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Photolysis of γ-(α-Carboxy-2-nitrobenzyl)-L-glutamic Acid Investigated in the Microsecond Time Scale by Time-Resolved FTIR

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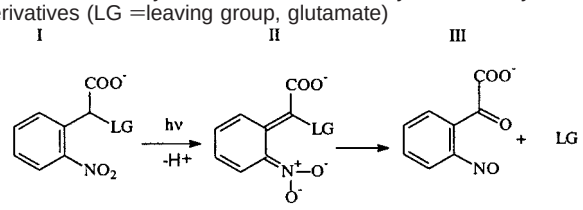
The photolytic release of biologically active substances from their photolabile inert precursors (caged compounds) has proven to be an extremely powerful technique for generating rapid concentration jumps.[1−7] This method allows for kinetic investigations of biological processes in the submillisecond time scale, which was previously limited by the millisecond time resolution achievable by the conventional mixing methods. One parameter that is critical to the use of the caged compounds in such kinetic investigations is the rate of the photolytic release of the substrate from these inert precursors. This rate has been indirectly determined using transient UV−visible absorption measurements.[1,2,8] However, such measurements are limited by the requirement for the presence of a chromophore that absorbs in the UV−visible region and hence have provided only an inferential estimate for this rate. For instance, in the case of the most commonly used photoremovable caging group, the 2-nitrobenzyl group, the only intermediate characterized by the transient absorption measurements has been the aci-nitro intermediate (shown in Scheme 1 as II).[2,8] On the basis of the mechanism

Scheme 1. Photolysis Reaction of α-carboxy-2-nitrobenzyl Derivatives (LG =leaving group, glutamate)



shown in Scheme 1 the rate of the photolytic release of the substrate from the caged compound has been assumed to be the rate of decay of the aci-nitro intermediate.

A more direct, noninferential method to determine the rate of release of the substrate requires a time-resolved spectroscopic investigation where the substrate exhibits specific spectroscopic signatures. Such spectroscopic signatures have been recently reported in the Fourier transform infrared (FTIR) spectra of one such caged compound, γ-(α-carboxy-2-nitrobenzyl)glutamate.[9] The spectra clearly revealed specific vibrational difference features that can be attributed to the caged glutamate as well as to the photolysis products, glutamate, and 2-nitrosophenylglyoxalic acid. A time-resolved spectroscopic investigation using these bands as markers would therefore allow for the direct characterization of the rates of photo release of the products. Previously, a time-resolved FTIR investigation using rapid scan techniques (with a time resolution of tens of milliseconds) has been reported for 1-(2-nitrophenyl)-ethyl ester of ATP.[10] However, this time resolution is not adequate to study a large number of caged compounds, such as caged glutamate, that are estimated to have a photolysis rate in the order

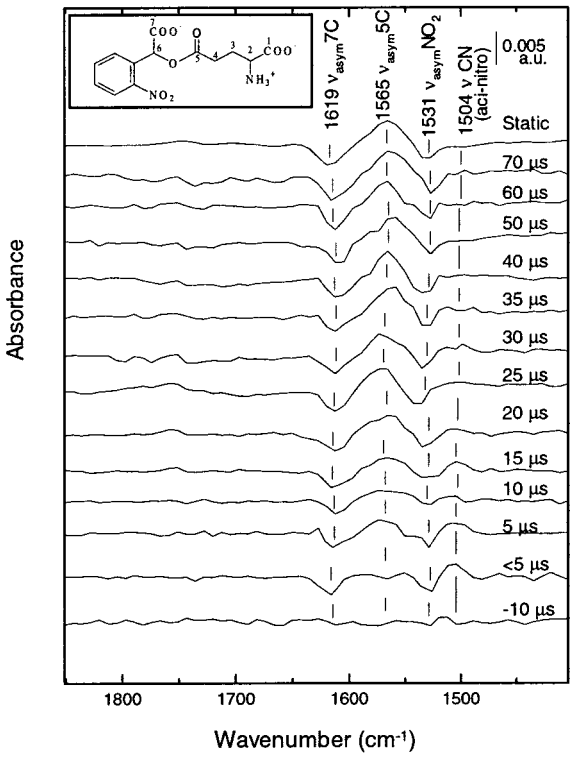


Figure 1. Representative time-resolved difference FTIR spectrum between 10 mM caged glutamate in 25 mM phosphate buffer after and before photolysis recorded in blocks of 5 μs.[12] Static difference FTIR spectrum between the completely photolyzed caged glutamate and caged glutamate is also shown in the Figure for comparison. A band at 1728 cm^{-1} observed in the static spectrum has not been labeled due to the lower level of signal-to-noise in the time-resolved spectra.

of tens of microseconds.[11] In the present report we use the step-scan FTIR method in combination with a homemade continuous flow sampling device to provide a microsecond time resolution, to study the photolysis of caged glutamate. The step-scan mode of a Nicolet Nexus FTIR spectrometer was modified such that the photolysis of the sample as well as each step of the mirror was initiated at the rate of 1 Hz. This rate ensured that the photolyzed sample was removed between the photolysis pulses, as monitored by performing rapid scan measurements (see Supporting Information).

The equilibrium difference FTIR spectra between the photolyzed caged glutamate and caged glutamate is shown in Figure 1 (labeled as static). The difference features have been assigned on the basis of model compound studies, and are discussed in detail by Jayaraman et al.[9] The negative features at 1619 and 1531 cm^{-1} arise from the caged glutamate, while the positive feature at 1565 cm^{-1} is assigned to the asymmetric 5C(γ)-carboxylate vibration

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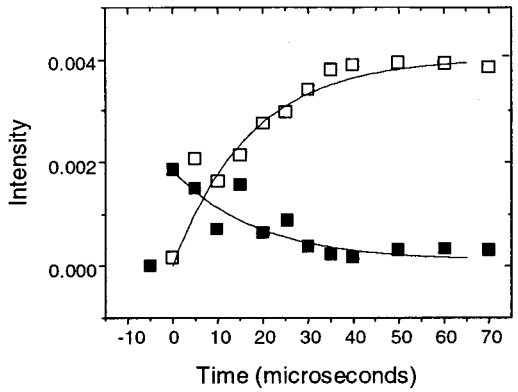

Figure 2. Change in the intensity of the difference feature at 1504 cm^{-1} (■) and 1565 cm^{-1} (□) plotted as a function of time. The decay of the 1504 cm^{-1} is well represented with a single exponential (solid line) with a time constant of 20 ± 8 μs, while the increase in the intensity of the 1565 cm^{-1} feature is well represented with a single-exponential rise with a time constant of 19 ± 4 μs.

of the photolyzed free glutamate. Since the glutamate is caged at the 5C(γ)-carboxylate position, the band at 1565 cm^{-1} is an excellent probe to study the release of the free glutamate from the caged glutamate.

The time-resolved difference FTIR spectra obtained at various times after photolysis are shown in Figure 1. The difference spectra represent the difference between the spectra obtained after photolysis and the spectrum before photolysis. The spectra were collected in blocks of 5 μs, which provides adequate signal-to-noise ratio in the difference spectra. The absence of difference features in the difference spectrum obtained prior to photolysis (−10 μs) was used as a monitor for sample integrity.

In the difference spectrum obtained between 0 and 5 μs after photolysis of the caged glutamate the predominant feature is the positive band at 1504 cm^{-1}. This positive feature is formed within the first 5 μs and decreases in intensity and disappears at longer times (Figure 2, filled squares), suggesting that the band arises from an intermediate in the photolysis reaction. The decay of the 1504 cm^{-1} signal is well represented by a single exponential with a time constant of 20 ± 8 μs. On the basis of the frequency, this band can be assigned to the C═N stretching vibration of the aci-nitro intermediate.[10]

To further confirm the assignment of the 1504 cm^{-1} feature to the aci-nitro intermediate, the rate of decay of the 1504 cm^{-1} was compared to the rate of decay of the aci-nitro intermediate probed with transient absorption measurements under the same experimental conditions.[12] The decay of the aci-nitro intermediate was probed at 425 nm, the characteristic absorbance of this intermediate,[11] and is shown in Figure 3. The decay of the absorption of the aci-nitro intermediate as a function of time could be well represented by a single-exponential decay with a time constant of 23 ± 1 μs. This value is in excellent agreement with the value obtained from the time-resolved FTIR spectra and similar to the 30 μs value published earlier,[11] thus providing further confirmation for the assignment of the 1504 cm^{-1} to the vibrational mode from the aci-nitro intermediate.

To investigate the rate of release of glutamate due to photolysis of the caged glutamate the time evolution of the intensity changes for the 1565 cm^{-1} band was examined (shown in Figure 2 as open squares). The increase in intensity of this band at 1565 cm^{-1} as a function of time is well represented by a single exponential increase, with a time constant of 19 ± 4 μs. This time constant for the

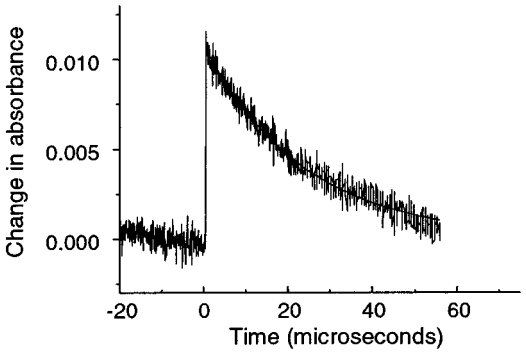

Figure 3. Transient absorption measurement at 425 nm of the aci-nitro intermediate decay produced by photolysis of 10 mM caged glutamate in 25 mM phosphate buffer (pH 7.4, room temperature) using the 355-nm light from a Nd:YAG laser. The decay is well represented by a single exponential with a time constant of 23 ± 1 μs.

formation of glutamate is similar to the values for the time constants obtained for the decay of the aci-nitro intermediate, thus suggesting that the rate-limiting step in the photolysis mechanism for the caged compounds (shown in Scheme 1) is the decay of the aci-nitro intermediate.

These results for the first time provide a direct measure of the rate of photo release of substrate from the photolysis of the commonly used caged glutamate derivative in an aqueous environment in the microsecond time scale. Furthermore, the results clearly indicate the feasibility of using step-scan FTIR to study noncyclic processes, and therefore FTIR can be extended to study a number of such processes that have previously not been studied extensively in the microsecond time scales.

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Supporting Information Available: Flow device, rapid scan FTIR, additional intermediates (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Conrad, P. G. I.; Givens, R. S.; Hellrung, B.; Rajesh, C. S.; Ramseier, M.; Wirz, J. *J. Am. Chem. Soc.* **2000**, *122*, 9346−9347.
(2) Gee, K. R.; Carpenter, B. K.; Hess, G. P. *Methods Enzymol.* **1998**, *291*, 30−50.
(3) Marriott, G., Ed. *Methods in Enzymology*; Academic Press: New York, 1998; Vol. 291.
(4) Hess, G. P.; Niu, L.; Wieboldt, R. *Ann. N.Y. Acad. Sci.* **1995**, *757*, 23−39.
(5) Adams, S. R.; Tsien, R. Y. *Annu. Rev. Physiol.* **1993**, *55*, 755−784.
(6) McCray, J. A.; Trentham, D. R. *Annu. Rev. Biophys. Biophys. Chem.* **1989**, *18*, 239−270.
(7) Kaplan, J. H.; Somlyo, A. P. *Trends Neurosci.* **1989**, *12*, 54−59.
(8) Hess, G. P.; Grewer, C. *Methods Enzymol.* **1998**, *291*, 443−473.
(9) Jayaraman, V.; Thiran, S.; Madden, D. R. *FEBS Lett.* **2000**, *475*, 278−282.
(10) Barth, A.; Corrie, J. E. T.; Gradwell, M. J.; Maeda, Y.; Mäntele, W.; Meier, T.; Trentham, D. R. *J. Am. Chem. Soc.* **1997**, *119*, 4149−4159.
(11) Wieboldt, R.; Gee, K. R.; Niu, L.; Ramesh, D.; Carpenter, B. K.; Hess, G. P. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 8752−8756.
(12) The time-resolved measurements were performed using 10 mM caged glutamate in 25 mM phosphate (D_2O) buffer at pH 7.4 and room temperature and photolyzed using 355-nm light from a Nd:YAG laser. The spectra were recorded with a Nicolet FTIR spectrometer in the step-scan mode. The sample holder, equipped with CaF_2 windows with a 50-μm path length, was modified into a flow device, and the flow rate was maintained such that the photolyzed sample was removed between each laser pulse. Spectra were collected at 8 cm^{-1} resolution, and the final difference spectra were obtained by co-adding five individual spectra.

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