

Study on curcumin-oligonucleotide conjugate as a probable anticancer agent: Its hybridisation with telomere target sequence 5'-GGGATTGGGATT-3'

Sanjay Kumar, Arvind Misra, Snehlata Tripathi and Krishna Misra

Nucleic Acids Research Laboratory, Department of Chemistry, University of Allahabad, Allahabad-211 002, India

ABSTRACT

Curcumin-oligonucleotide conjugate was synthesised by attaching diglycyl conjugate of curcumin to 12-mer complementary telomere sequence 5'-AATCCCAATCCC-3'. An enhanced T_m of 6°C was found, showing high affinity for the target strand. This may be exploited for the suppression of cancer i.e. by blocking the expression of telomere sequence [GGGATT] $_n$ repeats.

INTRODUCTION

The critical difference between normal and tumour cells lies at the end of their chromosomes. Telomeres are specialised heterochromatin structure that act as protective cap at the end of chromosomes. In human cells telomeres are made up of an average 5000-15000 base pairs of [GGGATT] $_n$ repeats and telomeres binding proteins. Because of an inherent flaw in the way the cells copy their DNA, each time cell divides, it loses 50-100 base pairs at the end of its telomeres. This is known as "end replication problems" as first described by Watson and is a consequence of the polarity of DNA strand and the mechanism of DNA synthesis¹. When a telomere loses a critical number of base pairs it triggers a signal for cell to stop dividing and apoptosis occurs².

The cells have evolved a number of strategies to counteract this progressive telomere loss, including complex recombination and retrotransposition scheme. However, the most common solution in higher eukaryotes is an enzyme complex called as telomerase³ a specialised reverse transcriptase that specially adds back telomere sequences that are lost during replication. This process maintains a dynamic equilibrium and prevents the chromosome from shortening to a critical length and prevent cells from receiving the signal to stop dividing. Cells that produce telomerase includes germ cells and cancer cells; these are essentially immortal, whereas normal somatic cells lack telomerase activity. The overall conclusion is one that cancerous cells fail to receive the signal to stop dividing either by activity of telomerase or by any other means, and nonstop cell division occurs. In present work we have

targeted the telomere sequences so that it may receive the signal to stop dividing or it may lose its ability for further cell division by hybridising with its complementary sequence [CCCTAA] $_n$ repeats, most probably through the antisense approach^{4,5}. We have earlier reported curcumin-bioconjugates with glycine and nucleosides^{6,7}. However, in the present work the conjugate of curcumin with two glycine units has been linked with an oligonucleotide sequence via its glycylic unit. The rationale behind selection of curcumin molecule was its therapeutic applications viz. anti-oxidant^{8,9}, anti-inflammatory^{10,11} and anti-cancer activity^{12,13}. The curcumin-oligonucleotide conjugate has been synthesised by phosphoramidite approach.

RESULT AND DISCUSSION

Starting with diglycyl conjugate of curcumin i.e. [1,7-Bis(4-O-glycinoyl-3-methoxy phenyl)-1,6-heptadiene-3,5-dione] (I) one of the terminal amino group of glycylic moiety was linked with ethylenechlorohydrin to generate a terminal hydroxy group i.e. 1-(4-O-glycinoyl-N-ethanol-3-methoxy phenyl)-7-(4'-O-glycinoyl-3'-methoxy phenyl)-1,6-heptadiene-3,5-dione (II) and this was phosphorylated with 2-cyanoethyl-N,N',N'-tetraisopropyl phosphoramidite (bis-reagent) to get 1-(4-O-glycinoyl-N-ethanol phosphoramidite-3-methoxy phenyl)-7-(4'-O-glycinoyl-3'-methoxy phenyl)-1,6-heptadiene-3,5-dione (III). The 12-mer complementary telomere sequence 5'-AATCCCAATCCC-3' was synthesised by amidite approach on solid support (CPG), while still attached to the resin by its 3'-prime, it was taken in a small vessel with medium porosity frit and stop cork. A regular flow of argon was maintained. The resin was washed with dry acetonitrile and treated with 3% TCA (trichloroacetic acid) to cleave the 5'-protecting dimethoxy trityl group, which makes 5'-prime free for coupling. The resin was again washed with acetonitrile and treated with the solution of (III) in acetonitrile along with 1H-tetrazole for coupling. The coupling reaction was allowed to proceed for 20 minutes and followed by another washing with acetonitrile. This was oxidised with I₂ solution and

finally treated with ammonia for 16 hours at 55°C to cleave curcumin-oligonucleotide conjugate (IV) from the resin and also to remove the amino protecting groups. The curcumin-oligonucleotide conjugate (IV) was obtained after removing ammonia and subsequent purification by HPLC, using linear gradient of acetonitrile and buffer. The hybridisation of (IV) with target telomere sequence 5'-GGGATTGGGATT-3' has now been studied by recording melting curve (T_m). The curcumin-oligonucleotide conjugate (IV) and target sequence were dissolved in buffer containing 0.1M EDTA, 1M sodium hydrogen phosphate and 1 M potassium dihydrogen phosphate with pH adjusted to 7.0. The melting curve were studied by recording the change in absorption at wavelength 260 nm, with respect to temperature which was raised from 5°C to 90°C at a rate of 0.5°C/min. The process was repeated (cooling and heating from 5°C - 90°C) to ensure the compatibility in the results. Alternatively the hybridisation of simple complementary and target strand was also studied in similar fashion for a through comparison.

The results shows that the melting temperature of curcumin-oligonucleotide conjugate and target sequence was 36°C whereas the hybrid with simple complementary strand and target strand show T_m as 30°C i.e. T_m in case of curcumin-oligonucleotide conjugate and target strand is 6°C more than that of reference. The result possibly are due to the fact that curcumin-oligonucleotide conjugate show very high affinity for the target strand as is evident from the higher T_m and hence can be exploited for the suppression of cancer i.e. by blocking the expression of telomere sequence (GGG ATT) repeats. If the expression of telomere sequence is blocked it will evidently direct the cell to receive the signal to stop dividing resulting in suppression in proliferation. This can also be helpful in ageing process.

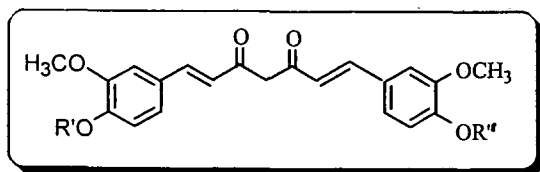


Fig. 1; I, R'=R''=-COCH₂NH₂
 II, R'=-COCH₂NH₂; R''=-COCH₂NH₂-CH₂CH₂OH
 III, R'=-COCH₂NH₂; R''=-COCH₂NHCH₂CH₂O- amidite
 IV, R'=-COCH₂NH₂; R''=-COCH₂NHCH₂CH₂O-oligo

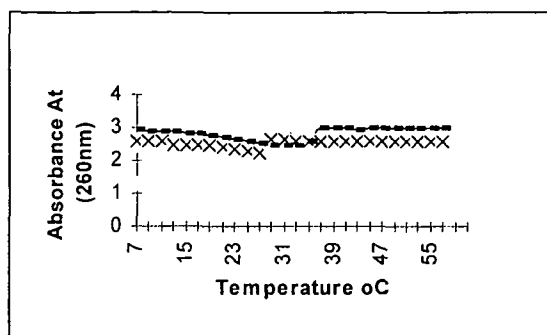


Fig 2; UV melting profile for (—) oligonucleotide-bioconjugate; and (x) Telomere target sequence.

ACKNOWLEDGEMENT

Financial assistance from ISIS Pharmaceutical USA, (SK and ST) and NVS Chemical Pvt. Ltd. (AM) is highly acknowledged.

REFERENCES

1. Watson, J. D. (1972) *Nat. New Biol.* **239**, 197.
2. Harley, C. B., Futcher, A. B. and Greider, C. W. (1990) *Nature*, **345**, 458.
3. Nugent, C. I. and Laundbland, V. (1998) *Genes Dev.* **12**, 1073.
4. Agrawal, S. (1992) *Trends in Biotechnol.* **101**, 192.
5. Tiwari, A. and Misra, K. (1992) *Ann. Neurosci.* **3**, 1.
6. Kumar, S., Narain, U. Tripathi, S. and Misra, K. (2001) *Bioconjugate Chemistry*, **12**(4),
7. Kumar, S., Dubey, K.K., Fujii, M., Tripathi, S. and Misra, K. (2000) *Nucleic acids Res. Symp. Ser.*, **44**, 75.
8. Sharma, O. P. (1976) *Biochem. Pharmacol.* **25**, 1811.
9. Toda, S., Mayase, T., Arichi, H., Tanizawa, H. and Takino, Y. (1985) *Chem. Pharm. Bull. (Tokyo)*, **33**, 1725.
10. Srinial, R. C. and Dhawan, B. N. (1973) *J. Pharm. Pharmacol.*, **25**, 447.
11. Satoskar, R. R., Shah, S. J. and Shenoy, S. G. (1986) *Int. J. Clin. Pharmacol. Ther. Toxicol.*, **24**, 651.
12. Nagbhusan, M., Amonker, A. J. and Bhide, S. V. (1987) *Food Chem. Toxicol.*, **25**, 545.
13. Singh, A. K., Sidhu, G. S., Deepa, T. and Maheshwari, R. K. (1996) *Cancer Lett.*, **107**, 109.