

Surface Enhanced Resonance Raman Scattering from Cyanocobalamin and 5'-Deoxyadenosylcobalamin

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Surface enhanced resonance Raman scattering (SERRS) from cobalamins is reported. To achieve efficient adsorption and strong SERRS, the molecule is adsorbed on a polylysine-treated silver colloid surface. This provides an organic barrier between the cobalamin and the metal, preventing denaturation, and gives positively charged groups for efficient adsorption. The technique can be used for *in situ* studies of changes in the metal coordination sphere, as is illustrated by reduction on the surface using dithionite for both cyanocobalamin and 5'-deoxyadenosylcobalamin. The efficient energy transfer at the surface appears to prevent photodegradation, which is a problem with resonance, and the efficiency of SERRS allows measurements at low effective concentrations.

INTRODUCTION

Surface enhanced resonance Raman scattering (SERRS) provides an extremely sensitive and selective probe of biologically important chromophores *in situ* in solution. The method requires that the molecule is adsorbed on a suitable metal surface (usually roughened silver, copper or gold electrodes or an aggregated colloid of these metals) so that an interaction between the molecule and the surface plasmon causes enhancement of between 10^9 and 10^{12} over normal Raman scattering.¹

In situ techniques with the specificity to define changes in cobalt oxidation state, corrin ring conformation and coordination in the fifth and sixth positions would be very valuable for studies of cobalamin chemistry and biochemistry. Previous solid-state FTIR Raman scattering indicated that Raman spectroscopy would be ideal for this purpose since there are bands sensitive to conformation changes in the molecule.² If visible excitation could be used, the additional advantage of resonance enhancement would provide information on both the electronic and the vibrational structure. However, resonance Raman studies have proved to be possible but difficult owing to the photolability of the cobalamins and to fluorescence in some cases.^{3,4} SERRS is ideal in these circumstances since it gives additional sensitivity and quenches fluorescence. In addition, in the colloidal form, the colloid moves repeatedly in and out of the beam, preventing prolonged exposure and reducing photodecomposition.

RESULTS AND DISCUSSION

Previous attempts to obtain SERRS spectra gave very poor results, but an understanding of the surface chem-

istry improves SERRS, making it a very effective and sensitive technique. For example, 25 azo dyes some of which fluoresce in resonance gave excellent SERRS at concentrations equivalent to 10^{-9} M in solution.⁵ In essence, the surface of the negatively charged colloid particles was altered by the addition of polylysine so that the surface was positively charged. Previously, in studies of sensitive proteins such as P-450, it was noted that the spin and oxidation state could be retained provided that the solution conditions were compatible with protein integrity and that a citrate-reduced colloid was used.⁶ In essence, the citrate-reduced colloid has a citrate layer on the surface providing negatively charged organic groups which give protection between the protein and the silver surface and prevent metal-induced denaturation. The addition of polylysine to this citrate-coated surface provides positively charged groups and a thicker, more effective layer of organic material between the metal and the absorbate, enhancing the protection against denaturation. This surface provides an ideal environment on which to adsorb negatively charged cobalamins.

The spectra of cyanocobalamin and 5'-deoxyadenosylcobalamin are shown in Fig. 1. There are some similarities with the solid-state FT Raman spectra and solution resonance spectra. In particular, the large band at 1498 cm^{-1} is present. As with the solid-state study but not with the previous solution Raman studies, a small shift in wavenumber between the 5'-deoxyadenosylcobalamin and the cyanocobalamin is observed. There are differences in intensity on some bands in the scattering from the two compounds, indicating sensitivity to the ligands in the fifth and sixth coordination positions. The equivalent concentration in solution of the molecule was 10^{-5} M. This is two orders of magnitude lower in concentration than resonance enhancement and the signal-to-noise ratio is still good, suggesting that lower concentrations will provide acceptable signals.

A surface reaction can be carried out by reducing

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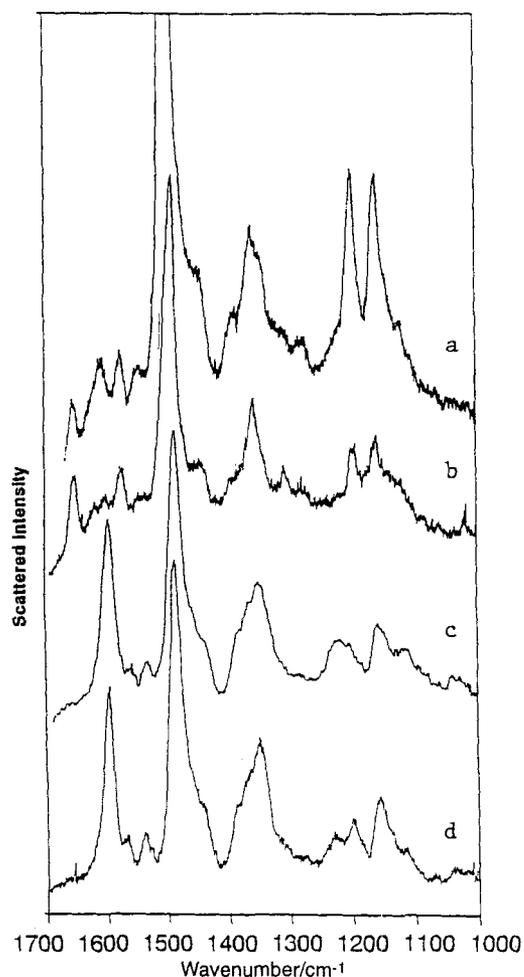


Figure 1. SERRS of cobalamins: (a) adenosylcobalamin; (b) cyanocobalamin; (c) reduced adenosylcobalamin; (d) reduced cyanocobalamin. 100 mW of 514 nm radiation was used and the scattering was recorded on an Anaspec modified Cary 81 instrument (5 cm^{-1} slits.)

each cobalamin on the surface with dithionite. The scattering from the reduced species is more similar than the scattering from the original compounds, suggesting that the sixth ligand position is similar owing to the dissociation of the 5'-deoxyadenosyl and cyanide moieties on production of cobalt(II). A similar effect has been seen in resonance.

In addition to providing better defined spectra at lower concentrations, the SERRS spectra provide unique information in terms of both relative intensities and the appearance of extra bands. For example, the IR-active carbonyl stretch is clearly seen at about 1650 cm^{-1} , but in SERRS it is readily identified at low concentration in contact with aqueous solution.

SERRS provides a good, sensitive probe for the study of cobalamins at low concentrations in contact with aqueous buffer and should assist more detailed studies of the chemistry of cobalamins at low concentrations.

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