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Design of amphiphilic oligopeptides as models for fine tuning peptide assembly with plasmid DNA

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Summary **We discuss the design of novel amphiphilic oligopeptides with hydrophobic and cationic amino acids to serve as models to understand peptide-DNA assembly. Biophysical and thermodynamic characterization of interaction of these amphiphilic peptides with plasmid DNA is presented. Peptides with at least +4 charges favor stable complex formation. Surface potential is dependent on the type of hydrophobic amino acid for a certain charge. Thermodynamically it is a spontaneous interaction between most of the peptides and plasmid DNA. Lys_7 and Tyr peptides with +4/+5 charges indicate cooperative binding with pDNA without saturation of interaction while Val_2-Gly-Lys_4, Val-Gly-Lys_5, and Phe-Gly-Lys_5 lead to saturation of interaction indicating condensed pDNA within the range of N/Ps studied. We show that the biophysical properties of DNA-peptide complexes could be modulated by design and the peptides presented here could be used as building blocks for creating DNA-peptide complexes for various biomedical applications, mainly nucleic acid delivery.**

Keywords: Plasmid DNA, amphiphilic peptides, hydrodynamics, thermodynamics, zeta potential

1. Introduction

Cationic peptides for non-viral gene delivery are being explored for a long time (*1-4*). Planck *et al*. (*5*), showed that the short-chain oligolysines - DNA complexes rather than polylysine is a better choice for receptor-mediated gene delivery due to minimal activation of the complement system. It was also shown that the compacting ability of arginyl (Arg) peptides > lysine (Lys) peptides > ornithyl peptides. However, complement activation by octaarginyl peptides was stronger than that induced by an octalysyl peptide. *In-vitro* gene delivery studies suggested that a minimum chain length of six to eight cationic amino acids is required to compact DNA into structures active in receptor-mediated gene delivery (*5,6*). Our studies showed that amphiphilic peptides with smaller hydrophobic domain form smaller complexes and their stability in solution depends on the nature of the hydrophobic amino acid (*7*). A short hydrophobic N-terminal of the peptide showed better condensing abilities compared with the peptides with larger hydrophobic N-terminal (*8*). In a recent study, factors like the presence of tryptophan and the overall hydrophobicity of the peptides as well as colloidal stability of the peptide-DNA complexes was analyzed (*9*). Such studies highlight the importance of understanding structure-stability relationships in development of novel nucleic acid delivery vectors. This work presents rational design of amphiphilic oligopeptides with distinct hydrophilic and hydrophobic domains that can confer confinement of interaction and bring different hydrodynamic properties and thermodynamics upon interaction with plasmid DNA.

2. Materials and Methods

2.1. *Materials*

Plasmid DNA (pDNA) used in this study was the 5.757 kb gWiZ GFP plasmid (obtained from Genlantis, San Diego, CA, USA). Reagents and protected amino acid residues for peptide synthesis were purchased from American Peptide Company (Sunnyvale, CA, USA). All the other materials such as ethidium bromide, monobasic sodium phosphate, sodium hydroxide, *N*,*N*-diisopropylcarbodiimide (DIC), 1-hydroxybenzotriazole

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hydrate (HOBT), piperidine, dichloromethane (DCM), methanol, hexane, trifluoroacetic acid (TFA), thianisole (TIS), dimethylformamide (DMF) and ether were obtained from Fisher Scientific Co (Fair Lawn, NJ, USA) or Sigma Chemical Co (St Louis, MO, USA) and were used without further purification.

2.2. *Peptide synthesis*

All the peptides used in the study were synthesized by solid phase peptide synthesis (*10*). The starting material was 1.653 g of Lys-wang resin with a resin load of 0.6 mEq/g. Lysine was deprotected with 20% piperidine solution in DMF. Fmoc amino acids (three equivalents excess relative to the resin) were activated using HOBt and DIC. The activated amino acids were then added to the deprotected resin and stirred for 1.5 h. 1.396, 0.884, and 1.009 g of lysine (Lys), glycine (Gly), and valine (Val) were activated for peptides containing valine. 1.173, 0.743, and 0.848 g of lysine, glycine and phenylalanine (Phe) were activated for peptides containing phenylalanine. The activated amino acids were then added to the deprotected resin and stirred for 1.5 h. Following the reaction, the peptide-resin slurry was drained and washed five times with DMF. After this step, the final peptide was recovered or the next amino acid was added after deprotection. After addition of the last amino acid, the resin was finally washed with DMF, DCM, methanol and hexane and dried. The peptides from the dry resins were cleaved by adding TFA/TIS (thioanisole)/water (95 mL/2.5 mL/2.5 mL) and stirring for 2 h. The peptides were collected and purified by repetitive washing and precipitation in cold ether until a white precipitate was obtained (*24*). The molecular weight of the peptides was determined by mass spectroscopy by direct injection of a 10 µL peptide solution (20 µg/mL) in methanol using 1,200 L Quadrupole MS/MS system (Varian Inc., Weston Parkway, Cary, NC, USA). Peptide solutions were dialyzed overnight against 10 mM phosphate buffer using dialysis membranes of MWCO 500.

2.3. *Dynamic light scattering (DLS)*

Hydrodynamic size and zeta potential of the complexes was determined by DLS. All peptide solutions and DNA were pre dialyzed using 500 and 3500 MWCO dialysis membranes respectively against 10 mM phosphate buffer for 12-8 h prior to preparing the complexes. DNA and peptide working solutions were mixed in predetermined proportions to obtain complexes at various N/P or +/- (*i.e.*, the ratio of positive charges from nitrogen of peptides to negative charges from the phosphate groups of DNA). The final concentration of DNA was maintained at 50 µg/mL for all the samples. The concentration of the peptides in these samples varied from 12.5 µg/mL to 500 µg/mL depending on the N/P ratio of the sample. These samples were vortexed for 10-15 sec and were kept at room temperature for 30 min for the complexes to be completely formed. Ten mM phosphate buffer of pH 7.4 that was used for this analysis was filtered through 0.45-micron filters. The size and zeta potential of peptide-DNA complexes as a function of N/P ratio were analyzed by DLS using Zeta Sizer Nano (Malvern Instruments, Westborough, MA, USA). The instrument was initially validated using the standards for size and zeta potential (ZP) provided by the manufacturer. Standard of 60 nm for size and −15mV for zeta potential were used for validation. Size and zeta potential of pDNA were also determined. Refractive index for these measurements was set at 1.335. Polydispersity index (PDI) of the samples was also estimated during size analysis. The size data from cumulant analysis and volume distribution has been compared to make final conclusions about size. Z-average diameter for peptide-DNA complexes has been reported in this study. The zeta potential was measured from the electrophoretic mobility of the samples. All the data has been expressed as mean ± standard deviation. Size analysis of peptide solutions (1 mg/mL) without pDNA was performed under similar conditions.

2.4. *Isothermal titration calorimetry (ITC)*

Thermodynamic analysis of complexation was performed by ITC (Microcal, Westborough, MA, USA). Plasmid DNA solution was diluted to get a concentration of 0.1 mg/mL using the dialysate (10 mM phosphate buffer, pH 7.4). Peptide solutions were dialyzed against 10 mM phosphate buffer, pH 7.4. Both DNA and peptide solutions were degassed before use. The buffer solution used for dilution was filtered through 0.45 micron filter. The sample cell of ITC was carefully washed with detergent solution and water. It was further rinsed with the dialysate buffer. A blank titration was performed for each peptide with the buffer in the sample cell and the peptide solution in the syringe. Final titrations were performed with DNA solution in the sample cell and the peptide solution in the syringe. Each titration was done thrice using the following ITC parameters – 20-25 injections were performed with 10 µL of peptide solution per injection. The injection duration was 12 sec and the time between injections was 120 sec. The stirring speed was set at 400 rpm. Two hundred and fifty µL syringe was used and the experiment was performed at 25°C (*11*). Data were analyzed to obtain the stoichiometry of binding, binding constant and change in enthalpy using the ITC software (MCS Origin 3.1) provided by Microcal.

3. Results and Discussion

This study involves analysis of interaction of DNA

and amphiphilic oligopeptides. Amphiphilicity was introduced by replacing some of the lysines with hydrophobic aminoacids so that the basic design of these peptides is $X_{(3-1)}$–Gly-$Lys_{(3-5)}$ where X is valine/phenylalanine/tyrosine. The presence of glycine between the charged and the hydrophobic domains gives flexibility to the peptide. Attaching neutral hydrophobic amino acids will preserve the existing positive charge of the peptide while altering the hydrophobicity. The hydrophobicity index values of valine, phenylalanine and tyrosine are 4.2, 2.8, and –1.3 kcal/mole respectively (*12*). The net charge on the peptides is +1/+3/+5. Lys_7 is the basic cationic peptide that served as a control. The peptide sequences, molecular weight, net charge are shown in Table 1. Based upon the hydrophobic nature of the attached amino acids, these peptides can be arranged in decreasing order of hydrophobicity, as shown below: Val_3-Gly-Lys_3 > Phe_3-Gly-Lys_3 > Val_2-Gly-Lys_4 > Phe_2-Gly-Lys_4 > Val-Gly-Lys_5 > Tyr_3-Gly-Lys_3 > Phe-Gly-Lys_5 > Tyr_2-Gly-Lys_4 > Tyr-Gly-Lys_5 > Lys_7.

Hydrodynamic size analysis of peptides by DLS indicates both monomodal and bimodal distribution depending on the peptide composition. Lys_7 that lacks hydrophobic amino acids forms smaller aggregates of 1.03 nm. It is very interesting to see that all the peptides with a single hydrophobic amino acid such as Val-Gly-Lys_5, Phe-Gly-Lys_5, Tyr-Gly-Lys_5, show a monomodal distribution of 219.0, 257.0, and 224.0 nm respectively. Val_3-Gly-Lys_3, which is the most hydrophobic peptide, also shows a monomodal distribution of size of 11.8 nm. All the other peptides show bimodal distribution. We believe that the propensity to form aggregates depends on the amino acid composition. The size and type of aggregates formed are the result of interplay between interactions within a single chain and interaction between different chains. The simultaneous presence of distinct interactions (*e.g.*, hydrogen bonding, electrostatic, and hydrophobic interactions) in the system leads to rich and subtle molecular self-assembly. In this study, it is important to understand the aggregation of these peptides, as it influences their interaction with pDNA and the colloidal stability of the complexes.

Some peptide-pDNA complexes show precipitation or turbidity at certain N/P (nitrogens from peptide/phosphates from pDNA) ratios. The time taken to precipitate and turbidity are discussed here along with the hydrodynamic size and zeta potential. Samples that were turbid or precipitated were not suitable for DLS measurements. In such cases, the supernatant solution of such samples was used to measure zeta potential. The hydrodynamic size and zeta potential of pDNA under the studied conditions are approximately 20 nm and –55 mV respectively.

DNA complexes of $Val_{(3-1)}$–Gly-$Lys_{(3-5)}$ type peptides exhibit a similar trend in change in size and PDI (Figure 1A-1C). DNA-(Val_3-Gly-Lys_3) complexes show a maximum size of 312.9 nm and a PDI of 0.9 at N/P 0.65 and 0.98 respectively (Figure 1A). Both size and PDI decrease gradually at higher N/P values. At N/P > 5.23 the solution of these complexes shows precipitation. At N/P 10.46 and 13.08 the sample looks turbid when prepared. The turbidity disappears when shaken and reappears with time. DNA complexes of (Val_2-Gly-Lys_4) (Figure 1B) show maximum PDI of 0.7 at N/P 3.36 and decreases drastically to 0.14 at N/P 6.72. The size increases gradually from N/P 1.26 to N/P 6.72. Precipitation is seen at N/P 10.0. These complexes do not show precipitation or visible aggregation between N/P 10.8 and16.8. However they are not stable in solution. The size of DNA-(Val–Gly-Lys_5) complexes remain almost stable between 100-150nm between N/P of 0.51 and 2.02 (Figure 1C). Then there is a drastic increase in the size to 320 nm at N/P 4.05. PDI of these complexes falls down from 0.59 to 0.39. Precipitation occurs at N/P 4.4. Val-Gly-Lys_5–DNA complexes show aggregation and precipitation within 15 min after preparation at N/P 16 and 20.5. Figure 2A shows zeta potential of DNA-Valine based peptides. At low N/P upto 4.0 the change in zeta potential is proportional to the charge of peptide. However, after the N/P at which the complexes show precipitation or turbidity, the clear supernatant was studied for zeta potential. At N/P > 5, ZP of DNA complexes of Lys_7 > (Val-Gly-Lys_5) > (Val_2-Gly-Lys_4) > (Val_3-Gly-Lys_3). We can say that for $Val_{(3-1)}$–Gly-$Lys_{(3-5)}$

Table 1. Properties of oligopeptides used in the study

Oligopeptides	Molecular weight (Calculated)	Net charge	Hydrodynamic Size (nm)	Molecular weight peak from mass spectroscopy
Val_3-Gly-Lys_3	757	+3	11.8 (99.0%)	756
Val_2-Gly-Lys_4	786	+4	42.9 (42.8%), 317.0 (57.2%)	786
Val-Gly-Lys_5	815	+5	219.0 (100%)	814
Phe_3-Gly-Lys_3	901	+3	149.0 (12.1%), 880.0 (73.0%)	900
Phe_2-Gly-Lys_4	882	+4	85.0 (19.3%), 391.0 (73.6%)	881
Phe-Gly-Lys_5	863	+5	257.0 (92.1%)	862
Tyr_3-Gly-Lys_3	949	+3	267.0 (25.3%), 26.1 (70.0%)	948
Tyr_2-Gly-Lys_4	914	+4	95.7 (30.9%), 341.0 (62.9%)	913
Tyr-Gly-Lys_5	879	+5	224.0 (100%)	878
Lys_7	914	+7	1.0 (99.00%)	913

Numbers indicated in the parenthesis for hydrodynamic size are the % volume distribution.

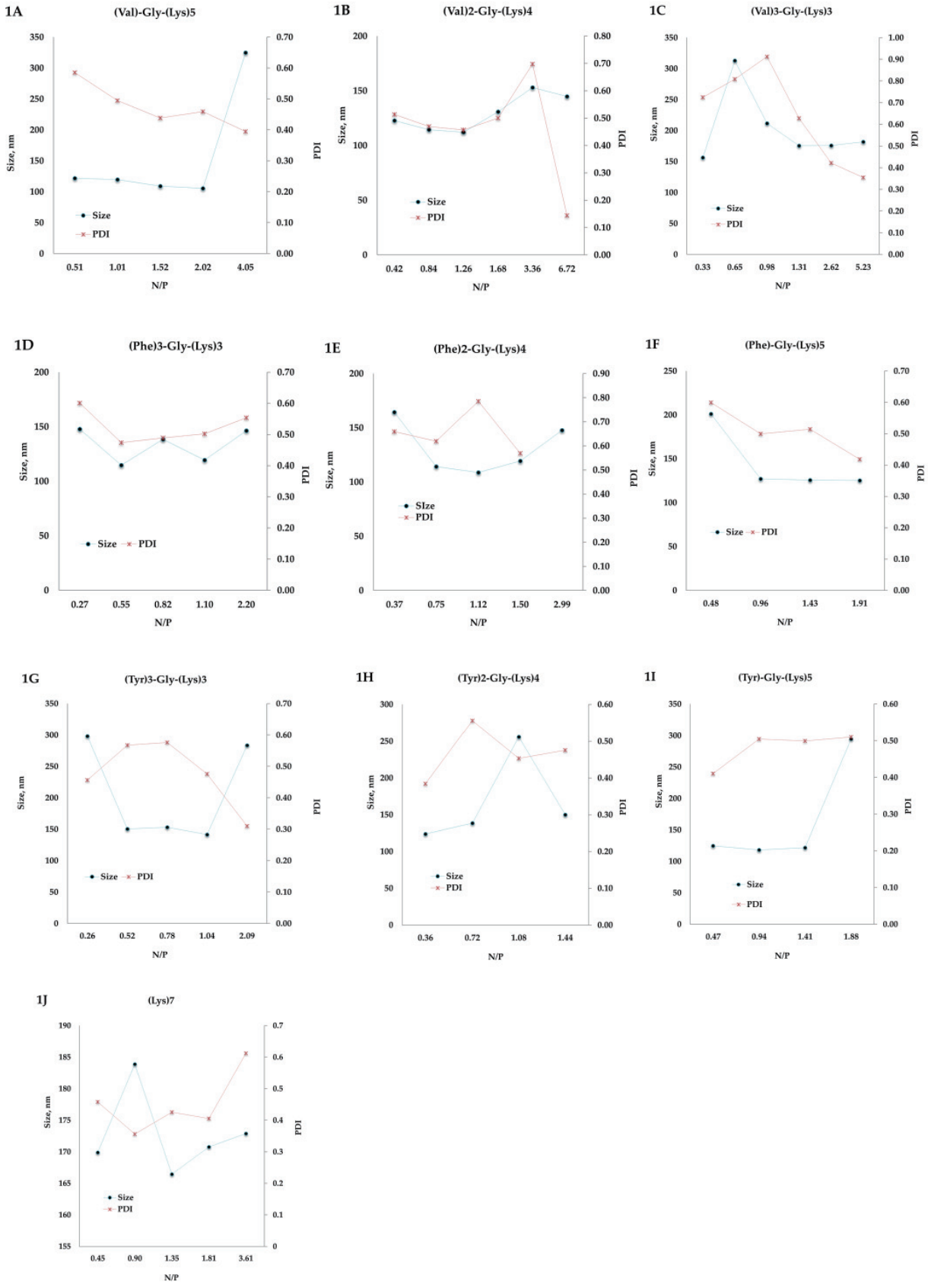

Figure 1. Hydrodynamic size and polydispersity index of plasmid DNA-peptide complexes as a function of charge.

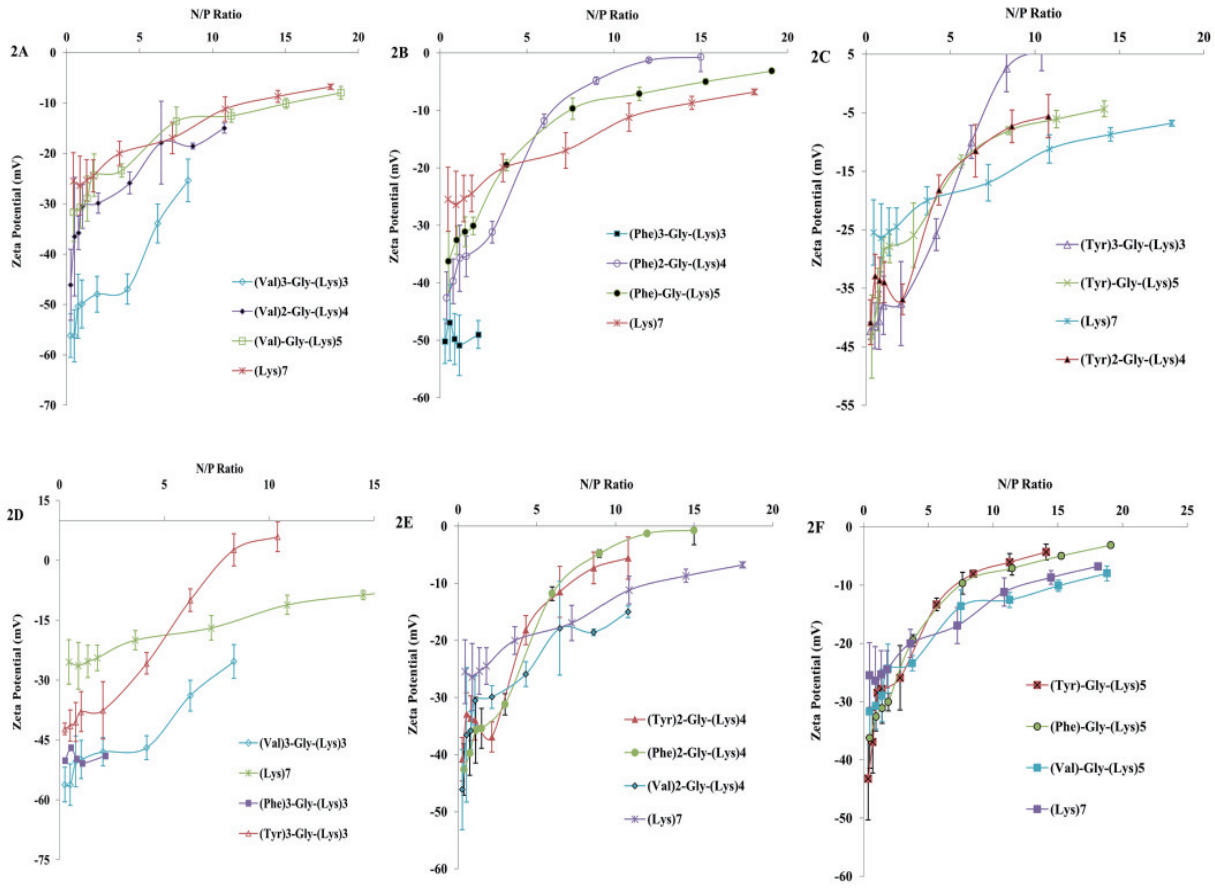

Figure 2. Change in zeta potential of plasmid DNA–peptide complexes as a function of charge.

type peptides, the size of the complexes is predominantly influenced by composition and the surface charge by charge of the peptide.

Phe_3-Gly-Lys_3–DNA complexes (Figure 1D) show a similar trend in size (100-150 nm) and PDI (0.5-0.65) between N/P 0.27 and 2.20. DLS measurements could not be made after N/P 2.20 due to turbidity that disappears by mixing but eventually returns. Precipitation is seen when the samples are left standing overnight. ΔH of 21.39 ± 6.04 cal/mole of Phe_3-Gly-Lys_3-DNA indicates predominance of hydrophobic interactions. Phe_2-Gly-Lys_4-DNA complexes (Figure 1E) show a size between 100-200 nm from N/P 0.37 to 2.99. PDI ranges between 0.5-0.7. The PDI was very high after N/P 1.50 making the sample unsuitable for DLS measurements indicating heterogenous sample. At higher N/P values, immediate precipitation of the complexes is seen. Phe-Gly-Lys_5–DNA complexes (Figure 1F) show a size between 100-200 nm from N/P 0.48 to 1.91. PDI ranges between 0.4-0.6 within the studied N/P. At N/P > 2, the samples are very turbid making DLS measurements impossible. Figure 2B shows zeta potential of DNA and $Phe_{(3-1)}$–Gly-$Lys_{(3-5)}$ type peptide complexes. Again, at low N/P the change in zeta potential is directly proportional to the charge of the peptide. DNA complexes of Phe_3-Gly-Lys_3 precipitate completely at N/P 2.0 and the solution is unsuitable for any further study. Though the net charge of Phe_2-Gly-Lys_4 is only +4, DNA-Phe2-Gly-Lys4 complexes reach near 0 zeta potential but this does not happen with Lys_7. This indicates that the surface charge and size of the DNA complexes depends on both the charge and composition of $Phe_{(3-1)}$–Gly-$Lys_{(3-5)}$ type peptides.

DNA complexes of 290 nm are formed at N/P 0.26 with Tyr_3-Gly-Lys_3 (Figure 1G) and it decreases to 141 nm at N/P 0.52 and then increases to 300 nm at N/P 2.09. The PDI increases from 0.46 to 0.6 and falls down at N/P 2.09. Samples show immediate precipitation at higher N/P values. DNA-Tyr_2-Gly-Lys_4 complexes show a maximum size at N/P 1.08 (Figure 1H). The PDI varies between 0.3 and 0.6 within the range studied. Tyr_2-Gly-Lys_4–DNA complexes, precipitate very slowly. Complete precipitation is seen after 60-75 min. Tyr-Gly-Lys_4–DNA complexes (Figure 1I) show a size of 100-150 nm between N/P 0.47 and 1.41 and drastically increase to 300 nm at N/P 1.88. The PDI also remains almost similar within N/P 0.47 and 1.88 and the complexes precipitate thereafter. DNA-Tyr_3-Gly-Lys_3 complexes even show a positive ZP after N/P 10.43. Figure 2C shows ZP of DNA and $Tyr_{(3-1)}$–Gly-$Lys_{(3-5)}$ type peptides. At very low N/P between 0.025 and 0.25, the zeta potential of DNA complexes of Lys_7 > (Tyr_3-Gly-Lys_3) = (Tyr_2-Gly-Lys_4) > (Tyr-Gly-Lys_5). A similar trend is seen until N/P 6.0 and thereafter, the

zeta potential of DNA complexes of (Tyr_3-Gly-Lys_3) > (Tyr_2-Gly-Lys_4) = (Tyr-Gly-Lys_5) > Lys_7. We can say that though the charge of the peptide influences ZP at lower concentrations of peptides, but the overall composition takes over at somepoint. This also suggests that peptide-peptide interactions play a very importnt role in modulating the properties of the complexes.

Lys_7-DNA complexes have a size bwteen 150-200 nm and PDI between 0.4 and 0.65 within the studies N/P values. Lys_7-DNA complexes (Figure 1J) show turbidity at N/P 7.22 immediately after preparation and precipitation is seen within 15-20 min after preparation between N/P 10 and 20. This indicates, that the presence of the hydrophobic aminoacids aids in keeping the complexes in solution as soluble aggregates due to peptide aggregates that might serve as surfactant micelles. This probably is the reason why there are soluble aggregates with peptides having hydrophobic amino acids. Zeta potential measurements serve as good starting point to understand this phenomenon. These differences in zeta potential combined with size analysis, of different peptides at different charge ratios suggest differing morphologies and composition of the complexes. At a very low concentration of the peptide, the complexes formed are not compact and are very heterogeneous. As the peptide concentration increases, the surfaces charge of the complexes increases, but at the expense of colloidal stability. There is an interplay between electrostatic repulsive forces and vander Waals attractive forces in colloidal stability. Assuming the attractive forces stay constant, if the electric repulsive forces are minimized by neutralization of charges on the surface of the particle, then the balance shifts in favor of net attraction and aggregation results (*13*). At low concentration of the peptide (when +/- charge ratio is less than 1), water soluble nonstoichiometric complexes form in which the content of the peptide units is lower than that of the DNA units, and that contain a net negative charge. Under these conditions the peptide chains are usually distributed among the DNA molecules. Initial ΔH of interaction of all the peptides with DNA (except Phe_3-Gly-Lys_3) is negative indicating electrostatic interaction. ΔH of interaction of most of the peptides is negative and ranges between -186.3 and -65.38 cal/mole indicating the predominance of electrostatic interactions (Table 2). The charge of the complex elevates with an increase in the peptide content, which in turn results in the amount of hydrophobic sites. At some point the complex reaches a critical composition, and the hydrophobicity increases to such an extent that further binding of the peptide should lead to precipitation of the complex. Under these conditions uneven distribution of the peptide molecules among DNA molecules occurs. This is called the disproportionation phenomenon. There is simultaneous formation of two types of complexes (non-stoichiometric and stoichiometric) that differ in the composition and solubility. At this stage, the surface charge is dependent on the interplay of the hydrophobic interactions due to Tyr/Phe/Val among peptides and with DNA. The non-stoichiometric complexes with a favorable charge composition remain in solution and the stoichiometric complex becomes insoluble because of higher content of the peptide in composition. This is evident from the precipitation seen in the peptide-DNA complexes. In the stoichiometry complex, the negative charge of the DNA is completely compensated (*14,15*). As the concentration of the peptide further increases, the portion of the stoichiometry complex grows, while the non-stoichiometric complex falls down. This is evident from precipitation and possibility of zeta potential measurement in the supernatant of the same sample for complexes at higher ratios. At higher N/P > 6.0, (Figure 2D-2F), Tyr peptides with +3/+5 charge have greater effect on zeta potential than Lys7 while those possessing +4 charge do not have a similar effect. Among peptides having +4 charges, Phe and Val containing peptides show greater impact than Lys_7 followed by Tyr peptide (Figure 2D-2F). Change in zeta potential is mainly related to neutralization of negative charges on pDNA. Zeta potential data shows that the interaction of the peptides with pDNA is dependent on both the charged and hydrophobic residues after a certain DNA-peptide composition is reached. At low concentration of the Tyr peptides, +3/+4 charges are favorable in neutralizing the positive charge on pDNA while at higher concentration of the peptides +3/+5 charge seems to be favorable. For peptides with +5 charges, presence of Phe and Val seems to be more favorable in neutralizing the charge on pDNA. This could be due to the differences in interaction of the hydrophobic amino acids with pDNA. This leads to an important conclusion that the though initial interaction of the peptide with pDNA is cationic charge dependent, eventually the orientation and interaction of the hydrophobic part of the peptide with pDNA controls the complex formation and its stability in solution.

Zeta potential was also studied at very low N/P between 0.025 and 0.25 (data not presented here). At a very low concentration of peptides, the interaction is mostly guided by the positive charge of the peptide. Lys_7-DNA complexes show maximum increase in zeta potential compared to all the other peptides. At

Table 2. Change in enthalpy for plasmid DNA-peptide interaction in pH 7.4 phosphate buffer.

Peptide	ΔH Cal/ mole
$(Val)_3$-Gly-$(Lys)_3$	-157.88 ± 14.02
$(Val)_2$-Gly-$(Lys)_4$	-279.00 ± 29.07
(Val)-Gly-$(Lys)_5$	-148.83 ± 6.46
$(Tyr)_3$-Gly-$(Lys)_3$	-65.38 ± 2.52
$(Phe)_3$-Gly-$(Lys)_3$	21.39 ± 6.04
$(Phe)_2$-Gly-$(Lys)_4$	-115.44 ± 8.18
(Phe)-Gly-$(Lys)_5$	-186.30 ± 5.09

Table 3. Thermodynamic parameters of peptide-pDNA interaction

Thermodynamic parameters	$(Val)_2$-Gly-$(Lys)_4$	(Val)-Gly-$(Lys)_5$	(Phe)-Gly-$(Lys)_5$
N	1.22 ± 0.23 (12.98)	1.67 ± 0.23 (13.91)	1.39 ± 0.04 (3.05)
K (M^{-1})	2.88×10^4 ± 2042.06 (7.09)	2.12×10^4 ± 251.66 (1.18)	2.01×10^4 ± 3394.1 (16.87)
ΔH ($Cal \cdot Mole^{-1}$)	-2.79×10^2 ± 2.07 (10.43)	-1.48×10^2 ± 6.46 (4.34)	-1.86×10^2 ± 5.09 (2.73)
ΔG ($Cal \cdot Mole^{-1}$)	-6.08×10^3 ± 41.38 (0.68)	-5.90×10^3 ± 7.05 (0.12)	-5.87×10^3 ± 100.46 (1.71)
ΔS ($Cal \cdot Mole^{-1} \cdot K^{-1}$)	19.5 ± 0.202 (1.039)	19.3 ± 0.035 (0.182)	19.1 ± 0.354 (1.86)

Standard deviations given in parenthesis.

such low N/P values, when compared to peptides with similar charges, DNA complexes show the following trend in zeta potential: Lys_7 > Tyr_3-Gly-Lys_3 > Phe_3-Gly-Lys_3 > Val_3-Gly-Lys_3; Lys_7 > Tyr_2-Gly-Lys_4 > Val_2-Gly-Lys_4 > Phe_2-Gly-Lys_4; Lys_7 > Val-Gly-Lys_5 = Phe-Gly-Lys_5 > Tyr-Gly-Lys_5. Below N/P 0.25, it can be seen that Lys7 dominates all the other peptides due to maximum cationic charge density. Among peptides having +3/+4 charges, Tyr peptides show the maximum effect on zeta potential while the presence of Tyr in +5 charged peptides leads to minimal effect on zeta potential. This indicates that the initial interaction of Tyr peptides possessing +3/+4 charges with pDNA is highly electrostatic and is favorable in neutralizing the charge.

ITC performed below the N/P of precipitation of Val_3-Gly-Lys_3, Phe_3-Gly-Lys_3, Phe_2-Gly-Lys_4, Tyr_3-Gly-Lys_3 do not show any indication of saturation of interaction with DNA. Titration of Tyr_2-Gly-Lys_4, Tyr-Gly-Lys_5 and Lys_7, into pDNA solution, shows varying binding heats indicating probability of cooperative binding. A further increase in peptide concentration results in involvement of most of the nucleic acid chains in complex formation. The ΔG values of interaction are negative indicating spontaneity of interaction and is similar for all the three peptides. The binding affinity (K) of Val_2-Gly-Lys_4 > Val-Gly-Lys_5 > Phe-Gly-Lys_5. The binding constants were found to be in the order of 10^4. This value represents moderate binding affinity. The stoichiometry for this interaction ranges from 1.22-1.67 amino group/phosphate. The change in entropy is positive. ΔS of interaction of these peptides with DNA is around 19 $cal \cdot mole^{-1}K^{-1}$ (Table 3). Stoichiometry (N) of interaction, binding constant (K), ΔG of interaction and ΔS were calculated for Val_2-Gly-Lys_4, Val-Gly-Lys_5 and Phe-Gly-Lys_5 (Table 3). Stoichiometry (N) ranges between 1.22 and 1.67. Negative ΔH and positive ΔS indicate a significant contribution of enthalpy and entropy towards free energy indicating the significance of both electrostatic and hydrophobic interactions in condensation of pDNA. Binding constants (K) of these peptides with pDNA are in the order of 2.01 to 2.88 × 10^4 indicating moderate interaction.

Conclusions

Amphiphilic peptides studied interact with pDNA to form complexes of 100-300 nm with varied stability in solution. Val and Phe peptides with +4 charges and Tyr peptides with +3/+5 charges could modulate surface potential of the peptide-pDNA complexes much better than Lys_7. Thermodynamic analysis shows spontaneous interaction between all the peptides and pDNA. Lys_7 and Tyr peptides with +4/+5 charges indicate cooperative binding with pDNA without condensation while Val_2-Gly-Lys_4, Val-Gly-Lys_5, and Phe-Gly-Lys_5 showed condensation of pDNA within the range of N/Ps studied. This study shows that separation of cationic and hydrophobic domains with a spacer amino acid can be used as a design strategy to modulate and fine-tune the hydrodynamic and thermodynamic properties of the resultant DNA complexes.

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