Caged compounds in time-resolved macromolecular crystallography

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Outline

- Introduction
- $o$-alkylated nitrophenyl compounds
- Potential problems and their possible solutions
What is a “caged compound”? 

Inactive precursor → Biologically active reagent + Blocking group

hv
Typical setup for photoinitiating a fast reaction and monitoring spectroscopically
Applications to physiology

Living cell

Focused laser pulse

Observe changes: $f(x,y)$

$f(t)$
Application to time-resolved X-ray crystallography
In-depth treatment:
o-alkylated nitrophenyl compounds

\[
\text{N} \quad \text{O} \quad \text{O}
\]

\[
\text{H} \quad \text{R}
\]

\[
\text{L}^- + \text{H}^+ +
\]

\[
\text{O} \quad \text{N}^- \quad \text{O}
\]

\[
\text{L} \quad \text{O}
\]

\[
\text{R}
\]
Mechanism for substrate release from \(o\)-alkylated nitrophenyl compounds

Pellicioli and Wirz *Photochem Photobiol Sci* 2002, 1, 441-458

\[
\text{L}^- + \text{H}^+ + \text{N} -> \text{L} + \text{H}_2\text{O}
\]

\[
\text{S}_1^- \tau < 10 \text{ ps}
\]
Example: photorelease of the neurotransmitter GABA

\[
\text{GABA (neurotransmitter)}
\]
Example: photogenerated pH jump
Barth and Corrie *Biophys J.* **2002**, *83*, 2864-2871

$$\text{SO}_4^{2-} + H^+ + \text{product}$$
Isocitrate dehydrogenase: reaction intermediate observed crystallographically on the ms timescale

![Chemical reaction diagram](image)
Strategy #1

Caged isocitrate

Isocitrate

Release rate = 10 s\(^{-1}\) (pH = 8)
= 200 s\(^{-1}\) (pH = 6.5)
Strategy #2

Caged NADP$^+$ type 1

Release rate $\sim 40 \text{ s}^{-1}$
Strategy # 3

Caged NADP$^+$ type 2

Release rate = 13,000 s$^{-1}$
Potential problem #1: side reactions of blocker group after uncaging

\[ \text{Reactive functional group} \]

\[ \begin{align*}
\text{H} & \quad \text{L} & \quad \text{R} \\
\text{L}^- & + \text{H}^+ + \end{align*} \]
Possible Solution
Cohen et al. *Biochemistry* 1997, 36, 9035-9044

Reactive functional group

\[
\text{O} \quad \text{N} \quad \text{O} \quad \text{+} \quad \text{SH} \quad \text{HO} \quad \text{H} \quad \text{H} \quad \text{SH} \quad \text{H} \quad \text{H} \quad \text{SH} \quad \text{HO} \quad \text{H} \quad \text{N} \quad \text{O} \quad \text{+} \quad \text{HO} \quad \text{N} \quad \text{H} \quad \text{O} \quad \text{+} \quad \text{HO} \quad \text{C}_6\text{H}_{12}\text{O}_6\text{S}_2 \quad \text{dithiothreitol}
\]
Potential problem #2: Self-screening prevents uniform sample photoactivation

Photoactivating laser pulse

Protein Crystal

Laue X-ray pulse

Protein crystal absorbs most laser light near the incident surface

Inhomogeneous photochemistry within the crystal
Possible solution:
2-photon excitation

• Requires caged compounds with significant 2-photon excitation cross sections
Example: Coumarins

Single-photon excitation: 368 nm
Two-photon excitation: 800 nm
Caged Nitric Oxide
(Roussin’s Red Salt)

Roussin’s Red Salt with attached 2-photon antenna complex (fluorescein)

\[\lambda_{\text{max}} = 500\ \text{nm}\]

1-photon excitation (436 nm)
2-photon excitation (800 nm)
Caged Complexes
Bibliography

Reviews


O-alkylated nitrophenyl compounds


**Coumarin compounds**


**Other photoactive systems**


