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Quantitative detection of dye labelled DNA using surface enhanced resonance Raman scattering (SERRS) from silver nanoparticles

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Abstract

The detection of dye labelled DNA by surface enhanced resonance Raman scattering (SERRS) is reported. The dye labels used are commercially available and have not previously been used as SERRS dyes. Detection limits using two excitation frequencies were determined for each label. This expands the range of labels which can be used for surface enhanced resonance Raman scattering with silver nanoparticles.

Keywords: Silver nanoparticles; SERRS; Oligonucleotides

1. Introduction

The direct analysis of DNA generally relies on labelling using specific dyes, which can then be detected in a sensitive and specific manner. The most common detection method involves the detection of the dye labels using fluorescence spectroscopy. However, fluorescence suffers from poor multiplexing ability due to the broad overlapping spectra, which are obtained. A more sensitive and molecularly specific technique is that of surface enhanced resonance Raman scattering (SERRS) [1–3]. SERRS has been shown to be generally three orders of magnitude more sensitive than fluorescence for the detection of dye labelled oligonucleotides [4]. Compared to fluorescence, SERRS has a simpler and more extensive labelling chemistry as it can be used for the detection of commercially available fluorescent dyes [5–7] as well as specifically developed SERRS dyes [8–10].

For an analyte to be SERRS active it must have a chromophore coincident with the laser excitation frequency and the ability to absorb onto a suitably roughened metal surface. Most compounds of interest do not possess these properties, however, the addition of a SERRS active label can achieve this by introducing both a chromophore and a surface seeking group into the molecule and this approach has been used successfully for the detection of DNA [6]. Commercially available fluorescent dyes can also be used as SERRS labels, since the metal surface quenches any fluorescence emitted by the dyes, which might otherwise swamp the signal [11]. In this case, the metal surface used takes the form of a colloidal suspension of silver nanoparticles made by the citrate reduction of silver nitrate [12].

Previously eight commercially available fluorescent dye labels (FAM, TET, HEX, TAMRA, R6G, ROX, FAM and TET) have been used to detect oligonucleotide sequences using SERRS and silver nanoparticles [6]. In this case, it was possible to detect the labels in a quantitative manner allowing detection limits to be obtained for each label. Gold nanoparticles with a silver coating have also been used for the detection of DNA by SERRS. SERRS spectra were obtained from oligonucleotides labelled with six different commercially available dyes: Cy3, TAMRA, Texas Red, Cy3.5, R6G and Cy5 [13]. These dyes were assessed for their multiplexing capabilities and show that it is possible to obtain spectra for each of these labels, although detection limits were not reported for each label. Vo-Dinh and co-workers have used a silver/PVA surface to detect labelled DNA corresponding to a gene indicative of breast cancer [14]. In these studies the dye used was Rhodamine B. Cresyl fast violet has also been used previously by Vo-Dinh as a SERRS label for a primer which was used in the PCR amplification of specific DNA

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sequences relating to HIV [15]. However, again detection limits were not reported in either case.

From the previously reported studies it is clear that SERRS offers significant advantages over other optical spectroscopies in terms of sensitivity and increased multiplexing. In order for SERRS to be used effectively, comparison of different labelling approaches is necessary to allow selection of the most appropriate labels for specific experimentation. In this paper, we show how to obtain SERRS signals from three dyes previously not used with DNA and report the detection limits for these dyes plus one other at two different excitation frequencies.

2. Experimental

2.1. Labelled oligonucleotides

The dye labelled oligonucleotides were purchased from Eurogentec (Belgium) and were purified by HPLC. The following oligonucletide sequences were used:

TCCACGTTTTCCCAGTCACGACGT for BODIPY TR-X 7C7C7C7C7C7CTCCACGTTTTCCCAGTCACGACGT for Cy3.5, Cy5.5 and Yakima Yellow, where 7 represents 5-propargylamine-2′-deoxyuridine.

2.2. Silver nanoparticle preparation

A colloidal suspension of silver nanoparticles was prepared by the citrate reduction of silver nitrate using a modified Lee and Meisel method [12].

2.3. Instrumentation

A Renishaw inVIA microscope system with a 514.5 nm argon ion laser and a Renishaw microscope system 1000 with a 632.9 nm helium–neon laser were used. Samples were analysed using a 20× objective to focus the laser beam into a microtitre plate containing the sample.

2.4. Sample preparation

All samples were prepared for SERRS analysis using the following amounts of reagents: 10 μl of dye labelled oligonucleotide, 10 μl of 0.1 mol dm^{-3} spermine tetrahydrochloride (Sigma), 250 μl of water and 250 μl of citrate reduced silver nanoparticles.

The concentration studies were carried out by diluting the oligonucleotides to various concentrations using sterile water and preparing the samples as outlined above. The samples were analysed within 1 min of the addition of the silver colloid and each concentration was analysed five times. The spectra obtained were the result of a 10 s accumulation time with the spectrometer grating centred at 1400 cm^{-1}. The spectra were baseline corrected using the GRAMS/32 software, and the average peak height of the strongest peak in the spectrum was plotted against the concentration.

3. Results and discussion

3.1. Nature of the labels

To obtain effective SERRS from DNA labelled with a fluorescent dye, we have found it best to promote strong surface adsorption by neutralising the phosphate backbone and adding functionality to promote this effect. When using silver nanoparticles produced by citrate reduction, the strongest SERRS signals are produced by aggregating the nanoparticles into discreet clusters. This maximises the enhancement through coupling of the surface plasmons and also averages out any surface anomalies, such as ‘hot spots’, to allow quantitation.

Spermine is a naturally occurring tetramine that neutralises the negatively charged phosphate backbone and provides controlled aggregation of silver nanoparticles [16,5]. This allows the DNA to adsorb onto the negatively charged citrate coated silver surface. The nature of the label also needs consideration. If the label is negatively charged we have found improved signal to noise ratios by using modified bases at the end of the oligonucleotide containing the label. These are 5-propargylamino-2′-deoxyuridines which provide the DNA with a positive charge once spermine is added. If the label is positively charged or neutral then these modifications are not necessary although increased sensitivity is obtained by using them.

The four dyes used in this study are shown in Fig. 1. The BODIPY TR-X has a thiophene function, which should promote surface binding and as such the propargyl amino modified bases were not used with this dye. The Yakima Yellow is a fluorescein analogue and is negatively charged necessitating the use of the propargyl amino modifications. Cy3.5 and Cy5.5 are both positively charged and in order to maximise surface adsorption, and hence the SERRS propargyl amino modifications were used with these dyes.

The adsorption maxima are also shown. This has a correlation with the resonance enhancement expected. Most enhancements arise from the surface interactions, however, improved discrimination and sensitivity can be obtained by considering the resonance component. When using silver nanoparticles maximum surface enhancement is obtained using excitation from green sources, such as 514.5 and 532 nm [17]. However, not all fluorophores provide resonance in this range and give poor SERRS, e.g. Cy5 [6]. Thus, we examined the four dye labelled DNA sequences at 514.5 and 632.9 nm to compare the SERRS obtained and the detection limits.

3.2. SERRS

The SERRS spectra from the four oligonucleotides using 514.5 and 632.9 nm excitation are shown in Fig. 2. At 514.5 nm, Cy5.5 only gave a SERRS spectra for the highest concentrations analysed, this is to be expected due to the fact that the excitation wavelength is far from being

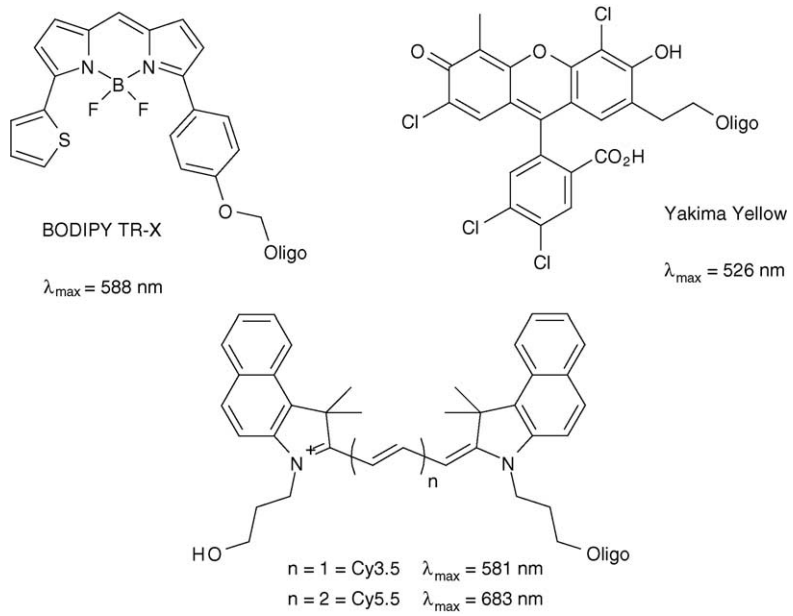

Fig. 1. The four dyes used in the study with their absorbance maxima.

in resonance with the dye (λ_{max} 683 nm). The other dye labelled oligonucleotides gave reasonable SERRS spectra at 514.5 nm, although the spectra obtained for Yakima Yellow displayed lower signal to noise ratios but with good people intensity. At 632.9 nm, good SERRS was obtained from all the labels, with the exception of Yakima Yellow, which did not give as good SERRS as the other labels. This is due to the lack of a resonance contribution at this frequency, but since poor spectra were obtained at both wavelengths it could be that Yakima Yellow is not as good a SERRS label as the other

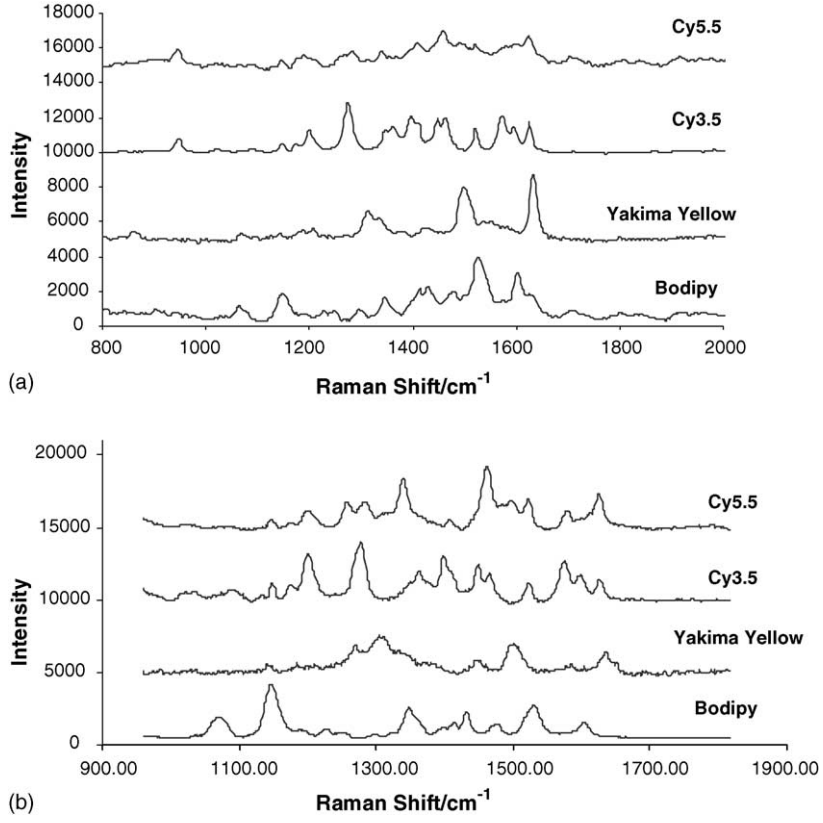

Fig. 2. The SERRS spectra from the four labelled oligonucleotides using a 10 s scan and excitation at (a) 514.5 nm and (b) 632.9 nm.

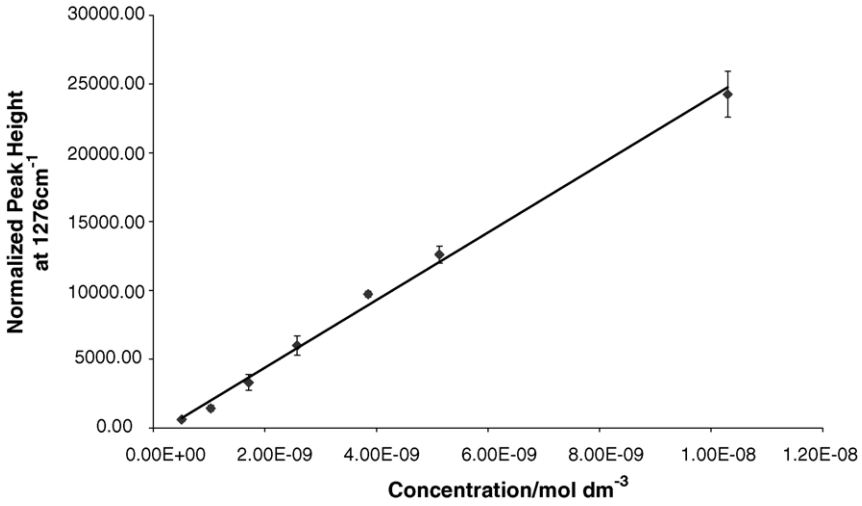

Fig. 3. Calibration graph obtained for Cy3.5 using 514.5 nm laser excitation. The error bars shown are ±1S.D. and each point is the average of five repeat samples.

dyes. This could be due to the nature of the dye, which even with the aid of the propargylamine modification may still not adsorb well onto the silver surface. Thus, Cy3.5 appears to be the only dye labelled oligonucleotide that works well at both frequencies.

3.3. Limits of detection

A dilution study for each dye labelled oligonucleotide was carried out using both 514.5 and 632.9 nm laser excitation. A typical calibration graph is shown in Fig. 3. The graph shows the linear concentration dependence that was obtained for Cy3.5 using 514.5 nm laser excitation. It can also be seen that the error in the measurements is relatively small with a R.S.D. of between 2 and 15% obtained for this set of data. Thus, it was possible to obtain quantitative and reproducible results.

The linear concentration dependence allowed detection limits for each dye labelled oligonucleotide to be obtained at both wavelengths and the results are shown in Table 1. It can be seen that the majority of the dyes analysed gave better detection limits when 632.9 nm laser excitation was used with the exception of Yakima Yellow where slightly better detection limits where obtained at 514.5 nm laser excitation. This is due to the fact that this dye is close to resonance with this wavelength. However, overall Yakima Yellow was a poorer SERRS dye than the other dyes and this is reflected in the higher detection limits obtained.

Table 1
The detection limits for the dye labelled oligonucleotides at each excitation frequency

Dye label	Detection limit (mol dm^{-3})	
	514.5 nm	632.9 nm
BODIPY TR-X	1.30×10^{-10}	7.85×10^{-12}
Yakima Yellow	1.72×10^{-11}	7.90×10^{-10}
Cy3.5	2.46×10^{-11}	7.48×10^{-13}
Cy5.5	–	5.52×10^{-12}

Lower detection limits for both Cy3.5 and BODIPY TR-X were obtained at 632.9 nm. This suggests that these dyes were more in resonance with the 632.9 nm laser excitation than 514.5 nm, even though their absorbance maxima lie between the two wavelengths, 581 and 588 nm, respectively. Also, the higher excitation wavelength meant that less fluorescence was observed in the spectra resulting in lower detection limits being obtained, as the signals were not lowered due to a background fluorescence signal. Cy3.5 proved to be an excellent SERRS dye, giving the lowest detection limit of all the dyes at 632.9 nm. In the case of Cy5.5, detection limits could not be obtained using 514.5 nm laser excitation as spectra could only be obtained with the highest concentrations. This is due to the fact that at 514.5 nm the dye is far from resonance, since its absorbance maxima is 683 nm. However, excellent detection limits were obtained when 632.9 nm excitation was use, which reflects the resonance contribution obtained at this wavelength.

4. Conclusion

The SERRS detection limits for four dye labelled oligonucleotides have been determined at two excitation frequencies using silver nanoparticles. The results are consistent with previous studies and show that the range of labels for use in quantitative DNA detection by SERRS has expanded. This will be of use for future studies involving SERRS and DNA.

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