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4(*R*/*S*)-Amino/Guanidino-substituted Proline Peptides: Design, Synthesis and DNA Transfection Properties

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Abstract: Collagen is a major structural protein found in the connective tissues of higher organisms and mammals and its biomechanical properties are related to the high thermal stability of its triple helical structure. The primary structure of collagen is composed of the repeating tripeptide motif of Pro-Hyp-Gly, where Hyp is 4*R*-hydroxy proline. Cationic collagen mimetics consisting of [Pro(X)-Pro(Y)-Gly]$_6$ where Pro(X) and Pro(Y) are 4(*R*/*S*)-amino/guanidine proline have been synthesized and shown to form triplexes more stable than the unmodified collagen peptide [Pro-Hyp-Gly]$_6$. The origin of hyperstability is due to conformational pre-organization of proline pucker arising from the electronegativity of the cationic group. These cationic collagen peptides are shown to be effective cell penetrating and plasmid DNA transfecting agents. The results have potential for design of new collagen mimetics for biomaterial applications and efficient cell penetrating agents for drug delivery applications.

Keywords: 4-Aminoproline peptides · Cationic collagen mimetics · Cell penetrating peptides · 4-Guanidino proline peptides

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1. Introduction

Collagen is a major structural protein found in the connective tissues of higher organisms and mammals and its biomechanical properties are related to the high thermal stability of its triple helical structure.[1] The primary structure of collagen (Fig. 1A) is composed of the repeating X–Y–Gly triplet motif, where X is predominantly proline and Y-position mostly occupied by *trans*-4*R*-hydroxyproline. The triple helical structure of collagen is constituted from three extended left-handed polyproline type II chains, intertwined in a parallel fashion with one residue shift to form a right-handed superhelix.[2] Interchanging Pro and 4*R*-Hyp in the X and Y-sites in the collagen chain or acetylation of the 4*R*-OH group in Hyp significantly decreases the thermal stability of collagen model peptides.[3] The two prolines at X and Y-sites in collagen do not have the same ring conformation and the X-site proline prefers C4-*endo* puckering while the Y-site proline prefers C4-*exo* puckering (Fig. 1) to allow optimal packing of three strands in triplex formation.[4] Interestingly collagen peptides with 4(*R*/*S*)-fluoroproline (Fig. 1B) in X/Y sites exhibited higher thermal stability compared to analogous 4(*R*/S)-hydroxyproline peptides.[5] Since the

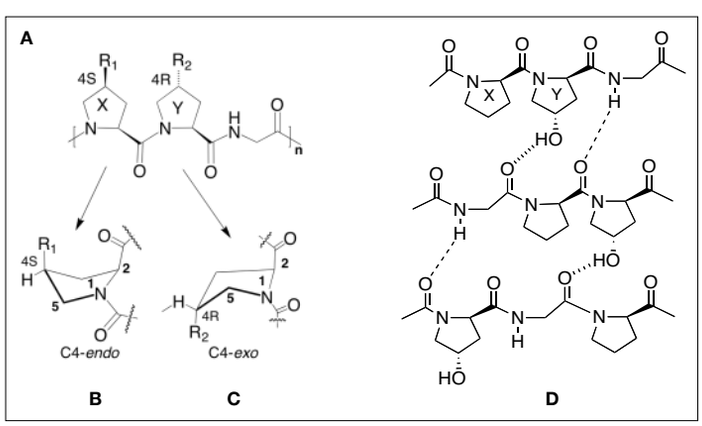

Fig. 1. Structure of collagen peptides with proline modifications and the preferred ring puckers for X and Y-sites (A) 4-hydroxyproline; R_1 = H, R_2 = OH (B) 4-fluoroproline R_1 = H/F, R_2 = F/H and (C) 4-aminoproline; R_1 = H, R_2 = NH_2 and (D) Interstrand H-bonding leading to triplex.

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4-fluro substituent cannot form H-bonds, the stabilizing effect was attributed to the electron-withdrawing inductive effect of F influencing the proline ring pucker.[5b] The recent crystal structures reported for the triple helical peptides $(Pro.Pro.Gly)_{10}$ and $(Pro.Hyp.Gly)_{10}$ indicated that the 4-OH group of Hyp had no direct structural effects on the hydration pattern and the resulting molecular structure.[6] The mechanical and thermal stability of collagen is attributed to 4*R*-Hyp residues involved in interchain H-bonding with amide carbonyls (Fig. 1D).[3] Hence understanding the molecular origin of triplex stability in collagen assumes importance.

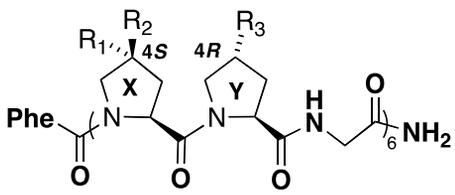
In view of these findings we embarked[7] on an investigation of the effects of 4(*R*/*S*)-amino (NH_2) substitution on proline, since it has the hybrid features of OH and F. The NH_2 function is similar to OH in size, but unlike F it has a hydrogen bonding ability. Due to its higher basicity (pk_a~10), it is predominantly protonated (NH_3^+) at physiological pH, making it electron withdrawing like F. The guanidinium [$NHC(=NH)NH_2$] group which is slightly bulky has a higher pK_a~13 and would even be better in terms of cationic and electron withdrawing characteristics. This article describes studies on the positional (X/Y) and stereochemical (*R*/*S*) preferences of 4(*R*/*S*)-amino/guanidino prolines in triplex stabilization of the collagen peptide $(X\text{-}Y\text{-}Gly)_6$. The collagen peptides incorporating such cationic substituted prolines possess many structural characteristics of cell-penetrating peptides (CPP).[8] In this context, we have studied the comparative cell permeating and transfecting abilities of the designed 4(*R*/*S*)-amino/guanidine proline collagen peptides.

2. Design and Synthesis of 4(*R*/*S*)-Hydroxy/Amino/Guanidino Proline Peptides

While 4*R*(OH/F)-substituents on proline are compatible in the Y position, 4*S*(OH/F)-prolines are not tolerated in Y-site for triplex formation.[4] However 4*S*(F)-proline, but not 4*S*(OH) is accepted in X-site to form a triplex.[2,5] In collagen peptide $(X\text{-}Y\text{-}Gly)_n$, proline at the X-site has C4'-*endo* pucker, while that at the Y-site possesses C4'-*exo* pucker[4] (Fig. 1) and this combination of puckering is needed for interstrand H-bonding and optimal packing of helical chains into a triplex. The preferred proline ring puckering in X and Y-sites is strongly dictated by the stereochemistry of the 4-substituent.[4]

To examine such stereochemical and site-dependent stability effects, chimeric cationic collagen peptides $(X\text{-}Y\text{-}Gly)_6$ having different combinations of 4*R*/*S*(OH/NH_2)-prolines in X/Y-sites were synthesized[9] (Table 1). The ionizable 4(*R*/*S*)-amino group with pK_a ~10.0 remains partially protonated at physiological pH. As a result, mixed ring puckers exist for different prolines giving rise to heterogeneous conformations for these peptides. In order overcome such a problem, the 4-amino group on proline was replaced by the 4-guanidino function that has pK_a~13 and remains fully protonated at physiological pH. This leads to single ring pucker for all the proline rings in peptide chain and therefore a well-defined conformation for the derived peptides. In addition to stabilizing the triplexes, the designed cationic 4(*R*/*S*)-amino/guanidino proline collagen peptides may have the potential as a new class of cell penetrating peptides.[8]

Table 1.



Peptide No	Title	X		Y
		R_1	R_2	R_3
1	4*R*-Hyp-Y	H	H	OH
2	4*R*-Amp-Y	H	H	NH_2
3	4*R*-Amp-X	NH_2	H	H
4	4*S*-amp-X	H	NH_2	H
5	Amp-Hyp	NH_2	H	OH
6	amp-Amp	H	NH_2	NH_2
7	4*R*-Gmp-Y	H	H	$NH(C=NH)NH_2$
8	4*S*-gmp-X	H	$NH(C=NH)NH_2$	H

The 18-mer collagen model peptides (**1**–**6**) having Phe at the N-terminus and end-capped at C/N-terminii were synthesized on MBHA resin by solid-phase techniques employing suitable monomer precursors as reported earlier.[7,9] The 4(*R*/*S*)-guanidinoproline peptides[10] (**7**,**8**) were synthesized on resin by deprotection of 4-NHFmoc group on-resin to 4-NH_2 at the end of synthesis of peptides **3** and **4** by piperidine treatment followed by reaction with the amidinylating agent N,N'-bis-Boc-1H-pyrazole-1-carboxamidine[11] to transform them to a guanidino function. Collagen forms parallel triplex wherein similar charged residues are present at termini (NH_3^+ at N-terminus and COO^- at C-terminus) leading to end-repulsion and hence destabilization. This is nullified by N-acetylation and C-amidation (end-capping), which leads to significant stabilization of the triplexes. The addition of Phe at N-terminus was done to enable the accurate determination of peptide concentrations by UV absorbance at 259 nm (ε = 200 $M^{-1}cm^{-1}$).[12] The sweet arrow peptide (SAP **9**) with arginine was synthesized as a reference peptide[13] for biological experiments. The synthesized peptides were cleaved from MBHA resin by treatment with TFA-TFMSA, which gave C-amidated peptides. The HPLC purified peptides were characterized by MALDI-TOF mass spectrometry.[7,9,10]

3. CD Spectroscopic Studies

Collagen-like triple helical structures having PPII conformation in each chain exhibit fingerprint CD spectra consisting of a weak positive band around 215–227 nm and a large negative band around 200 nm.[12] The ratio of the intensity of the positive to the negative band (R_{pn}) in the range 0.07–0.18 is an established criterion for triplex formation.[13] CD spectra were recorded at different pHs with a peptide concentration of 0.2 mM, which is higher than the critical triple helical concentration (0.15 mM) seen for these peptides.[7] Hence under the experimental conditions, all peptides exist in triplex form. Based on the characteristic CD profile and R_{pn} criterion[14] it is seen that i) the 4*R*-Y-peptides (**1**,**2**,**6**–**8**) formed triplex at all pHs (3.0, 7.0, 9.0 and 12.0), ii) 4*S*-Amp-peptide **4** formed a triplex at pHs 3, 7 and 9, but not at pH 12.0 and iii) 4*R*-Amp X-peptide **3** formed a triplex only at pHs 3.0 and 7.

The 4(*R*/*S*)-guanidino peptides (**7**,**8**) although exhibited CD characteristic of single-chain PPII-like conformation,[12] no change in CD profile (R_{pn}) was seen with an increase in peptide concentration suggesting that they remain in a single-chain helix form. The non-formation of collagen-like triplexes in 4-guanidinoproline peptides is perhaps a combined consequence of both

Table 2. CD – T_m (°C) of collagen peptides[a] Ac-Phe(X-Y-Gly)$_6$-NH$_2$

Entry	pH (X-Y-Gly)	3.0	7.0	9.0	12.0	EG:H$_2$O (3:1)
1	Pro-Hyp-Gly	27.0	28.0	27.0	27.0	31.0
2	Pro-Amp-Gly	60.0	54.7	26.0	45.0	23.0
3	Amp-Pro-Gly	36.0	33.0	ND	ND	35.0
4	amp-Pro-Gly	44.0	37.0	34.0	ND	39.0
5	Amp-Hyp-Gly	49.8	39.3	37.0	ND	42.0
6	amp-Amp-Gly	61.0	46.6	40.5	34.0	37.0
7	Pro-Gmp-Gly	58.1	58.0	57.5	57.8	41.6
8	gmp-Pro-Gly	50.8	50.6	50.7	50.8	49.0

[a]Values compiled from refs [7,9,10]. Entries 1–6 correspond to triplexes; Entries 7 and 8 correspond to single strand melting. *Buffers:* pH 3.0, 20 mM acetate; pH 7.0, 20 mM phosphate; pH 9.0 and 12.0, 20 mM borate buffers, all with 0.1 M NaCl. EG:H$_2$O; ethylene glycol-water (3:1); T_m values are accurate to ±0.5 °C.
Substituted prolines: Hyp; 4*R*-OH; Amp: 4*R*-NH$_2$; amp; 4*S*-NH$_2$; hyp: 4*S*-OH; Gmp: 4*R*-guandino; gmp: 4*S*-guanidino. The peptides with triad motifs hyp-Amp-Gly, Pro-amp-Gly, Hyp-Amp-Gly did not form triple helix at any of the pHs.

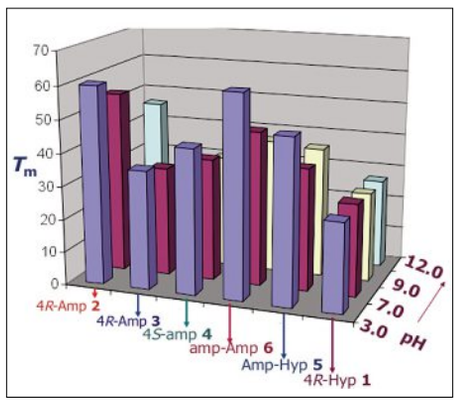


Fig. 2. Comparative CD-T_m of triplexes from cationic collagen peptides **1**–**6** as a function of pH.

interstrand charge repulsion and unfavorable steric factor in interchain association.

The stability of triplexes derived from different peptides having 4(*R*/*S*)-substituted prolines at various pHs were evaluated from temperature-dependent CD ellipticity data. Successful formation of triplex was confirmed by a sigmoidal transition indicative of cooperative melting and mere linear decrease in ellipticity suggested failure to form a triplex.[7,9] The T_m values for triplex melting of peptides **1**–**6** were obtained from the maximum in the first derivative curves of the sigmoidal transitions and summarized in Table 2. It is seen that the T_m of non-ionizable 4(*R*/*S*)-hydroxyproline peptide **1** was invariant with pH (entry 1). In contrast, the T_m of 4(*R*/*S*)-aminoproline peptides **2**–**6** was pH-dependent with T_m decreasing with increase in pH up to 9.0, followed by increase again till pH 12.0 (entry 2–6). Some peptides (**3**–**5**) did not show triplex formation in alkaline pH (entry 3–5). Since the ionizable terminal groups NH$_2$ and COOH have been capped, the pH dependence of T_m implies that the ionizable 4-NH$_2$ groups on prolines in peptides (**2**–**6**) play a direct role in determining the triplex stability *via* electrostatic interactions, hydrogen bonding, or a combination of both.

The thermal stability of 4*S*-Amp X-peptide **4** (entry 4) at pH 3.0 and 7.0 was higher compared to that of 4*R*-Amp X-peptide **3** (entry 3) by 5–8 °C. At pH 9.0, only the 4*S*-amp X-peptide **4** formed a triplex and at pH 12.0, the X-peptides **3**–**5** failed to show any triplex. This is in contrast to the 4*R*-Amp Y-peptide **2** that formed a triplex at all pH ranges. The triplexes from X-peptides **3** and **4** were however of lower stability than that of the 4*R*-Amp-Y-peptide **2** at pH 3 and 7, while 4*S*-amp X-peptide **4** was better than **2** at pH 9.0. Since the pK_a of the 4-NH$_2$ group is ~10.5 and the X-peptides do not form triplexes at pH 12, these results suggest that protonation of the 4-amino group is essential for triplex formation of X-peptides.

The overall results demonstrate that the 4*R*/*S*-aminoprolines are one of the rare examples of proline derivatives that stabilize the collagen triplex when present in the X-position and the 4*S*-amp is better than 4*R*-Amp. To examine the reason, we analyzed the relation of pyrrolidene ring conformation with the *R*/*S* stereochemistry of 4-NH$_2$/NH$_3^+$ by vicinal ^{1}H–^{1}H-coupling constants in ^{1}H-NMR of 4*R*-*trans* and 4*S*-*cis* aminoproline monomers.[9] The analysis done according to previously established methods,[9,15] indicated that 4*R*-*trans*-aminoproline **1** prefers a C(4)-*exo* pucker (Fig. 1) for the pyrrolidene ring as in 4*R*-*trans* hydroxyproline, while the preferred conformation for 4*S*-*cis*-aminoproline **2** is C(4)-*endo*. In case of proline with no C(4) substituent, these two puckers are isoenergetic and theoretical calculations,[4a,16] indicated that C(4)-*exo* form is preferred at Y-site and not favored at the X-site wherein C(4)-*endo* form is inherently favored. Taking advantage of these attributes, the chimeric peptide **6**, which has 4*S*-NH$_2$ at X-site and 4*R*-NH$_2$ at Y-site was synthesized, to get the best stability. The T_m (entry 6, Table 2) results indicated that the chimeric peptide **6** showed higher stability than both the 4*R*-Amp Y-peptide **2** and 4*S*-amp X-peptide **4** in the pH range 3.0–9.0. Interestingly, at alkaline pH 12.0, where X-peptides do not form triplex, the chimeric X-Y peptide showed triplex formation but with a lower stability than the Y-peptide **4**. The comparative T_m values of various 4(*R*/*S*)-amino/guanidinoproline peptides as a function of pH are shown graphically in Fig. 2, which indicates that the best stabilities are shown by the 4*R*-Amp Y-peptide **2** and the chimeric X-Y peptide **6**.

In case of 4(*R*/*S*)-guanidino proline (Gmp) peptides **7** and **8**, which do not form triplexes, in aqueous buffer, T_m of the single chain helix for 4*R*-Gmp Y-peptide **7** was higher than that of 4*S*-gmp X-peptide **8** by 8.6 °C (Table 2, entry 7 and 8). While the guanidino peptides formed PPII single chain helix with significant stability (>50 °C), there was no pH-dependence of stability. Since the 4-guanidino substituent remains in cationic form at all pH range, the proline puckering is retained and hence no change in the stability is seen as a function of pH.

4. Effect of Solvent

The three strands of collagen triplex are held together by interchain H-bonds. Ethylene glycol amplifies the effects of H-bonding compared to water and hence a useful solvent for detecting weak triple helical propensities.[17] In EG:H$_2$O (3:1), the CD-T_m for 4*R*-Hyp peptide **1** is higher by 3–4 °C compared to that in aqueous buffer (entry 1), since the interstrand H-bonding that is key to stabilization of triplex by Hyp peptide is more favored in EG:H$_2$O compared to H$_2$O. The T_m of 23 °C for 4*R*-Amp peptide **6** in EG:H$_2$O (entry 2) is 8 °C less than that of 4*R*-Hyp peptide **7** (T_m = 31 °C) and lower by 31 °C compared to that in aqueous buffer pH 7.0. Even the 4*R*-Amp X-peptide **3** showed destabilization by 2 °C in EG:H$_2$O (entry 3) but introduction of 4*R*-Hyp in Y position significantly enhanced triplex stability (ΔT_m = +7 °C) of the chimeric peptide **5**. In contrast to the 4*R*-Amp peptides **2** and **3**, the 4*S*-amp-X peptide **4** exhibited significantly higher stability (ΔT_m =16 °C) in EG-H$_2$O compared to that of 4*R*-Amp Y-peptide **2**.

The higher stability of Hyp peptides **1** and **5** in EG:H$_2$O originates from the favorable intrastrand H-bonding. The ob-

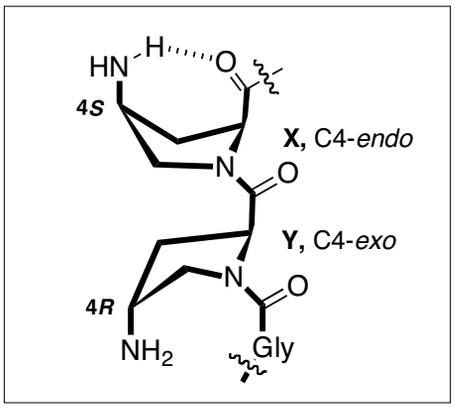


Fig. 3. Position and stereochemistry dependent preferred proline puckering in collagen peptide X-Y-Gly.

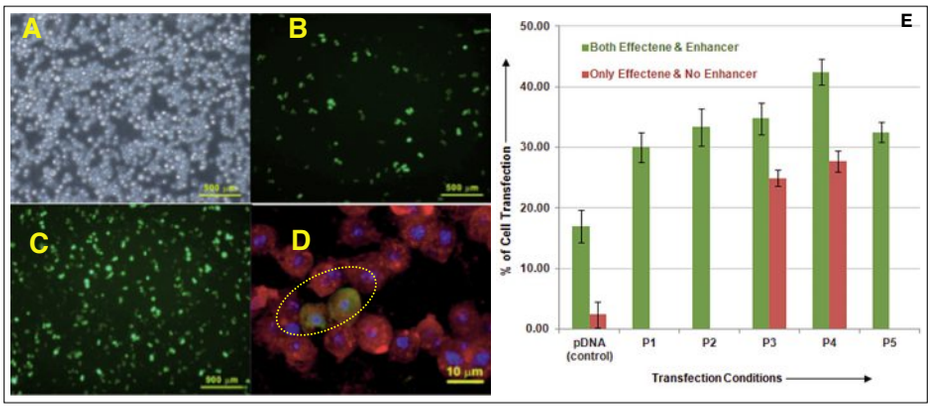


Fig. 4. Expression of GFP in Drosophila S2 cells in presence of cationic peptides. (A) DIC image of cells. (B) Cells transfected with pRmHa3-GFP plasmid using Qiagen reagents. (C) Cells transfected in the presence of peptide **8** and absence of enhancer. (D) Superimposed confocal images of Texas red labeled fluorescent peptide **9** along with DAPI stained nuclei (blue) and the expressed green fluorescence (encircled). (E) Relative transfection efficiency of 4(*R*/*S*)-amino/guanidine proline (P1=2, P2=3, P3=7, P4=8 and P5=9) and SAP (P5) peptides in the presence and absence of *enhancer* in Qiagen *Effectene*™ transfection kit.

served triplex destabilization of cationic 4*R*-Amp peptides in EG:H_2O suggests that H-bonding is not the dominant stabilizing force but the electrostatic repulsions of interstrand 4*R*-NH_3^+ groups that cause the destabilization. These remain unscreened in EG:H_2O in the absence of salt and the cationic substituent may also endow an unfavorable ring pucker. On the other hand, the triplex stabilization seen for 4*S*-amp X-peptide in EG:H_2O may arise from a favorable intramolecular H-bonding between the 4*S*-amino and the Cα-amide carbonyl possible in this proline which prefers C(4)-*endo* conformation, and hence a *trans*-geometry for the amide bond (Fig. 3) similar to that invoked in 4*S*-N-acetyl proline.[18] We have recently demonstrated the importance of such intramolecular hydrogen bonding in 4*S*-aminoproline in the derived homopolypeptide to favor a PPII conformation in water that switches to intermolecular (interstrand) mode in a hydrophobic solvent such as trifluoroethanol and leading to the novel β-structure.[19]

5. Transfection of GFP by 4(*R*/*S*)-Amino/Guanidine Collagen Peptides

The guanidinium groups in peptides are known to recognize the anionic sulfate of heparin on the plasma membrane[20] and efficiently translocate through cell membranes. The transfection efficiency of plasmid DNA (pRmHa3-GFP) encoding green fluorescent protein GFP expressed in Drosophilia S2 cells was examined using the Qiagen transfection reagent kit,[21] which contains two reagents, 'effectene' and 'enhancer', along with EC buffer. The cationic enhancer condenses the plasmid DNA, while the surfactant effectene assists in the internalization of the complex into cells. The transfection experiments were done by replacement of either or both of the reagents with the individual peptides

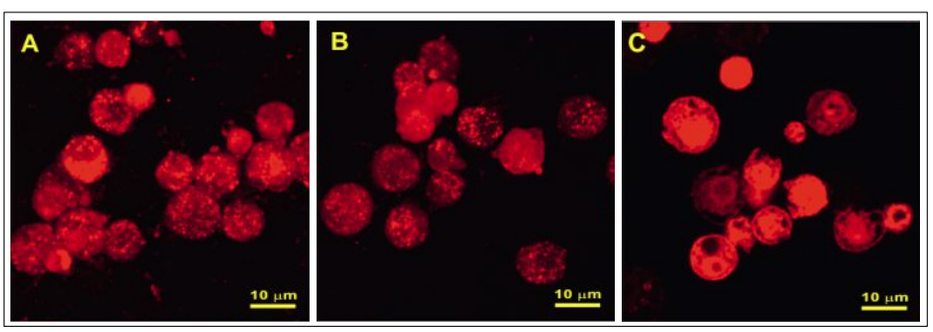


Fig. 5. Confocal images of fluorescent peptides inside S2 cells. Cells were incubated with 1 μM of Texas red labeled (A) peptide **7** (B) peptide **8** (C) peptide **9**. Punctuation of cells seen in (A) and (B).

2,**3**,**7**–**9** at a plasmid/peptide ratio of 1:25 (w/w) to investigate the role of the guanidine peptides in the transfection process.[10] The results of the expression of GFP are shown in Fig. 4. In comparison to the GFP fluorescence obtained by the Qiagen kit with both reagents (Fig. 4B), the cationic guanidine peptides **7** (Fig. 4C) and **8** are able to specifically replace the 'enhancer' component of the Qiagen transfection kit. The higher intensities of the green fluorescence in the presence of **8** indicated improved transfection efficiencies with the guanidinyl peptides.

The transfection efficiencies quantitated from the count of green fluorescent cells are shown in Fig. 4E. It is seen that i) in the absence of Qiagen enhancer, the transfection efficiency of plasmid DNA decreases by 8-fold (control), ii) peptides **2**,**3**,**7**–**9**) considerably enhance the transfection efficiencies of plasmid DNA by more than 2-fold compared to control (green bars), and iii) transfection efficiencies for peptides **2**,**3** and **9** are negligible in the absence of enhancer (red bars). Most importantly, the guanidinyl peptides **7** and **8** show high transfection efficiency even in the absence of enhancer (red bars), about 12 times more than the control. Thus, not only is the transfection efficiency of Qiagen reagents boosted in the presence of cationic 4-aminoprolyl (**2**,**3**)/4-guanidinyl (**7**,**8**) peptides but also the guanidinyl peptides exhibited a better enhancer effect in the absence of the equivalent Qiagen reagent. The 4*S*-guanidino peptide **8** was better than the 4*R*-guanidinyl peptide **7** in the GFP expression in the cells. Further, the 4(*R*/*S*)-guanidinylproline peptides **7** and **8** transfected the plasmid DNA (red bars) in the absence of enhancer with efficiency appreciably higher by 2 to 2.5 fold compared to that with Qiagen kit. These results clearly suggest that the 4(*R*/*S*)-guanidinylproline peptides are efficient functional enhancers in transfection of DNA.

The Texas Red-tagged cationic peptides **10**–**12** were used to examine the post-uptake localization sites of peptides in S2 cells. These were obtained by reacting the resin-bound peptides with fluorescent Texas Red-X succinimidyl ester reagent followed by cleavage.[10] The fluorescent guanidinyl peptides **10** and **11** exhibited a punctuated pattern in cytoplasm, while the fluorescent peptide SAP **12** showed a red diffused spread in the cytoplasm (Fig. 5).

Such punctuation may suggest that the peptides **10** and **11** localize themselves into cytoplasmic organelles/endosomes,[22] while the fluorescent SAP peptide **12** destabilizes the endosomes with the peptide spread all over the cytoplasm. In an experiment involving transfection of GFP encoding plasmid with fluorescent peptide, the expressed GFP protein could be visualized amidst the excess red fluorescent peptide in the cytoplasm (Fig. 4D). The fluorescent peptides taken up by the cells induced no significant toxicity since the size distribution of the S2 cells remained essentially identical with that of the untreated cells. We[23] have recently demonstrated a similar better cell permeability of cationic peptide nucleic acids in HeLa cells and these peptides have the ability to localize in nucleus as well. Further these cationic peptides do not exhibit toxicity even at mM concentration levels as seen by MTS assay.[10]

6. Conclusions

In conclusion, it is shown that the replacement of 4*R*-OH prolyl residues in collagen peptides by 4*R*-NH_2 proline in Y site and/or 4S-proline in X-site leads to significant stabilization of the derived triple helices. The observed pH and salt effects suggest different mechanisms to be responsible for enhancing the triplex stability at different conditions. The higher stability at lower pH could arise from increased electronegativity and the hydrogen bonding potential of the protonated amine moiety. At higher pH, the stabilizing effect may be a consequence of hydrogen bonding and the absence of electrostatic repulsion in non-protonated 4-NH_2 groups. In a hydrophobic solvent such as EG:H_2O where H-bonding is favorable compared to water, 4*S*-NH_2 proline in X-site which assumes the inherently favored C4-*endo* pucker due to formation of intramolecular H-bond shows better triplex stability. In comparison, 4(*R*/*S*)-guanidino prolyl peptides (**7**,**8**) that are more cationic remain in single chain PPII helix and do not form triplexes, due to interstrand electrostatic repulsion. The 4(*R*/*S*)-aminoprolyl (**2**/**4**) and 4(*R*/*S*) guanidino proline (**7**,**8**) cationic collagen peptides boost the transfection efficiencies and the highly cationic guanidinyl peptides are functional enhancers in transfecting the gene-encoded plasmids. The cationic peptides condense DNA very efficiently, leading to enhanced DNA transfection.

The results have a direct bearing on the current interest in collagen structure[4] and mimetics.[24] The properties of 4-NH_2 analogue with potential cross linking abilities may have significance in the design of new collagen-based biomaterials[25] as new scaffolds for tissue engineering. The cell permeating and transfection properties of the cationic peptides prompt future studies on peptide uptake in different cell lines to understand the mechanistic aspects. Conjugation with lipid chains may impart hybrid functions of both DNA compaction and cell permeability to a single reagent leading to rational design of much needed nonvirals for gene and drug delivery.[26]

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