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Label-free Rapid Semi-quantitative Detection of Proteins Down to Submonolayer Coverage by Using Surface-Enhanced Raman Scattering of Nitrate Ion

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A novel heat-induced SERS method was used to selectively enhance a band of NO_3^- at 1049 cm^{-1} for semi-quantitative detection of lysozyme and insulin down to 10^{-9} and 10^{-8} M, respectively, and bell shape variations of SERS intensities was observed for the concentration dependency of the proteins

We have recently proposed a heat-induced SERS sensing method for rapid detection of a peptide, glutathione.[1] In this method, 30 μL glutathione solution was heated on an aluminum pan plate at 100°C for 3 min to obtain a dry film of glutathione, and then, its SERS signal was acquired. Comparing to conventional SERS methods, this protocol enables one to obtain much larger SERS enhancement without loss of vibrational information about an analyte. In the present study, the application of this heat-induced SERS method is extended to protein detection. Firstly, a citrate buffer containing 6 mM $NaNO_3$ was mixed with a Ag colloid solution. Then, a protein was added to the mixture followed by drying the mixture at 100°C for SERS measurement.

Figure 1（a）and (b) compares SERS spectra of lysozyme (10^{-5} M in citrate reduced silver colloid) measured by use of the heat-induced method and without heat treatment (solution sample). It shows that the heat-induced method enhances the signal significantly, especially enhances the signal at 1049 cm^{-1}. To confirm whether the peak at 1049 cm^{-1} originates from NO_3^-, normal Raman spectra of lysozyme solutions containing 0 and 40 mM $NaNO_3$ are compared (Figure 1(e), (f)), and the band is found at 1049 cm^{-1}, revealing that it arises from the NO_3^- vibration. This peak has seldom been reported in SERS references, however, Mrozek et al. confirmly assigned it to the NO_3^- mode in their SERS study of saccharide using electrochemically roughed silver surface.[2] Both their and our works suggest that a dry film method selectively enhances the peak of NO_3^- at 1049 cm^{-1}.

The strength and sharpness of the NO_3^- peak at 1049 cm^{-1} lead us propose that this band may be suitable for quantitative analysis of the proteins. Figure 2(A) displays concentration-dependent (10^{-5}-10^{-9} M) SERS spectra of lysozyme. Comparison to previous studies, the results in Figure 3(A) demonstrate two novelties. First, the detection limit is much lower; down to a submonolayer coverage of 10^{-9} M, which is more sensitive than the method proposed before by which the detection limit is 10^{-6} g/L (equivalent to 7×10^{-8} M). [3] Second, a concentration-dependent curve shows a bell shape rather than a sigmoidal one. [1] Similar results of insulin were displayed in Figure 3 (C) and (D). The bell shape was supported by a UV-Visible study (data not shown) which suggests that when the protein concentration is above monolayer coverage, a further increase in protein concentration would cause a negative effect against silver aggregation and decrease the SERS signal. This phenomenon was not found when a tripeptide, gluthionine was used as an analyte [1]. The difference leads the proposal that the crowdness of the silver colloid environment is responsible for silver colloid aggregation. As lysozyme is a large and hard molecule (129 amino acids) comparing to small and soft gluthionine (3 amino acids), it is reasonable to observe that concentrated lysozyme inhibits silver colloid aggregation while the small peptide does not.

The adsorption mechanism of protein and NO_3^- possibly follows the most common co-adsorption scenario, which has been well established as a double-layer adsorption rule.[4] Namely, there is a layer of electrolyte such as citrate and NO_3^- between an adsorbed layer of proteins and Ag colloid. The results in Figure 3 provide some support to this speculation, suggesting that the intensities of protein bands including those at 761 and 853 cm^{-1} are positively correlated with the intensity of the NO_3^- band, which may be an evidence for the co-adsorption manner of the protein and NO_3^-. A detailed depiction on the adsorption mechanism of protein and electrolyte was published recently. [5]

Peaks due to acid radicals are usually considered as undesirable background in a SERS study, and additional caution should be paid to avoid these peaks. Here, however, we have developed a novel method which can selectively enhance the peak at 1049 cm^{-1} originating from NO_3^- to make it valuable for semi-quantitative detection of proteins without any additional resonant effect. This method is simple, rapid (dry time is 210s), reproducible (see Figure 4 (C)) and label-free. The laser power is low (6 mW) and the exposure time is short (20 s), which meets the need for routine analyses. The intensity and the sharpness of the NO_3^- peak suggest its potential for more extended applications, especially for those biomolecules with low Raman cross sections and bad quality spectra by normal SERS protocol. Also the bell shape indicates the potential of this method for exploring absorption phenomenon of protein on colloidal interface. These studies are ongoing in our group.

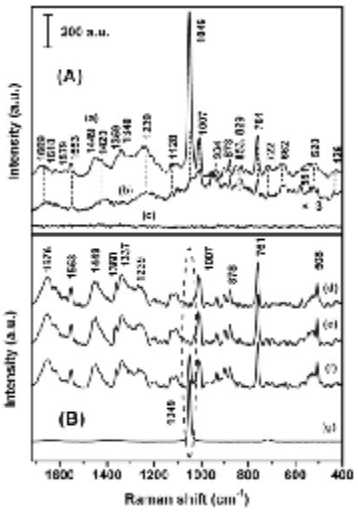

Figure 1. (A)(a) A SERS spectrum of a dry film of the solution containing 10^{-5} M lysozyme and 6 mM $NaNO_3$; (b) A SERS spectrum of a solution containing 10^{-5} M lysozyme and 6 mM $NaNO_3$; (c) A normal Raman spectrum of a dry film of 10^{-5} M lysozyme solution. (B)(d) A normal Raman spectrum of 7×10^{-3} M lysozyme in pure water; (e), (f) Normal Raman spectra of 7×10^{-3} M lysozyme in a 5 mM citrate buffer (pH 4.0) containing 0 mM $NaNO_3$ and 40 mM $NaNO_3$; (g) A normal Raman spectrum of 1M $NaNO_3$. The intensity bar is applied only for (A) while band intensities in (B) have been adjusted artificially for display.

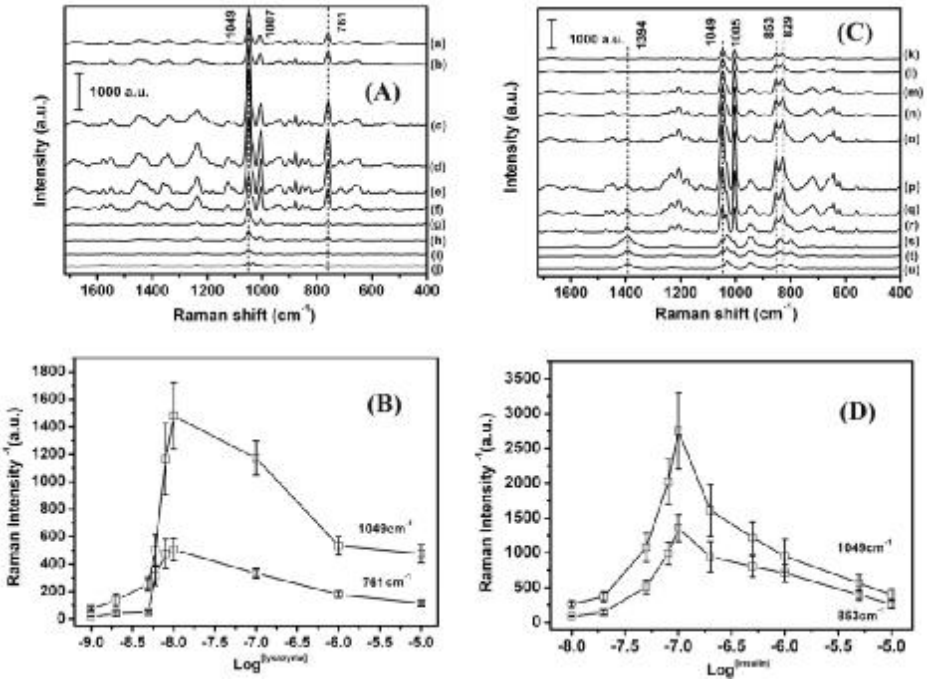

Figure 2. (A) Concentration-dependent (10^{-5}-10^{-9} M) SERS spectra of lysozyme (a) 1×10^{-5} M (b) 1×10^{-6} M (c) 1×10^{-7} M (d) 1×10^{-8} M (e) 8×10^{-9} M (f) 6×10^{-9} M (g) 4×10^{-9} M (h) 2×10^{-9} M (i) 1×10^{-9} M (j) 1×10^{-10} M; (B) Raman intensity at 1049 (NO_3^- band) and 761 cm^{-1} (Tyr band) of the SERS spectra in (A); (C) Concentration dependent (10^{-5}-10^{-8} M) SERS spectra of insulin (k) 1×10^{-5} M (l) 5×10^{-6} M (m) 1×10^{-6} M (n) 5×10^{-7} M (o) 2×10^{-7} M (p) 1×10^{-7} M (q) 8×10^{-8} M (r) 5×10^{-8} M (s) 2×10^{-8} M (t) 1×10^{-9} M; (u) 0 M control; (D) Raman intensity at 1049 and 853 cm^{-1} (Trp band) of the SERS spectra in (C).

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