Surface-Enhanced Infrared Absorption and Amplified Spectra on Planar Silver Halide Fiber

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We have discovered surface-enhanced infrared absorption (SEIRA) on a planar silver halide surface, the first time this effect has been observed on a *nonmetallic substrate*. The enhancement is not due to the presence of metallic silver on the surface as ruled out by X-ray photoelectron spectroscopy (XPS). In addition, the fibers show 10-fold spectral amplification due to the increased coupling of evanescent waves through an increased number of internal reflections to the samples on the surface of the thin planar element. A special cell for the fiber permits deposition of small quantities of solution followed by slow evaporation of the solvent with dry nitrogen and allows measurements to be made on much less than a monolayer of sample. The combination of easy access to the sample and high sensitivity promises many useful applications to biological, chemical, and physical problems in the mesoscopic and nanoscopic domains.

Novel techniques can often be used in ways that are not anticipated in the initial stages of the research. Our efforts to develop the use of planar silver halide polycrystalline fibers to carry out infrared measurements on small quantities of biological materials has led to important advances in this area. In the course of examining the dependence of the spectra on the quantity used, we have discovered surface-enhanced infrared absorption (SEIRA) on the silver halide surface, the first time this effect has been observed on a *nonmetallic substrate*. The enhancement is not due to the presence of metallic silver on the surface as ruled out by X-ray photoelectron spectroscopy (XPS). In addition, the fibers show 10-fold spectral amplification due to the increased coupling of evanescent waves through an increased number of internal reflections to the samples on the surface of the thin planar element. A significant improvement in the sensitivity and convenience of microscale infrared spectroscopy is thus achieved using planar silver halide fibers, allowing measurements to be made on much less than a monolayer of sample.

Surface-enhanced Raman scattering (SERS) by molecules on metal surfaces provided the opportunity for the study of surface layers in detail due to the large enhancements in signal (10^6 or more,[1,2] with surface-plasmon enhanced techniques, $\sim10^{10}$)[3] in comparison to the usual Raman measurement. Surface-enhanced infrared absorption (SEIRA) was discovered soon after the discovery of SERS for surface layers on metal films[4] and has attracted growing attention in the past few years despite the relatively small enhancements (3−1000). More recently, surface enhanced magnetooptical effect (SEMO) has been reported.[5]

We developed a cell for FTIR-ATR studies on cylindrical silver halide fiber [AgX, usually AgBr:AgCl, 0.6:0.4][6,7] and described the spectra of small organic molecules in amounts as small as 90 ng, but requiring 10 μg for reasonable quality spectra.[8,9] A solution of the sample was applied with a micropipet to the fiber and the solvent removed by a stream of dry nitrogen.

Spectral Amplification

Not long ago, we demonstrated that the IR absorbance due to ethanol on a flattened fiber increased with decreased thickness (approximately as $1/d$, with d as the thickness) without studying the spectra or concentration dependence.[10] We therefore embarked on a study of the infrared spectrum of the enzyme, trypsin, on a planar fiber (d, 155−220 μm) installed into our IR cell. A schematic of the cell and the planar fiber is shown in Figure 1A.

We found that the absorbances in the spectra were greater than those obtained on a cylindrical fiber by a factor of 5−13. This amplification is a considerable advance in the fiberoptic technique, illustrated by the comparison of the IR spectra of 1 μg of trypsin on an AgX cylindrical fiber and on a planar fiber. (Figure 1B) The signal-to-noise ratio in the spectrum on the planar fiber is also improved.

Discovery of Surface Enhanced Infrared Absorption (SEIRA)

Previous studies had shown that absorption increased linearly with amount almost up to the limit of evanescent wave penetration.[9,11] In an examination of the relationship between absorbance and quantity on the planar fiber and to test the penetration limit, we found that trypsin in amounts between 1 and 10−12 μg showed a moderately rapid rise in absorbance between 0 and 3−4 μg, changing to a linear rise between 3−4 and 8−9 μg, with the penetration depth limit about 10 μg. Increasing the sample size well above 10 μg to 60 μg (not shown) had very little effect on the spectroscopic intensity, suggesting that the penetration depth [12] limit was reached at around 10 μg, corresponding to a layer of less than approximately 0.3 μm thickness under these conditions. Plots of absorbance versus quantity of trypsin are shown for three wavelengths in Figure 2A. The linear region extrapolated to an absorbance well above zero; so clearly the absorbance was anomalously high in the range below 1−2 μg.

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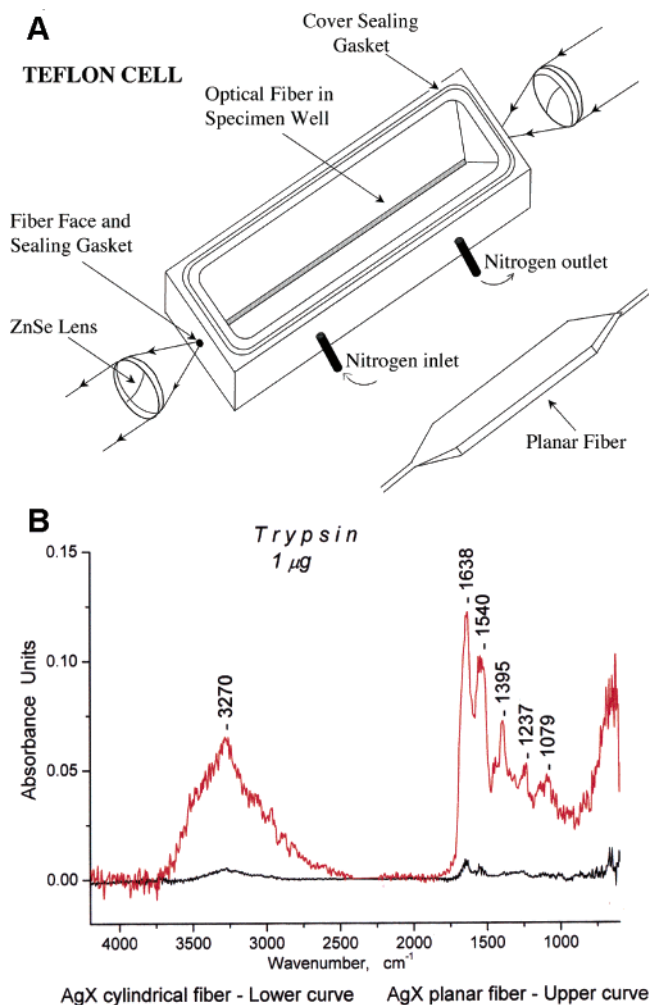
Figure 1. Fiberoptic IR cell and spectra. (A) Schematic of the planar AgX fiber next to the cell into which it is placed. Planar portion: *l* 3.9−4.0 cm, *w* 0.4 cm, *d*, 155−220 μm. Cylindrical portion: *d*, 0.9 mm. A sample of 4 μL is distributed (11−12 drops) as uniformly as possible on the planar portion of the fiber. After closing the cell, the sample is exposed to a slow flow of dry N_2 (1 mL/min). Infrared spectra are recorded (300 scans) continuously over the range from 4250 to 600 cm^{-1} to follow the removal of the water. (B) Spectra of 1 μg of trypsin on a cylindrical AgX fiber (lower curve) and on a planar fiber (170 μm) (upper curve). The spectrum on the planar fiber is less noisy than that previously reported for 10 μg of trypsin on a cylindrical fiber in ref 9. The ratio of absorbances is approximately 13.

We then examined the spectra of trypsin in amounts between 25 and 1000 ng. The linear behavior of the absorbance between about 3 and 8 μg (Figure 2A) permitted an estimate of the absorbance expected for smaller quantities. From 25 to 2000 ng, the spectral intensities were stronger than expected. For the smallest amount, 25 ng, the spectrum was 10−35 times stronger than expected on the basis of the absorption changes observed between 3 and 8 μg. As the amount of trypsin was increased, the enhancement ratios decreased, reaching a limit somewhat above 1. Thus the planar silver halide surface gave rise to a surface enhanced infrared absorption (SEIRA) effect. The enhancement is defined as the absorption observed in place of that expected from the linear absorption increases for amounts between 3 and 8 μg, that is, for protein located away from the surface. The expected absorption *already includes* amplification due to the planar element.

In the course of many series of experiments with trypsin, three regimes of spectroscopic behavior were found, the first
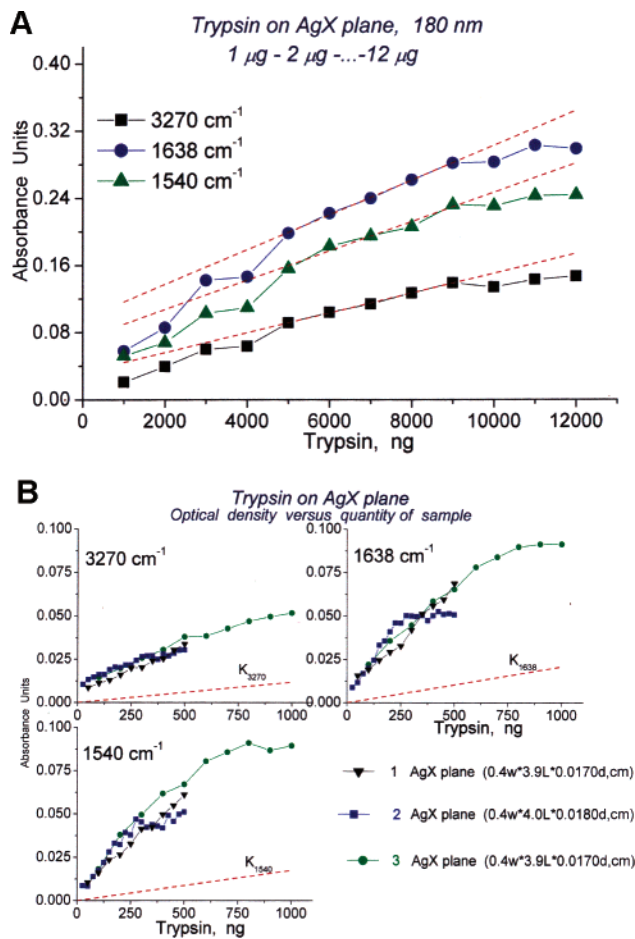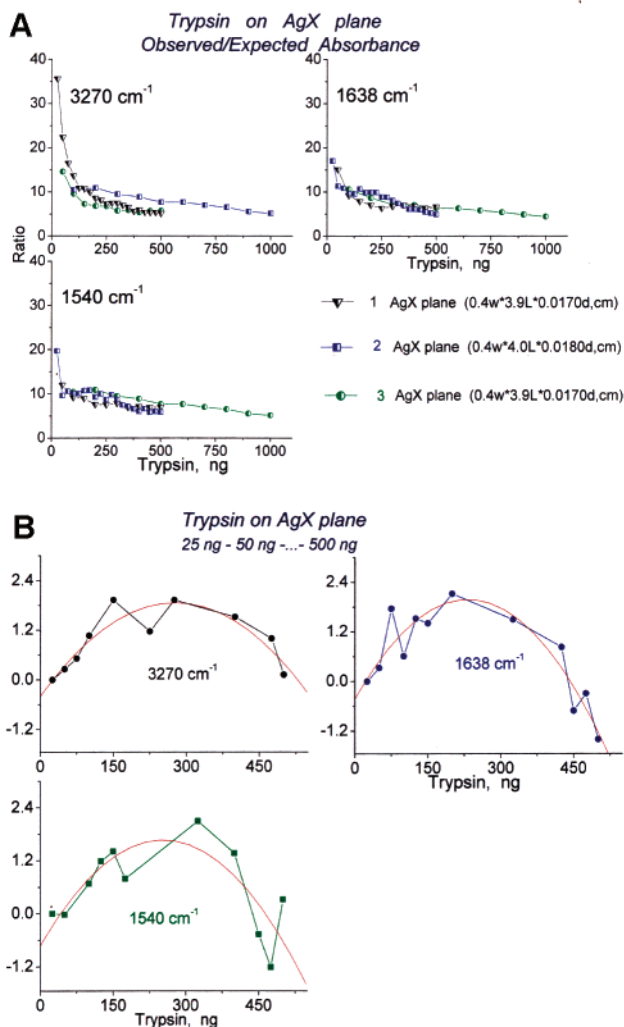

Figure 2. (A) Plots of absorbance (*A*) versus quantity of trypsin (T) (1000−12000 ng) for three different wavelengths in spectra from a series of measurements on a planar fiber (planar region 0.4 × 3.9 cm, *d* 180 μm). The reference slopes (linear region) from this series are $A_{3280} = 0.0325 + 1.18 \times 10^{-5}T$, $A_{1638} = 0.0958 + 2.072 \times 10^{-5}T$, and $A_{1540} = 0.0725 + 1.746 \times 10^{-5}T$. (B) Plots of absorbance versus quantity of trypsin (25−1000 ng) for three different wavelengths in spectra from three different series of measurements on a planar fiber. The reference slopes ($A_{calc} = K_\lambda T$) are those derived from the plots in Figure 2A.

for quantities between 50 and 2000 ng, the second, from 3 to 10 μg, and the third for quantities above 10 μg.

Range of SEIRA Effect

There are several ways to examine the enhanced intensities found for small amounts. The simplest is to plot the ratios of the experimental absorbance to the expected absorbance against the quantity of sample (Figure 3A). Such plots show clearly the magnitudes of the enhancements but do not reveal the changes with increasing amounts of sample. What is needed are the enhancements for each increment of sample, shown by plotting the increase in absorbance for a given pair in quantity divided by the increment for the previous pair. Because the spectra are moderately noisy, the points are scattered. However, polynomial fits describing most of the points have broad maxima between 250 and 600 ng (Figure 3B illustrates the point). From data for *Bos taurus* trypsin 1 in the MEROPS protease database[13] the trypsin [Sigma T-0303 porcine pancreatic trypsin] was estimated to have a volume of 2.85×10^5 A^3/molecule, requiring 5.47×10^{12} molecules or 214 ng (MW 23 545) for the volume of a monolayer (height 100 A) on the flat AgX surface (0.4 × 3.9 cm). The degree of surface enhancement reaches a peak for

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Figure 3. Enhancement analysis. (A) Ratios of observed /expected absorbance versus quantity of trypsin (25−1000 ng) for three different wavelengths for three series of spectroscopic measurements on a planar fiber. See Figure 2B. (B) Absorbance increment fractions versus quantity of trypsin for a series of measurements from 25 to 500 ng on an AgX plane The fractions were derived by taking the difference A_{n+1} − A_n and dividing by the previous difference A_{n-1} − A_{n-2}. Polynomial fits (excluding a few outliers) [Y_{3270} = −0.39412 + 0.01633T − 2.94875 × $10^{-5}T^2$, Y_{1638} = −0.41892 + 0.0207T − 4.46046 × $10^{-5}T^2$, and Y_{1540} = −0.72366 + 0.01895T − 3.75067 × $10^{-5}T^2$] suggested that the maximum effect was attained at about 250 ng, an amount that should be sufficient to fill one layer. Similar analyses for two other series yielded maxima at 325 and 600 ng.

approximately one to three surface layers (5−15 nm) and thus must be a short-range effect.

Some of the variations in absorbance may be traced to uneven distribution of the sample on the surface. Atomic force microscopy (AFM) measurements show that a 200 ng sample of trypsin is initially unevenly spread on the surface. (The surface is somewhat hydrophobic, judged from the contact angles of the droplets deposited on the planar surface.) Addition of 4 μL of water followed by the usual drying procedure produced a more even distribution of trypsin. (Figure 4)

Two Effects: Amplification and Surface Enhancement

The two effects reported here are **amplification**, in which the spectroscopic intensity is raised by almost an order of magnitude, and **surface enhancement** (SEIRA), which produces almost another order of magnitude increase in absorbance. The
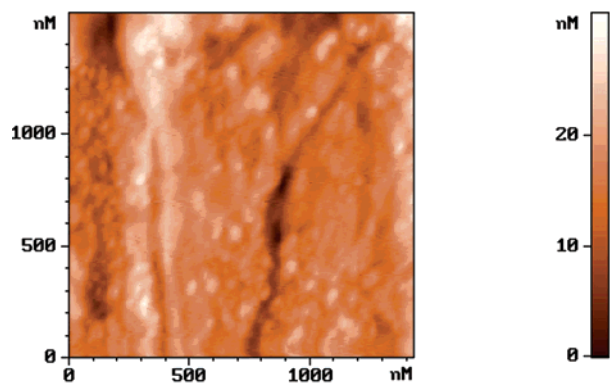
Figure 4. Tapping mode AFM image of the AgX fiber surface with a small amount of trypsin adsorbed. The trenches occur occasionally on the fiber surface; the granular material seen is the protein with roughness on the order of 5−10 nm, indicating one to two layers of material.

simplest explanation for amplification is that the thin planar fiber permits many more interactions of the evanescent wave with the sample on the surface through an increased number of internal reflections, resulting in stronger absorbance even though the signal strength is lower. The penetration depth limit is diminished in thinner cells, a potentially valuable property for the examination of the surfaces of external samples. Symmetrically tapered planar IR waveguides have been fabricated from epoxide-embedded single-crystal Ge by grinding and polishing to a thickness at the taper minimum of <30 μm, yielding spectra comparable to or better than those obtained with a regular Ge ATR crystal.[14] For water, an amplification of 37 was obtained.

We have also shown that small organic molecules also display SEIRA using the AgX planar fiber. Results for the polar molecule, *p*-nitrobenzoic acid, which has been the subject of numerous SEIRA measurements on silver (and other metallic) substrates and for the nonpolar molecule, *n*-dodecane, will be reported separately.[15]

A tentative model for the unexpected SEIRA effect on AgX may be formulated on the basis of the following points. The range of the effect is small, perhaps 10−15 nm, reminiscent of the distances found for SEIRA on metal substrates. Chemical changes, such as formation of silver clusters, are not responsible for the effect. We have confirmed the *absence* of metallic silver from the surface (both sides of the planar element) after it was used for the IR spectroscopy, by XPS. The metallic Ag Auger line (MVV) (binding energy of 1128 eV) is adjacent to the peak of AgX (1131 eV) but is completely missing in the spectrum. The atomic ratio of Ag$^+$ to X$^-$ (Cl$^-$, Br$^-$) is the expected 1:1 without traces of silver oxide (lacking in the high-resolution spectrum of the oxygen peak).

One should note the apparent lack of a wavelength effect in the SEIRA spectra. Our working hypothesis at this time is that an image dipole induced at the surface by the molecular dipole of a proximate molecule can be invoked to explain the electromagnetic enhancement.[4,16] We shall consider these matters further in subsequent communications.

Conclusions

Planar AgX fibers represent a significant advance in fiberoptic spectroscopic technology and can be easily used in a nitrogen flow cell for samples of 25 ng. They are much less expensive than the elegant tapered Ge elements produced by Braiman and co-workers.[14] Both the AgX and Ge cells yielded amplifications

of at least an order of magnitiude in spectroscopic intensity. The novel surface enhancement found for AgX planar fibers opens a new frontier in the nanoscopic and mesoscopic domains, raising questions about solid−liquid interfaces, molecular structure of liquids in confined environments, and the interaction of samples with the surface. Although it may be difficult to find other materials with the favorable properties of AgX, it would be worth searching for SEIRA in other ionic solids.

The relative ease with which samples may be quantitated goes part way in satisfying the worry that SEIRA effects are not adequately quantitative.[17] The combination of amplification and surface enhancement has allowed many interesting studies to be undertaken and these will be reported in subsequent articles. The easy access to the sample and high sensitivity promises many other applications to biological, chemical, and physical samples in the mesoscopic and nanoscopic domains.

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