above results demonstrate that both peptides are easy to handle and do not misfold if they are first dissolved in HFIP. However, the peptides are not helical in all solvents. For instance, Fig. 4 shows the spectra of TM_{wt} and TM_{KK} in MeOH. The spectrum shows a single minimum at around 215 nm, characteristic of a β-sheet. We note that once MeOH is evaporated and the peptides are redissolved in HFIP, they become helical again. Therefore, β-sheet formation in MeOH is reversible.

The two CD spectra in Fig. 4 are similar. Therefore, both TM_{wt} and TM_{KK} form β-sheets in MeOH, and the flanking Lys–Lys segments do not affect the secondary structure. However, the lysines have a profound effect on peptide solubility in MeOH: TM_{KK} is soluble at ~5 mg/mL in MeOH, while TM_{wt} solubility is only 0.2 mg/mL.

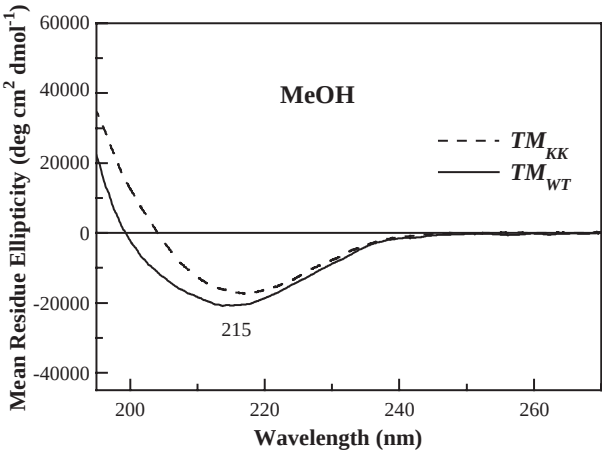

Fig. 4. Circlular dichroism spectra of FGFR3 TM_{WT} (solid line) and TM_{KK} (dashed line) in methanol. The spectra, exhibiting a single minimum at around 215 nm, are characteristic of β-sheets.

β-Sheet-like CD spectra are observed in 4% SDS if the dry peptides are dissolved directly in SDS. For instance, Fig. 5 compares TM_{KK} CD spectra in SDS when TM_{KK} is dissolved directly in SDS (dashed line), and when it is first dissolved in HFIP, HFIP is removed, and then the peptide is dissolved in SDS (solid line). Interestingly, the solubility of the peptides in 4% SDS, if dissolved directly, is less than 0.1 mg/mL, quite different from the solubility in SDS after HFIP removal (2 mg/mL). Therefore, sample preparation can have a profound effect on secondary structure and solubility.

Results for TM_{wt} are identical to the TM_{KK} data shown in Fig. 5. Therefore, the flanking Lysines do not seem to contribute to the observed behavior in Fig. 5.

Based on these experiments, it is recommended that stocks of long hydrophobic peptides are prepared in HFIP at a known concentration. Aliquots of this solution can be then used after HFIP is removed under a stream of nitrogen. If needed, the dry sample can be placed in a vacuum chamber to remove any traces of the solvent. This protocol will ensure correctly folded α-helical hydrophobic peptides, with solubility in detergent that is suitable for biophysical investigations.

The presented data suggest that identifying an appropriate structure preserving solvent system is crucial for the biophysical investigations of long hydrophobic peptides. The TM domains of RTKs are helical in the plasma membrane, and it is therefore the responsibility of the investigator to ensure that the TM domains are helical in hydrophobic environments and in lipid bilayers in particular as the best model mimetic of the plasma membrane. We have found that dissolving the FGFR3 TM domain in HFIP ensures that the peptide is correctly folded into an α-helix. Furthermore, premixing peptides and lipids in the presence of HFIP ensures that the membrane-incorporated proteins are helical. These findings will likely hold true for the TM domains of other RTKs.

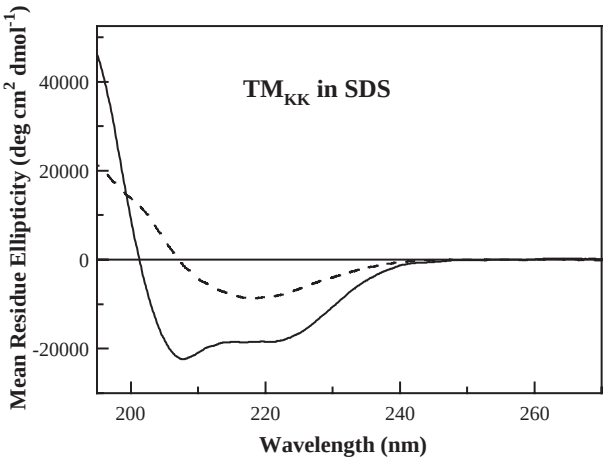

Fig. 5. Circular dichroism spectra of TM_{KK} in 4% SDS. TM_{KK} was first dissolved in HFIP, dried, and redissolved in 4% SDS (solid line), or it was dissolved directly into 4% SDS (dashed line). The peptide is helical only if dissolved in HFIP first. Results for TM_{wt} are identical to the shown ones.

An important observation is that the CD spectra of TM_{wt} and TM_{KK} are always similar in a given solvent system. Therefore, the flanking Lysines do not affect the helicity of the TM domain. This finding is consistent with a previous report that flanking Lys do not perturb the secondary structure of hydrophobic TM peptides [24].

3.2.1. Orientation of TM_{wt} and TM_{KK} in bilayers

Next we investigated if the Lysines in TM_{KK}, which are immediately adjacent to the predicted TM domain, change the disposition of the helix in the bilayer. In particular, we assessed the tilt of the incorporated TM_{wt} and TM_{KK} in POPC bilayers using OCD. OCD provides a relatively easy and quick way to assess the helix tilt, with precision ~10°.

The OCD samples were oriented POPC multilayers containing 5 mol% TM_{wt} and TM_{KK}, deposited on a quartz slide from an organic solvent. The multilayers were placed in the spectropolarimeter in such a way that they were normal to the optical path. The sample was rotated around the optical path in increments of 45°, and spectra were collected and averaged. A background spectrum of a lipid multilayer was subtracted from the OCD spectra of the proteins in the lipid matrix.

Fig. 6A shows the predicted OCD spectra for helices that are normal and parallel to the bilayer plane [35]. These spectra are calculated using the parameters given in [35] as previously reported [36]. Fig. 6B shows the experimental OCD spectra of TM_{wt} and TM_{KK}. A Comparison of Fig. 6A and Fig. 6B suggests that both peptides adopt transmembrane orientations. The observed difference in amplitude is not statistically significant; the OCD signal of a TM helix is low and thus difficult to measure above the lipid background, and the amplitude of the signal depends on the thickness of the sample, which is hard to control. The shape of the spectrum, and the observed positions of the maximum and minimum, however, are consistent with TM helix orientation. Based on the OCD data, we conclude that both TM_{wt} and TM_{KK} incorporate as TM helices, normal to the bilayer plane. Therefore, the flanking Lysines do not induce a measurable (by OCD) change in helix tilt with respect to the bilayer normal.

3.3. FGFR3 TM domain dimerization in SDS

We have used SDS-PAGE to monitor the dimerization propensity of TM_{wt} and TM_{KK}. SDS-PAGE is routinely used to probe the occurrence of TM helix dimerization and the effect of a single amino acid substitution on dimer stability [21,37]. The peptide samples were subjected to SDS-PAGE using 10–20% tricine precast gels (Novex, San Diego, CA). Samples were reduced, boiled for 5 min prior to loading, and visualized using Coomassie blue or silver staining. Fig. 7 shows that at concentrations ~10–30 μg, two distinct bands, corresponding to a monomer and a dimer, were observed for TM_{wt}. The dimer band is weak, but is always observable for peptide loads ≥10μg. Therefore,

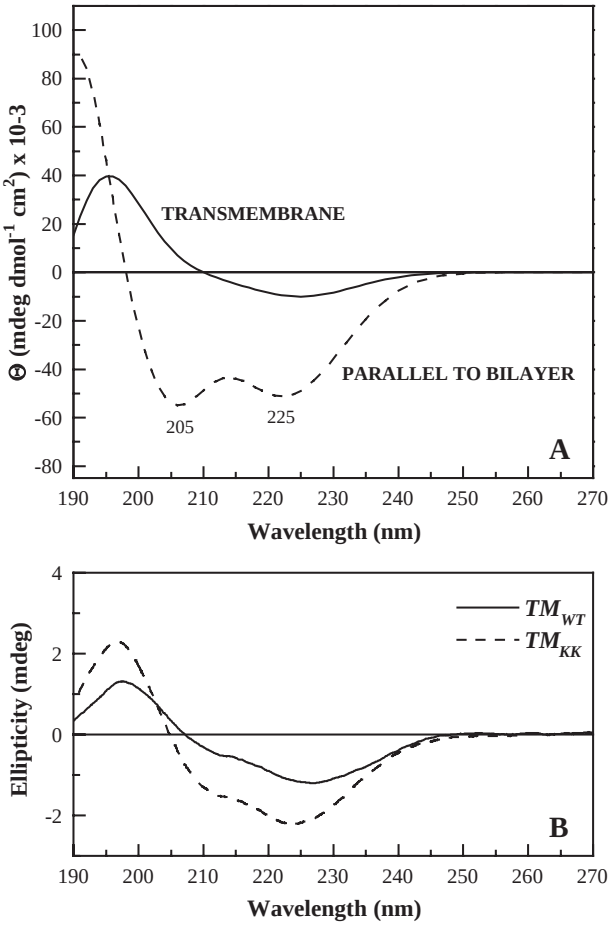

Fig. 6. Effect of flanking Lysines on the oriented CD spectrum. (A) Expected spectra for peptides that are normal and parallel to the bilayer plane. (B) Experimental data for FGFR3 TM_{wt} (solid line) and TM_{KK} (dashed line) in POPC multilayers. Peptides and lipids (molar ratio 1:20) were mixed in HFIP/chloroform, deposited on a quartz slide, and hydrated to form multilayers. Comparison of the experimental data (B) and the expected spectra (A) suggests that both peptides have transmembrane orientations (see text). Therefore, the flanking Lysines do not perturb the transmembrane orientation of the peptides.

TM_{wt} dimerizes in SDS. TM_{KK}, however, runs as a 100% monomer under identical conditions. This finding demonstrates that the flanking Lys–Lys segments destabilize the FGFR3 TM dimer in SDS.

As seen in Table 1, TM_{wt} has a single Cys (Cys 396), while TM_{KK} does not. A question may therefore arise if the observed difference in dimerization may be due to disulfide bonds between the two Cys in the TM_{wt} dimer. Disulfide bonding is not expected to contribute to dimer formation because samples are reduced and boiled prior to loading. To further examine this possibility, we "capped" Cys396 in TM_{wt} by derivatizing it with fluorescein-5-maleimide, and we subjected the labeled and the unlabeled TM domains to SDS-PAGE. We could follow the colored bands of the labeled peptides, and we could observe monomer/dimer separation in real time. The dimer band was yellow, suggesting that even peptides with capped Cys396, which cannot participate in disulfide bonds, form dimers. Furthermore, Coomassie blue was used to stain this gel and gels containing underivatized peptides. The Coomassie-stained bands corresponding to labeled and unlabeled peptides appeared identical, thus suggesting that Cys396-mediated disulfide bonding does not play a role in TM_{wt} dimer formation, provided that the samples are reduced.

A question may also arise if the observed TM_{wt} dimer is stabilized via electrostatic interactions. This could occur if non-biological anti-parallel dimers are formed, such that the positive charges on the C-terminus interact with the negative charges at the N-terminus. To address this possibility, we synthesized a third peptide, NH_2-RRAGS-*VYAGILSYGVGFFLFILVVAAV*TLCRLR-$CONH_2$. In this peptide, the two negative charges at the N-terminus were substituted with two Arg, such that both the N- and the C-termini have positive charges. We subjected this peptide to SDS-PAGE, and we observed a dimeric band similar

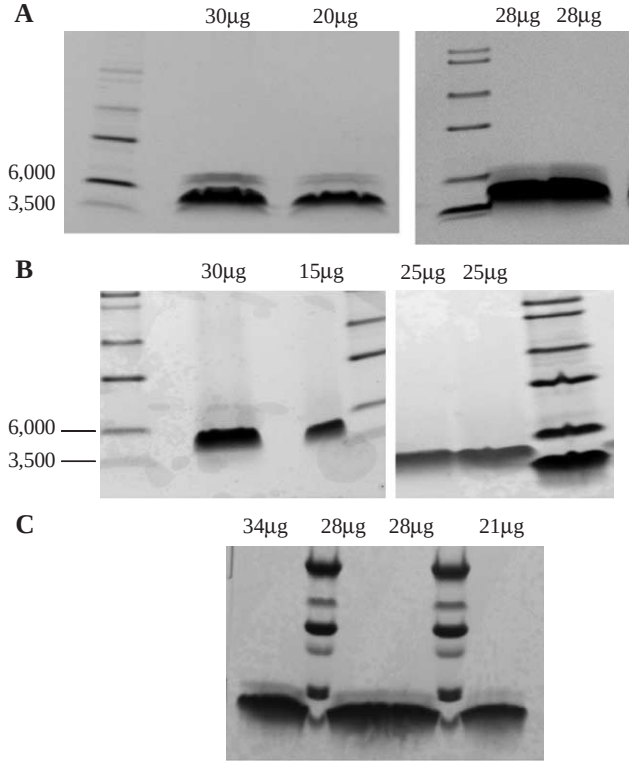

Fig. 7. SDS-PAGE of TM_{wt} and TM_{KK}. 10–30 µg of peptide, dissolved in sample buffer, was reduced with NuPAGE reducing agent and boiled for 5 min prior to loading onto Novex precast tricine gels. The two lowest molecular weight standards (molecular weight in kDa) are marked. The peptides were visualized with Coomassie blue. (A) Typical gels of TM_{wt}. (B) Typical gels of TM_{KK}. Two distinct bands, corresponding to the monomeric and dimeric states, were observed for TM_{wt}. TM_{KK}, however, runs as a monomer. Therefore, the flanking Lysines destabilize the dimer in SDS. (C) SDS-PAGE results for NH_2-RRAGS*VYAGILSYGVGFFLFILVVAAV*TLCRLR-$CONH_2$. In this peptide the two negative charges at the N-terminus were substituted with two Arg. The peptide dimerizes, suggesting that the wild-type dimer that we observe in (A) is not stabilized via electrostatic interactions between the positive charges on the C-terminus interact and the negative charges at the N-terminus.

to wild-type (Fig. 7C). Therefore, the TM_{wt} dimer that we observe in Fig. 7A is not stabilized via electrostatic interactions.

Our findings that the flanking Lysines can disrupt FGFR3 TM domain dimers in SDS differ from the results of a previous study of Melnyk et al. [24]. Melnyk et al. demonstrated that flanking lysines do not destabilize the glycophorin A dimer in SDS [24]. The difference between the two results is easy to explain in view of the very different dimerization propensities of GpA and FGFR3 TM domain: FGFR3 dimerizes weakly, while GpA is a very stable dimer (it runs as a 100% dimer on a SDS gel) [38–40]. If the Lysines have a weak destabilizing effect, they will not have a visible effect on the monomer–dimer equilibrium of GpA in SDS. On the other hand, proteins with weak dimerization propensities, such as FGFR3 TM domain and maybe all RTK TM domains, may be affected.

The findings that flanking residues can affect TM helix interactions in a hydrophobic environment are consistent with previous work by Lew et al. [41]. Using TM helices flanked by either two Lys or AspLys as models, Lew et al. [41] have demonstrated that TM helix oligomerization in the membrane is affected by charges at the N- and C- termini.

The exact mechanism of Lysine-induced dimer destabilization is unclear and may be specific for a given sequence. One possibility is that the bulky Lys residues, positioned close to the dimerization motif, may sterically hinder assembly, thus inhibiting dimerization. Furthermore, it has been shown that Lys residues at the C-terminus can form a "cap" and fold back, interacting with backbone carbonyl groups [42]. Such interactions can also inhibit dimerization. While the effect of the flanking Lysines on FGFR3 TM domain dimerization is of substantial biophysical interest, Lys-induced effects are a consequence of sequence modification only, and do not, in any way, shed light on the role of RTK TM domains in signaling.

The results presented here suggest that wild-type sequences of RTK TM domains should be always synthesized and studied, such that the behavior of sequence variants can be properly assessed against wild-type. When modifying a sequence, we need to confirm that we are investigating wild-type behavior, rather than effects arising due to sequence modification.

4. Conclusion

We have demonstrated that FGFR3 TM domain can be produced with high yield via a modified solid phase synthetic strategy. We have further shown that substitution of the flanking residues with Lysines do not alter the secondary structure of the peptide and the transmembrane orientation of the helix in the bilayer. However, the lysines affect protein–protein interactions in SDS and abolish dimerization. These findings suggest that caution needs to be exercised when modifying a TM sequence; modified sequences should be always compared to wild-type in order to assess the effects of sequence modifications.

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