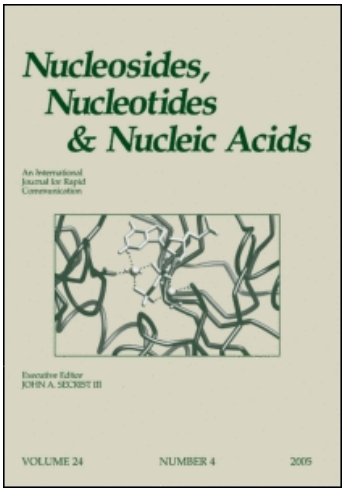

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Optical Tweezers as a Probe for Oligodeoxyribonucleotide Structuration

Nicola Borbone[a]; Giorgia Oliviero[a]; Jussara Amato[a]; Gennaro Piccialli[a]; Luciano Mayol[a]; Lisa Miccio[b]; Giuseppe Pescen[b]; Antonio Sasso[b]
[a] Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli "Federico II,", Napoli, Italy [b] Dipartimento di Scienze Fisiche, Universitá di Napoli "Federico II,", Napoli, Italy

To cite this Article Borbone, Nicola , Oliviero, Giorgia , Amato, Jussara , Piccialli, Gennaro , Mayol, Luciano , Miccio, Lisa , Pescen, Giuseppe and Sasso, Antonio(2007) 'Optical Tweezers as a Probe for Oligodeoxyribonucleotide Structuration', Nucleosides, Nucleotides and Nucleic Acids, 26: 10, 1295 — 1299
To link to this Article: DOI: 10.1080/15257770701530541
URL: http://dx.doi.org/10.1080/15257770701530541

Nucleosides, Nucleotides, and Nucleic Acids, 26:1295–1299, 2007

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770701530541

Taylor & Francis
Taylor & Francis Group

OPTICAL TWEEZERS AS A PROBE FOR OLIGODEOXYRIBONUCLEOTIDE STRUCTURATION

Nicola Borbone, Giorgia Oliviero, Jussara Amato, Gennaro Piccialli, and Luciano Mayol □ *Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli "Federico II," Napoli, Italy*

Lisa Miccio, Giuseppe Pescen, and Antonio Sasso □ *Dipartimento di Scienze Fisiche, Università di Napoli "Federico II," Napoli, Italy*

□ *The aim of this work is to investigate if the optical tweezers (OT) are suitable as a diagnostic tool for monitoring the oligodeoxyribonucleotide (ODN) structural behavior in solution. Preliminary experiments, performed on the quadruplex formed by the ODN sequence TGGGGT, showed that the OT can be used as a probe for ODN structuration by monitoring the medium viscosity changes associated with ODN folding-unfolding processes.*

Keywords Optical tweezers; DNA quadruplexes; viscosity changes; structural studies

INTRODUCTION

Oligodeoxyribonucleotides (ODNs) can form an unexpected number of secondary structures. Quadruple helices based on G-quartets (G-quadruplexes) have aroused widespread interest not only for their substantiated presence in many biologically important regions of the genome, but also because such structures form the scaffold of several aptamers provided with useful biological properties.[1–6] ODNs are commonly characterized by means of several spectroscopic techniques such as UV, NMR, CD, and x-ray. Furthermore, several articles describe the use of OT for studying the interactions between nucleic acids and the other biomolecules.[7,8]

In this article, we describe a novel potential application of the OT equipment aimed to the analysis of ODN structural changes in solution such as,

This work is supported by Italian MURST (PRIN 2005). The authors are grateful to Centro di Servizi Interdipartimentale di Analisi Strumentale (CSIAS), for supplying the NMR facilities.

Address correspondence to Nicola Borbone, Dipartimento di Chimica delle Sostanze Naturali, University of Naples, Faculty of Pharmacy, via Domenico Montesano 49, Napoli 80131, Italy. E-mail: borbone@unina.it

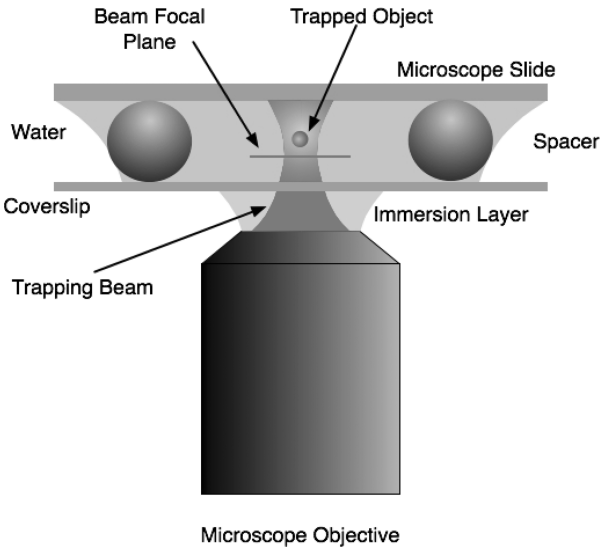

FIGURE 1 The optical tweezers working principle. The laser beam is tightly focused by a microscope objective inside a small sample cell. The sample cell, built with a microscope slide and a coverslip separated by a thin spacer, is filled with the solution to be investigated. Small polystyrene bead are dilute in the solution. Once trapped the bead continues to move due to the collisions with the surrounding molecules.

for example, the folding of ODN single strands into quadruplex structures in the presence of K^+ containing buffer.

Optical tweezers are a particular trapping scheme based on a strongly focused laser beam through a microscope objective lens (Figure 1).[9,10] The optical trap is useful to capture and manipulate neutral dielectric particles as well as many biological objects like bacteria, cells, organelles, chromosomes, etc. The typical forces exerted by an OT are in the range from tens of fN up to tens of pN.[11] These forces are typical for the macromolecular bounding, and for the interactions between neutral micro-objects and surfaces. During the last two decades OT have been used as a powerful tool in an impressive number of experiments covering a wide variety of fields, as biology, medicine, and soft matter.[12–14] Among the many OT applications, microrheology is certainly one of the research area where such technique is particularly promising. Indeed, OT has been revealed as a powerful tool to investigate diffusional behavior on micron- and nano-size scales that is particularly relevant for simple and complex fluid analysis.[15,16] On the contrary, traditional rheometers provide only average values over bulk samples, and they completely fail when small amount of samples are available as, for instance, in molecular and cell biology.

A trapped spherical bead continues to move inside the optical potential well due to the continuous collisions with the solvent molecules (well known as Brownian motion). Since the particle explores volumes comparable to its size during this motion, studying the resulting thermal noise represents a

powerful method for local viscosity measurements of the host medium. The analysis of the particle motion in the frequency domain[11] is very useful in order to get hold of these properties. In fact the power spectrum of a bead, trapped in an optical tweezers, can be exactly calculated and it turns out to have a Lorentzian shape:

$$S_X\langle f\rangle = \frac{k_B T}{3\pi^3 \eta d\left(f_C^2 + f^2\right)}; \quad f_C = \frac{\kappa}{6\pi^2 \eta d} \tag{1}$$

where f_C is called *corner frequency*. The bead positions can be measured using a quadrant photodiode (QP)[17] which collects the laser light scattered and unscattered from the trapped particle. The output signal is, for small displacements from the equilibrium position, linearly proportional to the displacement itself. The QP signal is acquired with a digital oscilloscope. The power spectral density (PSD) is easily obtained from the position data, by means of a standard numerical FFT routine. The output of the position detector is a voltage signal, so far the PSD is $S_V\langle f\rangle = \beta^2 S_X\langle f\rangle$ where β is the calibration factor required to convert the voltage units in length units. Unfortunately the calibration procedures are based either on the comparison of known forces, that is, the viscous drag (Stoke's drag method), or by moving a bead stuck on the coverslip surface across the laser beam. These calibration procedures provide a calibration factor whose accuracy is usually larger than 13–15%, which is a limiting factor if the measure of small viscosity changes represents the aim of the experiment. However, if these small changes can be measured with respect to an arbitrary reference value, that is, the initial viscosity of the solution, the accuracy is limited only by the instrumental noise, by the numerical routine to compute the PSD, and by the statistical errors from the best fit. As reported below the final accuracy is smaller or at least equal to 10%.

From Equation (1) it is evident that the f_C is inversely proportional to the viscosity of the medium. For two different media, M1 and M2, the viscosity is related to the respective corner frequency by the relation:

$$\frac{f_C^1}{f_C^2} \propto \frac{\eta_2}{\eta_1}$$

It is now clear that from a set of measurements of the corner frequencies in several media it is possible to estimate the viscosity variation with respect to a particular reference medium, such as distilled water, provided the temperature of the sample is kept constant during the measurements.

TABLE 1 Corner frequency ratios between ON or ON + 80 mM K^+ solutions (superscript 1) and pure water (superscript 2)

f_C^1/f_C^2	ON 0.0 mM	ON 1.2 mM	ON 2.4 mM
ON/water	1	0.971(7)	0.945(7)
(ON + K^+ 80 mM)/water	0.995(6)	0.951(7)	0.830(7)

RESULTS

In order to understand if this technique can be used to observe the ON structuration by monitoring the viscosity changes in the ON medium, preliminary measurements have been performed on a ON sample (TGGGGT) at two different concentrations (1.2 and 2.4 mM) in 80 mM potassium buffer. To estimate the neat contribution to the medium viscosity due to the change of ON structure, we had to take into account the different effects exerted by the addition of salts and ONs alone to pure water. The values reported in Table 1 show that the viscosity change between pure water and 80 mM potassium buffer is negligible at this concentration (column 2, Table 1), and that, as expected, a slight increase of water viscosity is observed when the ON is added to pure water (row 2, Table 1). On the other hand, a clear increase of viscosity is observed when the ON is added to the 80 mM K^+ buffer (row 3, Table 1). The marked decrease of the f_C ratio (i.e., higher viscosity) should be correlated to the ON structuration (single strands → quadruplex structure).

In another set of experiments we measured the f_C ratios for a series of 1.6 mM TGGGGT solutions in K^+ buffer at increasing concentration. The second row of Table 2 shows once again that the f_C ratio resulted to be almost unaffected by the buffer concentration (at least in the range 0–80 mM); on the other hand when the ON is added to the buffered solution a marked change in the f_C ratios is observed (row 3, Table 2). The more the salt concentration, the smaller the ratio. One more time the data are in agreement with the correlation between the rise of viscosity and the ON structuration.

We conclude that the OT could be used as a novel technique—alternative to the classical CD and NMR—for the fast analysis of ODN structural behavior in solution. The main advantage of OT over CD and NMR is

TABLE 2 Corner frequency ratios between salt or salt +1.6 mM ON solutions (superscript 1) and pure water (superscript 2)

f_C^1/f_C^2	K^+ 0 mM	K^+ 20 mM	K^+ 40 mM	K^+ 60 mM	K^+ 80 mM
K^+/water	1	1.02(6)	1.00(4)	1.01(6)	0.99(5)
(K^+ + ON 1.6 mM)/water	0.98(5)	0.95(5)	0.89(6)	0.72(6)	0.65(7)

represented by the very tiny amount of sample required for the analysis—about 2 μL of ODN sample solution compared to the hundreds of μL required for CD and NMR.

REFERENCES

1. Shafer, R.H.; Smirnov, I. Biological aspects of DNA/RNA quadruplexes. *Biopolymers* **2001**, 56, 209–227.
2. Arthanari, H.; Bolton, P.H. Functional and dysfunctional roles of quadruplex DNA in cells. *Chem. Biol.* **2001**, 8, 221–230.
3. Jing, N.; Marchand, C.; Guan, Y.; Liu, J.; Pallansch, L.; Lackman-Smith, C.; De Clercq, E.; Pommier, Y. Structure-activity of inhibition of HIV-1 integrase and virus replication by G-quartet oligonucleotides. *DNA Cell Biol.* **2001**, 20, 499–508.
4. Smirnov, I.; Shafer, R.H. Effect of loop sequence and size on DNA aptamer stability. *Biochemistry* **2000**, 39, 1462–1468.
5. Pileur, F.; Andreola, M.L.; Dausse, E.; Michel, J.; Moreau, S.; Yamada, H.; Gaidamarov, S.A.; Crouch, R.J.; Toulmé, J.J.; Cazenave, C. Selective inhibitory DNA aptamers of the human RNase H1. *Nucleic Acids Res.* **2003**, 31, 5776–5788.
6. Chinnapen, D.J.F.; Sen, D. Hemin-stimulated docking of cytochrome c to a hemin-DNA aptamer complex. *Biochemistry* **2002**, 41, 5202–5212.
7. Harada, Y. Studies on biomolecules using single molecule imaging and manipulation techniques. *Science and Technology of Advanced Materials*, **2004**, 5, 709–713.
8. Smith, D.E.; Gemmen, G.J.; Millin, R.; Rickgauer, J.P.; Schweitzer, A.L.; Fuller, D.B N. Using optical tweezers to study protein-DNA interactions. *Proc. SPIE* **2005**, SPIE-5930, 274–283.
9. Ashkin, A.; Dziedzic, J.M.; Bjorkholm, J.E.; Chu, S. Observation of a single-beam gradient force optical trap for dielectric particles. *Opt. Lett.* **1986**, 11, 288–290.
10. Malagnino, N.; Pesce, G.; Sasso, A.; Arimondo, E. Measurements of trapping efficiency and stiffness in optical tweezers. *Opt. Commun.* **2002**, 214, 15–24.
11. Gittes, F.; Schmidt, C.F. *Methods in Cell Biology*; New York: Academic Press, 1998, vol. 55, p. 129.
12. Mehta, A.P.; Rief, M.; Spudich, J.A.; Smith, D.A.; Simmons, R.M. Single-molecule biomechanics with optical methods. *Science* **1999**, 283, 1689–1695.
13. Tanaka, H.; Ishijma, A.; Honda, K.; Yanagida, T. Orientation dependence of displacements by a single one-headed myosin relative to the actin filament. *Biophys. Journ.* **1998**, 75, 1886–1894.
14. Zander, C.; Enderlein, J.; Keller R.A. *Single Molecule Detection in Solution*; New York: Wiley-VCH, 2002.
15. Addas, K.M.; Schmidt, C.F.; Tang, J.X. Microrheology of solutions of semiflexible biopolymer filaments using laser tweezers interferometry. *Phys. Rev. E* **2004**, 70, 21503–21516.
16. Pesce, G.; Sasso, A.; Fusco, S. Viscosity measurements on micron-size scale using optical tweezers. *Rev. Sci. Instr.* **2005**, 76, 1151051–1151155.
17. Gittes, F.; Schmidt, C.F. Interference model for back-focal-plane displacement detection in optical tweezers. *Opt. Lett.* **1998**, 23, 7–9.