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Synthesis and Properties of Enkephalin Oligonucleotide Conjugates

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Peptide-oligonucleotide conjugates (POCs) is a kind of potential gene therapy drugs. It has several applications, including improving antisense agents for interfering with the RNA function and targeting disease-causing mRNA within cells. In this paper, an efficient synthesis of six enkephalin-oligonucleotide conjugates had been accomplished by azide-alkyne cycloaddition, in which the oligonucleotide and peptides were directly appended by azide and alkyne groups. Meanwhile, the hydrolytic stability of enkephalin-oligonucleotide conjugates was studied by reversed phase HPLC and the interaction of the products with pUC18 DNA also conducted by agarose gel electrophoresis. The results obtained showed that the triazole linkage is much more stable in slightly acidic environment than in slightly alkaline, the interaction between enkephalin-oligonucleotide conjugates and pUC18DNA is stronger than that between enkephalin and pUC18 DNA.

Keywords: Enkephalin, Oligonucleotide, Conjugates, Synthesis.

INTRODUCTION

Oligonucleotide derivatives constitute a class of potential therapeutic agents that can be used to target double-stranded DNA, mRNA sequences and even proteins through specific recognition of base sequences and paired with the target sequence. However, the systemic therapeutic use of oligonucleotide is hindered by their poor cellular uptake, lack of stability in intracellular fluid, lack of target specificity and low binding affinity to the target[1-4]. Overcoming these obstacles can be realized by many different ways. One approach is conjugation of the oligonucleotide to a peptide that has been suggested as cell delivery vehicles[5-8]. Significantly, the oligonucleotides were shown to be taken up by cells when they functioned as noncovalent complexes or covalent conjugates of suitable peptides. Peptide fragments also gain some advantages upon conjugation to oligonucleotides, such as improvement of a certain peptide's ability to fine-tune the oligonucleotide cleavage activity[9]. The chosen oligonucleotide CGCACACACGC was a synthetic apurinic undecamer contain 11-basepair, the well-defined system for structural studies[10].

Among the miscellaneous peptides, the selected opioid-enkephalin has many important physiological functions in animals, such as increase the resistance of immune system and antitoxic of lymphocytes[11-14]. Tyrosine was the main amino acid residue for the degradation of enkephalin. Of the degraded enkephalin by enkephalinergic neuroblastoma cells, 66 % was accounted for by the formation of tyrosine, 30 % by the formation of Tyr-Gly-Gly[15]. β-Alanine was the most readily liberated amino-acid with time in fixed acid concentrations[16]. Tyr-Gly-Gly was an extraneuronal metabolite of opioid peptides derived from proenkephalin, concentrations of which in brain were used to provide an index of enkephalin release *in vivo*[17]. Tyr-β-Ala-Phe-Gly can be recognized by antisera[18]. Tyr-Gly-Gly-Phe-Leu was cyclic prodrug of the opioid peptide, which was shown to have high lipophilicity and low hydrogen bonding potential[19]. According to the above study, six peptides sequences Tyr-β-Ala, Tyr-β-Ala-Phe-Gly, Tyr-Gly-Gly-Phe-Leu, Tyr-Gly-Gly-Phe-Lys, Tyr-Gly-Gly-Phe-Leu-β-Ala and Tyr-Gly-Gly-Phe-Leu-Lys were chosen.

Conjugates of peptides with oligonucleotides have numerous and diverse applications in biology, biotechnology and medicine. Therefore, the development of an efficient synthesis of peptide-oligonucleotide conjugates is an important subject. In this paper, six enkephalin-oligonucleotide conjugates had been synthesized by using a fragment coupling approach[20,21]. In our studies, the oligonucleotide and peptides were directly appended by azide and alkyne groups. Oligonucleotide and peptides were assembled separately on their own supports. After cleavage from the support, the conjugate and the peptides was purified and analyzed. The cycloaddition reaction between the azide and alkyne allows the synthesis of

the desired conjugates in high purity and yields (Fig. 1). The studies of peptide-oligonucleotide conjugates properties[22,23] are scarce, however, the application of which is decided by their properties. As a kind of therapy drug, the stability and absorptivity of which is as important as its function in body. Herein the hydrolytic stability of peptide-oligonucleotide conjugates was studied in conditions of different time, temperature and pH by RP-HPLC. Further studies of the interaction between peptide-oligonucleotide conjugates and pUC18 DNA will make a certain contribution to biology and pharmacology. The synthesis and properties of peptide-oligonucleotide conjugates will assist to their exploitation and application.

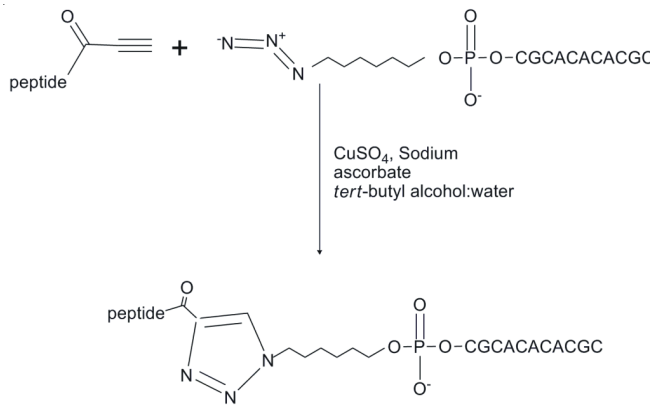

Fig. 1. N-alkyne-peptide conjugation with 5'-azidohexyl oligonucleotide using click chemistry in solution

EXPERIMENTAL

Molecular mass of peptides and the sequence of oligonucleotide were performed on Bruker Esquire type 3000 electrospray ionization-ion trap mass spectrometer (Bruker, Germany). The oligonucleotide sequence was synthesized on an Applied Biosystems DNA synthesizer model 3400 (Applied Biosystems, USA); High performance liquid chromatograph was conducted on a Agilent 1200 (Agilent, USA); Agarose gel electrophoresis was conducted on DYY-8C electrophoresis system (Beijing Liuyi Instrument Factory, China).

Fmoc-Tyr (tBu)-Wang Resin, Protected Fmoc-amino acids and N,N',N'-tetramethyluronium, hexafluorophosphate (HBTU), N-hydroxybenzotrizole (HOBT), N-ethyldiisopropylamine (DIEA) were purchased from GL Biochem (Shanghai) Ltd.; pyridine, TFA, TEA, NaN_3, NaI, MoO_3, $CuSO_4{\cdot}5H_2O$, sodium ascorbate were purchased from Aladdin reagent; DMT-dGiBu, DMT-dCBz, DMT-dABz, 5'-DMT-nucleoside-LCAA-CPG, Diisopropylsilane, Propiolic acid, 6-Bromo-1-hexanol were purchased from Sigma-Aldrich; pUC18 DNA was obtained from Fermentas Company. All chemicals were of analytical or biological grade.

Synthesis of N-alkyne-enkephalin: Synthesis of peptide fragments was carried out on Fmoc-Tyr (tBu)-wang resin (1 g, 0.38 mmol/g) following the standard procedures of solid-phase peptide synthesis. 20 % piperidine in N,N-dimethyl formamide, HOBT/HBTU/DIEA as condensation reagent, were used for the deprotection and coupling steps, respectively. The last coupling was done with propiolic acid instead of amino acids using HBTU, HOBt and DIEA as the coupling agent. The peptide was cleaved from the resin by treatment with TFA/water/TIS (9.5:0.25:0.25) for 3 h. The resulting peptide was precipitated by addition of cold ether after suction filtration.

The products were analyzed on a 9.4 × 250 mm column with a 300-A pore size and a 5 μm particle size (C18; Agilent, USA) using the solvent systems: A (H_2O containing 0.1 % TFA) and B (acetonitrile containing 0.1 % TFA). A linear gradient of 10-50 % B in 0.5 h was applied at a flow rate of 2.5 mL/min and detection at 275 nm. ESI (ionspray) mass analyses were performed on a Bruker Esquire type 3000 electrospray ionization-ion trap mass spectrometer. The potential of the electrospray needle was placed at 4000 V and the potential of the orifice leading into the mass analyzer was set at 131.5 V to extract negative ions. The flow rate for nitrogen was set at 7 psi. Samples were injected at 4.5 μL min^{-1}.

Synthesis of 5'-azido-hexyl-oligonucleotide: The oligonucleotide sequence was synthesized using commercially available monomeric units on an Applied Biosystems 3400 DNA Synthesizer following the manufacturer's recommendations with the phosphoramidite procedure. The last coupling was done using 6-azido-hexyl with no modifications to the normal procedures. The oligonucleotide sequence was deprotected and cleaved from the solid support. It was further purified by RP-HPLC at 260 nm with a linear gradient of 1-25 % B in 25 min (condition as above) and characterized by ESI-MS with the potential of the orifice leading into the mass analyzer was set at 79.6 V to extract negative ions, the flow rate for nitrogen was set at 11 psi (condition as above).

Synthesis of peptide-oligonucleotide conjugates: General procedure: 5'-Azido-hexyl-oligonucleotides and enkephalin were dissolved in 900 μL water: *tert*-butyl alcohol (1:1). 10 μL $CuSO_4{\cdot}5H_2O$ (1 equivalent, 100 mmol L^{-1}) and 8 μL freshly prepared solution of sodium ascorbate (4 equivalents, 500 mmol L^{-1}) was then added. The mixture was stirred in a vortex at room temperature and the progress of the reaction was monitored by RP-HPLC at 245 nm with a linear gradient of 1-25 % B in 25 min (condition as above).

Properties of peptide-oligonucleotide conjugates

Hydrolytic stability of peptide-oligonucleotide conjugates

Reaction temperature: 0.5 OD peptide-oligonucleotide conjugates aqueous solution was added into phosphate buffer (pH 7.4) containing 10 mmol L^{-1} sodium phosphate, 0.1 mmol L^{-1} sodium chloride and 0.1 mmol L^{-1} EDTA at 25, 36.5, 38.5, 42 °C for 24 h. The degree of hydrolysis was detected by RP-HPLC at 245 nm.

Reaction time: 0.5 OD peptide-oligonucleotide conjugates aqueous solution was added into phosphate buffer (pH 7.4) containing 10 mmol L^{-1} sodium phosphate, 0.1 mmol L^{-1} sodium chloride and 0.1 mmol L^{-1} EDTA at 36.5 °C for 12, 24, 48 and 96 h. The degree of hydrolysis was detected by RP-HPLC at 245 nm.

Solution pH: 0.5 OD peptide-oligonucleotide conjugates aqueous solution was added into phosphate buffer containing 10 mmol L^{-1} sodium phosphate, 0.1 mmol L^{-1} sodium chloride and 0.1 mmol L^{-1} EDTA at pH 6.5, 7.4 and 8.6, at 36.5 °C for 24 h. The degree of hydrolysis was detected by RP-HPLC at 245 nm.

Interaction between peptide-oligonucleotide conjugates and pUC18 DNA: The interaction between peptide-oligonucleotide conjugates and pUC18 DNA was analyzed by comparing with the interaction between peptide and pUC18 DNA. A certain amount of pUC18 DNA was mixed with aqueous peptide-oligonucleotide conjugates and peptide solutions of different concentration, at 36.5 °C for 2 h. Then the results were detected by 1 % agarose gel electrophoresis with ethidium bromide (EB) as chromogenic agent under 60 V voltage 15 min.

RESULTS AND DISCUSSION

Synthesis of peptide-oligonucleotide conjugates: Herein we describe the synthesis of six enkephalin-oligonucleotide conjugates using a fragment coupling approach. The peptide was modified by an alkynyl group at the N-terminus and oligonucleotide was labeled by an azido group at the 5'-end, alkynyl group and azido group was used for alkyne-azide click reaction, an attractive methodology for the conjugation of biomolecules, in aqueous conditions to produce enkephalin-oligonucleotide conjugates with high purity and high yields. 1,2,3-Triazole will be produced during the reaction process, which has a strong UV absorption at 245 nm. The progress of the reaction was monitored by RP-HPLC at 245 nm. Results obtained showed that a total of 95-100 % conjugate were formed in reaction times 2 h (data not shown).

The crude products were analyzed and purified by RP-HPLC (Table-1). Prior to MS analysis, HPLC purified samples were desalted by SepHadex G-25 and then lyophilized and redissolved in 50-200 µL water/acetonitrile (1:1, v/v). 2 % TEA was added for improving its detection sensitivity. ESI (ion spray) mass analysis was performed on a Bruker Esquire type 3000 electrospray ionization-ion trap mass spectrometer. The potential of the electrospray needle was placed at 4000 V and the potential of the orifice leading into the mass analyzer was set at 135.2 V to extract negative ions. The flow rate for nitrogen was set at 11 psi. Samples were injected at 5 µL min^{-1}. Negative ESI mass analysis of the six enkephalin-oligonucleotide conjugates generated signals corresponding to their molecular mass (Table-2, Figs. 2-7).

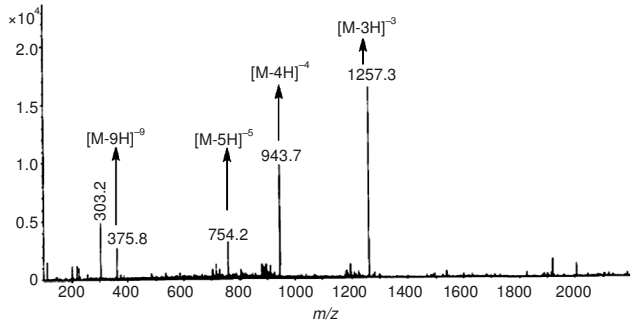

Fig. 2. Negative ion ESI-MS spectrum of Tyr-β-Ala-CGCACACACGC

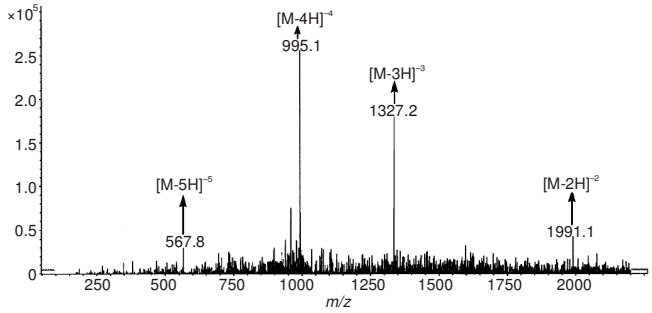

Fig. 3. Negative ion ESI-MS spectrum of Tyr-β-Ala-Phe-Gly-CGCACA-CACGC

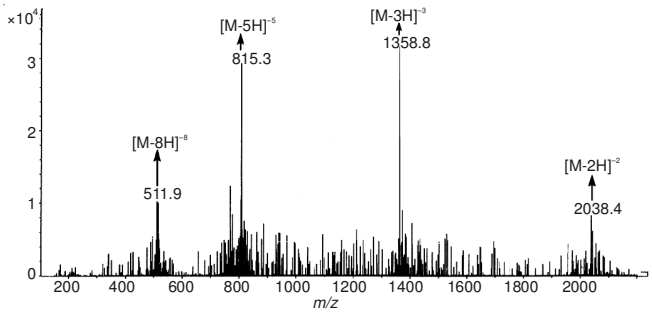

Fig. 4. Negative ion ESI-MS spectrum of Tyr-Gly-Gly-Phe-Leu-CGCACA-CACGC

Properties of peptide-oligonucleotide conjugates

Hydrolytic stability of peptide-oligonucleotide conjugates: Being a qualified gene therapy drugs not only required low toxicity, preferable permeability and specificity of peptide-

TABLE-1
RP-HPLC ANALYSIS DATA OF THE SYNTHESIZED CONJUGATES

Conjugates	Peptide-oligonucleotide conjugates	t_R (min)	Purity
1	Tyr-β-Ala-CGC ACA CAC GC	12.837	54.8
2	Tyr- β-Ala-Phe-Gly-CGC ACA CAC GC	14.097	68.1
3	Tyr-Gly-Gly-Phe-Leu-Lys-CGC ACA CAC GC	11.310	67.2
4	Tyr-Gly-Gly-Phe-Leu-CGC ACA CAC GC	13.282	70.6
5	Tyr-Gly-Gly-Phe-Leu- β-Ala-CGC ACA CAC GC	12.909	76.0
6	Tyr-Gly-Gly-Phe-Lys-CGC ACA CAC GC	14.451	57.9

TABLE-2
MASS SPECTROMETRIC CHARACTERIZATION DATA OF THE SYNTHESIZED CONJUGATES

Conjugates	Peptide-oligonucleotide conjugates	Molecular mass	ESI-MS
1	Tyr-β-Ala-CGC ACA CAC GC	3777.8	3777.9
2	Tyr-β-Ala-Phe-Gly-CGC ACA CAC GC	3984.1	3984.2
3	Tyr-Gly-Gly-Phe-Leu-Lys-CGC ACA CAC GC	4209.8	4210.6
4	Tyr-Gly-Gly-Phe-Leu-CGC ACA CAC GC	4081.7	4082.4
5	Tyr-Gly-Gly-Phe-Leu-β-Ala-CGC ACA CAC GC	4154.9	4156.5
6	Tyr-Gly-Gly-Phe-Lys-CGC ACA CAC GC	4096.8	4097.4

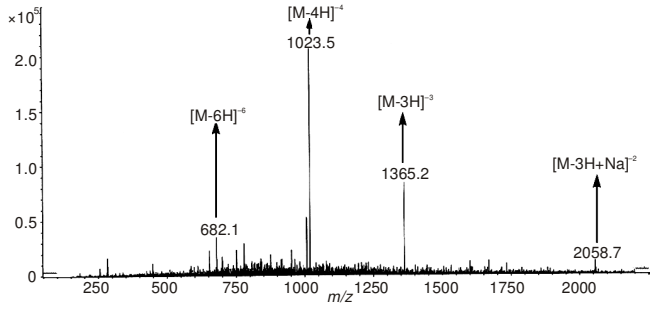

Fig. 5. Negative ion ESI-MS spectrum of Tyr-Gly-Gly-Phe-Lys-CGCACA-CACGC

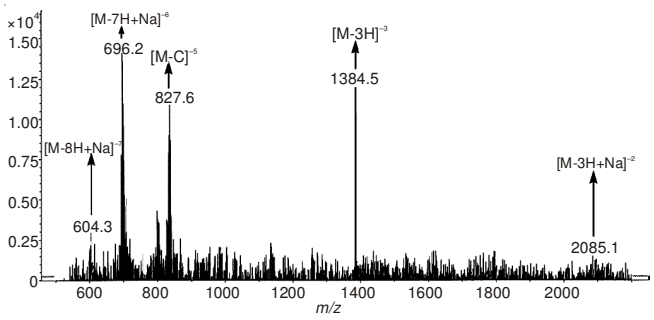

Fig. 6. Negative ion ESI-MS spectrum of Tyr-Gly-Gly-Phe-Leu-β-Ala-CGCACACACGC

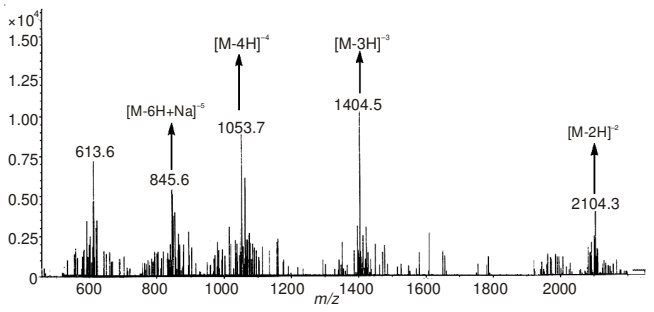

Fig. 7. Negative ion ESI-MS spectrum of Tyr-Gly-Gly-Phe-Leu-Lys-CGCACACACGC

oligonucleotide conjugates and required the stability in human physiological pH and temperature conditions within a certain period of time. In conditions of simulation environment of human body, the effect of varying reaction temperature, reaction time and solution pH on the hydrolytic stability of peptide-oligonucleotide conjugates was assessed by carrying out studies *via* RP-HPLC.

The effect of reaction temperature show that in case of conjugates **1**, **2**, **3** and **5**, there is singular peak in chromatogram (Figs. 8-13), which indicate that the hydrolysis degree was very insignificant in different reaction temperatures. For conjugates **4** and **6**, there is a small interfering peak on the side of major peak, indicating that there is a certain degree of hydrolysis. But the hydrolysis degree of both conjugates **4** and **6** were all below 1 % (Figs. 12-13). The effect of reaction time also shows the similar results. The results of conjugates hydrolysis in different pH show that extremely obscure hydrolysis took place for the conjugates **1**, **2**, **3** and the hydrolysis degree of conjugates **4**, **5** and **6** were all below 3 %. It can be seen from the above analysis that the stability of conjugates was decent in simulated environment of human body (Tables 3-5).

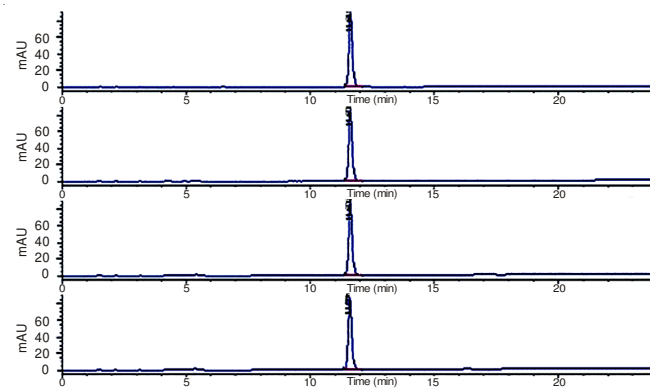

Fig. 8. Liquid chromatogram of the stability of conjugates 1's hydrolysis. The top to down was conjugate **1** hydrolyzed for 24 h at 25, 36.5, 38.5, 42 °C, respectively

Interaction between peptide-oligonucleotide conjugates and pUC18 DNA: As the basic genetic material and genetic information carrier, DNA is a kind of very important

TABLE-3
HYDROLYSIS STABILITY OF ENKEPHALIN-OLIGONUCLEOTIDE CONJUGATES UNDER DIFFERENT TEMPERATURES (pH = 7.4, HYDROLYSIS TIME IS 24 h)

Hydrolysis temperature (°C)	22.0	36.5	38.5	42.0
Tyr-β-Ala-CGC ACA CAC GC	0.01	0.09	0.34	0.98
Tyr-Gly-Gly-Phe-Lys-CGC ACA CAC GC	0.01	0.06	0.21	1.10
Tyr-Gly-Gly-Phe-Leu-CGC ACA CAC GC	0.01	0.37	1.86	3.86
Tyr-Gly-Gly-Phe-Leu-β-Ala-CGC ACA CAC GC	0.01	0.17	0.46	0.98
Tyr-β-Ala-Phe-Gly-CGC ACA CAC GC	0.01	0.02	0.28	0.81
Tyr-Gly-Gly-Phe-Leu-Lys-CGC ACA CAC GC	0.01	0.22	0.67	1.28

TABLE-4
HYDROLYSIS STABILITY OF ENKEPHALIN-OLIGONUCLEOTIDE CONJUGATES UNDER DIFFERENT TIMES (pH = 7.4, AT 36.5 °C)

Hydrolysis time (h)	12	24	48	72
Tyr-β-Ala-CGC ACA CAC GC	0.07	0.09	0.10	0.93
Tyr-Gly-Gly-Phe-Lys-CGC ACA CAC GC	0.03	0.06	0.05	0.74
Tyr-Gly-Gly-Phe-Leu-CGC ACA CAC GC	0.22	0.37	0.37	1.37
Tyr-Gly-Gly-Phe-Leu-β-Ala-CGC ACA CAC GC	0.31	0.17	0.67	2.87
Tyr-β-Ala-Phe-Gly-CGC ACA CAC GC	0.01	0.02	0.07	0.61
Tyr-Gly-Gly-Phe-Leu-Lys-CGC ACA CAC GC	0.11	0.22	0.20	0.71

TABLE-5
HYDROLYSIS STABILITY OF ENKEPHALIN-OLIGONUCLEOTIDE CONJUGATES UNDER DIFFERENT pH (THE TEMPERATURE IS 36.5 °C, HYDROLYSIS TIME IS 24 h)

Hydrolysis pH	6.5	7.4	8.4
Tyr-β-Ala-CGC ACA CAC GC	0.12	0.10	0.10
Tyr-Gly-Gly-Phe-Lys-CGC ACA CAC GC	0.09	0.03	0.08
Tyr-Gly-Gly-Phe-Leu-CGC ACA CAC GC	1.00	0.32	0.71
Tyr-Gly-Gly-Phe-Leu-β-Ala-CGC ACA CAC GC	0.77	0.56	2.01
Tyr- β-Ala-Phe-Gly-CGC ACA CAC GC	0.11	0.06	0.13
Tyr-Gly-Gly-Phe-Leu-Lys-CGC ACA CAC GC	0.10	0.20	0.27

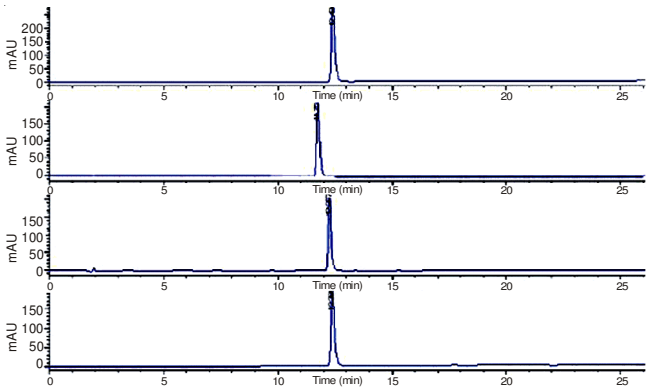

Fig. 9. Liquid chromatogram of the stability of conjugates 2's hydrolysis. The top to down was conjugate **2** hydrolyzed for 24 h at 25, 36.5, 38.5, 42 °C, respectively

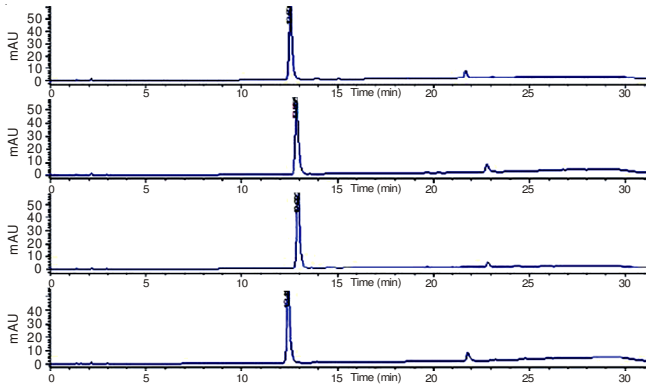

Fig. 10. Liquid chromatogram of the stability of conjugates 3's hydrolysis. The top to down was conjugate **3** hydrolyzed for 24 h at 25, 36.5, 38.5, 42 °C, respectively

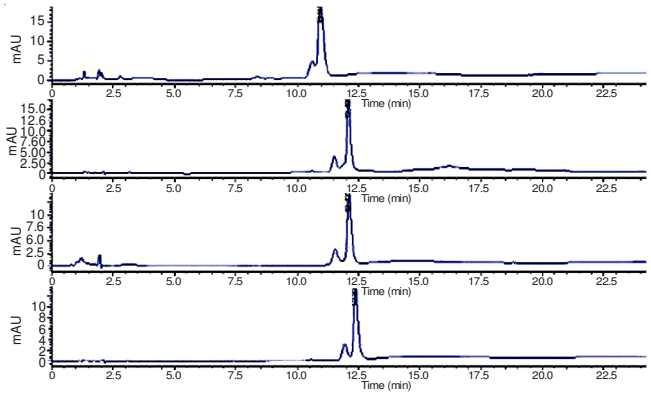

Fig. 11. Liquid chromatogram of the stability of conjugates 4's hydrolysis. The top to down was conjugate **4** hydrolyzed for 24 h at 25, 36.5, 38.5, 42 °C, respectively

biological macromolecule. For that reason, it becomes a prerequisite to understand the interaction between peptide-

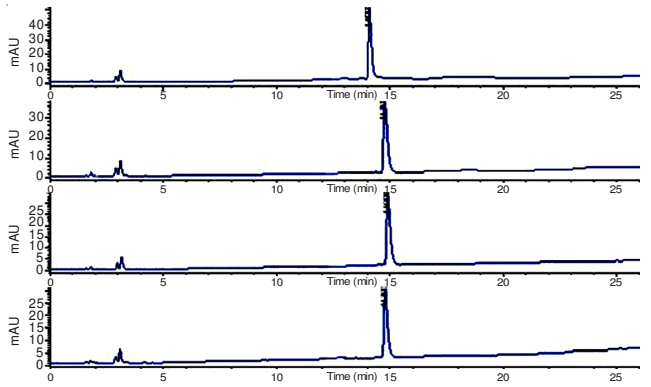

Fig. 12. Liquid chromatogram of the stability of conjugates 5's hydrolysis. The top to down was conjugate **5** hydrolyzed for 24 h at 25, 36.5, 38.5, 42 °C, respectively

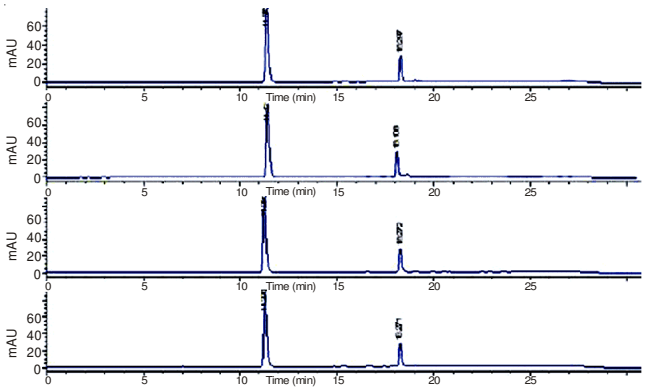

Fig. 13. Liquid chromatogram of the stability of conjugates 6's hydrolysis. The top to down was conjugate **6** hydrolyzed for 24 h at 25, 36.5, 38.5, 42 °C, respectively

oligo nucleotides and pUC18 DNA. The studies of interaction between conjugates and pUC18 DNA will conducive to under standing the pharmacological mechanism and provide the in struction for design more effective drugs. pUC18 DNA with a negative charge in the electric field, it is migrating toward the positive. There are three main conformations of pUC18 DNA, super tight spiral structure is run at the top; linear run in the second; nicked due to the loose structure hindered the largest migration rate minimum. Complete pUC18 DNA is super-coiled configuration. When one strand is cut, it becomes nicked configuration; when cutting the two strands in the same location, it becomes a linear configuration. Electrostatic effects generally do not cause conformational changes of pUC18 DNA[24].

When pUC18 DNA interacts with peptide-oligonucleotide conjugates, it is possible that pUC18 DNA may be unwind-inged or conjugate may embed in pUC18 DNA form biological macromolecule complexes. The despiralization of pUC18

DNA or the formation of biological macromolecule complexes will decrease its migration rate, on the basis of which the results of interaction could be detected by 1 % agarose gel electrophoresis.

As shown in Fig. 14a, there is no interaction between 5'-CGC ACA CAC GC-3' and pUC18DNA. However, either the interaction between pUC18 DNA and conjugate Tyr-Gly-Gly-Phe-Leu-CGC ACA CAC GC or peptide Tyr-Gly-Gly-Phe-Leu-alkyne, we can see a significant migration electrophoretic band. It is possible that pUC18 DNA may be unwindinged or conjugate may embed in pUC18 DNA form biological macromolecule complexes. The despiralization of pUC18 DNA or the formation of biological macromolecule complexes will decrease its migration rate. The interaction between pUC18 DNA and conjugate Tyr-Gly-Gly-Phe-Leu-CGC ACA CAC GC is stronger than that of peptide Tyr-Gly-Gly-Phe-Leu-alkyne.

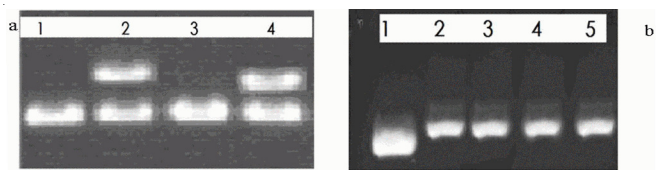

Fig. 14. (a) Gel electrophoregram of Tyr-Gly-Gly-Phe-Leu-alkyne, 5'- CGC ACA CAC GC-3' and conjugate **4** interact with pUC18 DNA. Lane 1, 0.03 μg μL^{-1} pUC18 DNA; Lane 2, 0.03 μg μL^{-1} pUC18 DNA + 0.2 mmol L^{-1} conjugate **4**; Lane 3, 0.03 μg μL^{-1} pUC18 DNA + 0.2 mmol L^{-1} 5'-CGCACACACGC-3'; Lane 4, 0.03 μg μL^{-1} pUC18DNA + 0.2 mmol L^{-1} Tyr-Gly-Gly-Phe-Leu; (b) Gel electrophoregram of different concentration conjugate 5 interact with pUC18 DNA. Lane 1, 0.03 μg μL^{-1} pUC18 DNA + 0.4 mmol L^{-1} conjugate **5**; Lane 2, 0.03 μg μL^{-1} pUC18 DNA + 0.3 mmol L^{-1} conjugate **5**; Lane 3, 0.03 μg μL^{-1} pUC18DNA + 0.2 mmol L^{-1}, conjugate 5; Lane 4, 0.03 μg μL^{-1} pUC18 DNA + 0.1 mmol L^{-1} conjugate **5**; Lane 5, 0.03 μg μL^{-1} pUC18 DNA

From the Fig. 14b, we can see that pUC18 DNA mobility is decreased with the increasing concentration of Tyr-Gly-Gly-Phe-Leu-β-Ala-CGC ACA CAC GC, indicated the formation of complexes between them. The molecular weight increases, resulting in reduced rate of migration, according to its structure and experimental phenomena can speculate the groove effect is happened between pUC18 DNA and conjugate Tyr-Gly-Gly-Phe-Leu-β-Ala-CGC ACA CAC GC.

Conclusion

In this paper, six enkephalin-oligonucleotide conjugates were successfully synthesized by using a fragment coupling approach. The products were purified by RP-HPLC and characterized by ESI-MS. The hydrolytic stability of enkephalin-oligonucleotide conjugates was studied by RP-HPLC and the interaction with pUC18DNA also had been studied by agarose gel electrophoresis. The results obtained showed that the triazole linkage is much more stable in slightly acidic environment than in slightly alkaline and the interaction between peptide-oligonucleotide conjugates and pUC18 DNA were all stronger than peptide and pUC18 DNA (Fig. 14). Other conjugates were the same with conjugate Tyr-Gly-Gly-Phe-Leu-β-Ala-CGC ACA CAC GC (data not shown). The properties studies of peptide-oligonucleotide conjugates can be used for the further study of their exploitation and application.

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