Sample Analysis Report
Number 51

Photodegredation of Protein Light Harvesting Complexes In 4ul Microvolume Samples

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Introduction

The recent introduction of a new Microvolume capability for High-performance UV/Vis/NIR spectrophotometers has opened up the possibility of a range of investigations into the behavior of samples which were previously thought to be too concentrated, or which are impossible to prepare in large volumes.

The ability of the Cary 4000/5000/6000i series instruments to measure samples with volumes as small as 0.8ul with the precision normally expected of research-grade spectrophotometers in 1-3ml samples means that research can be carried out on smaller, more highly absorbing samples than has ever been previously possible. One example of the possibilities is in the measurement of bacterial porphyrins or light-harvesting complexes, which may only be available in very small amounts, yet can be studied in this system without loss of precision.

This study shows that it is possible to measure the absorbance characteristics of 4ul of protein and also that it is possible to measure changes arising from photobleaching of the sample during measurement.

By coupling the powerful performance of the Cary 4000 reference grade spectrophotometer with the small sample capability and sample path precision of the Hellma Traycell the problem of quantitation of small quantities of Protein in small volumes is solved. Noise levels as low as 0.0002 Abs with samples of 1ul mean those protein concentrations of as low as 100ug/ml can be measured in a single microlitre. (Or down to approx 4 ng DNA.) The instrumentation also offers the significant advantage of being able to take a scan of the sample at this concentration, which can verify purity. If 4ul of sample can be spared, the increase in path-length from 0.2 to 1mm means that as good or better results can be obtained with samples containing as little as 20ug/ml

The Hellma TrayCell (below) allows the powerful Cary 4000 to deliver results of astonishing precision in samples as small as 0.8uL

The dynamic range of the Cary 4000, to in excess of 9 Abs in normal cuvettes (see sample Analysis report Number 37) or about 7 Abs in the TrayCell, allows Protein concentrations from about 20 ug/ml-100,000 ug/ml to be measured. (Rear beam attenuation is required with readings above 4A)

Instrumentation

The Cary 6000i spectrophotometer (below) fitted with manual and automated rear beam attenuators and a 1 cm cell holder modified to provide maximum precision with a Hellma Traycell.

Materials and Reagents

Bacterial chromophores purified by gel permeation chromatography in the University of Sheffield department of Molecular Biology and Biochemistry was analyzed. (Professor C N Hunter)

Conditions

The Cary 6000i was used for this experiment but in the UV/Vis region where the same results could be expected from a Cary 4000, between 200 and 800 nm. The Traycell was mounted and aligned to 4.8%T using the precision cell holder. A bandwidth of 2 nm, scan speed of 60 nm/min with an averaging time of 2 seconds and data interval of 2 nm were used. Rear beam attenuation to 1.3 Abs was used to offset the internal attenuation of the Traycell

Discussion

The sample was initially scanned to reveal the absorbance profile, and then repeat scans were made under identical conditions, which revealed a change in maximum absorbance with successive scans. As there was no baseline absorbance shift, or very minimal baseline absorbance shift, the conclusion drawn is that the energy of the light used to perform the analysis was photobleaching the sample at peak absorbance. Confirmation of this result could be obtained by illumination of the sample with an external light source between readings.

Conclusion

Although this is a very simple experiment, the results show that there is a physical phenomena taking place in the solution which could provide the basis for further investigation. The sample size requirement is so small that even precious and difficult to purify proteins can be analyzed.

Graphics and Tables

Figure 1 *Repeat scans of Bacterial porphyrin which show complete stability at 500 nm (+-0.0008A) yet significant change at 400 nm suggesting photo bleaching is occurring*

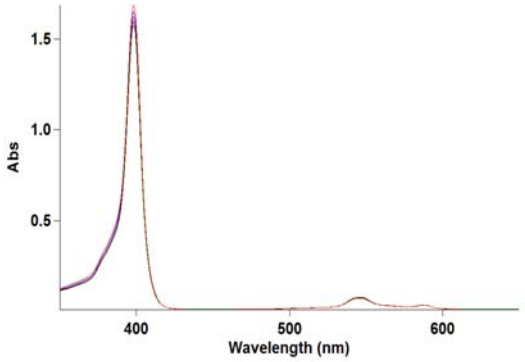


Figure 2 Detail from the scan at 400 nm showing changes of reading (0.02Abs (equivalent to 0.2 Abs in 1cm) with successive scans

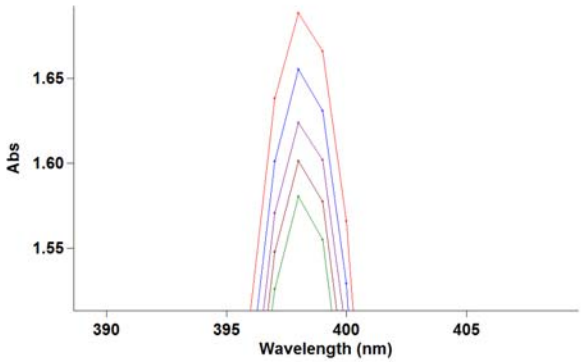
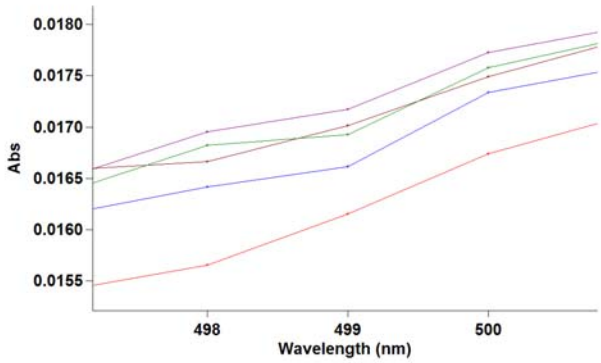


Figure 3 Detail from the scan at 500 nm showing reproducibility of reading and low variance (0.0008 Abs (equivalent to 0.008Abs in 1 cm Path) of less than 1% of the 398 nm peak change



These data were observed using standard Varian Cary UV instruments Collection Time: 03/06/05 12:33:51

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