View Article Online / Journal Homepage / Table of Contents for this issue
Dynamic Article Links

Photochemical & Photobiological Sciences

Cite this: *Photochem. Photobiol. Sci.*, 2012, **11**, 620

www.rsc.org/pps

COMMUNICATION

A caged cyanide

Markus Knipp,*[a] Johanna J. Taing,[a] Chunmao He[a] and Cristiano Viappiani*[b]

Received 26th October 2011, Accepted 23rd February 2012
DOI: 10.1039/c2pp05359d

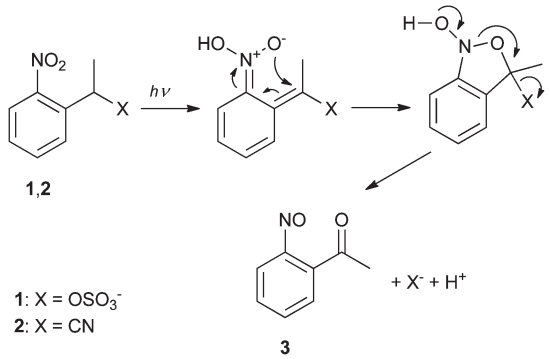
Published on 12 March 2012. Downloaded by The University of Manchester Library on 30/10/2014 10:52:32.

A photoactivatable caged cyanide, 1-(2-nitrophenyl)ethyl (NPE) cyanide, was synthesized, which upon irradiation in the near UV releases cyanide. It is demonstrated that the compound can be used to induce formation of the Fe^{III}–CN^- complex in the heme protein nitrophorin 4 from *Rhodnius prolixus*.

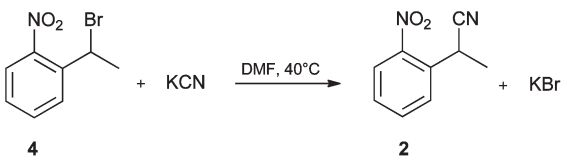
Rapid mixing techniques allow an insight into the kinetics of fast bimolecular reactions down to the millisecond time-scale. However, when reactions should be observed that are faster than this limit, more advanced and specialised techniques are required. Photoactivatable caged compounds represent a method to generate an active compound *in situ* from an inactive precursor by flash photolysis.[1] Here we describe a novel caged cyanide for studies of fast kinetics of heme proteins. Cyanide is an interesting effector for studies with heme proteins, as it can readily form very stable Fe^{III}–CN^- complexes which provide a useful model for retrieving structural information on liganded species.[2] More relevant, exposure of {FeNO}^{6}† (or other complexes) to excess cyanide can be used to displace NO (or other ligands) to follow the associated kinetics.

The preparation of a caged cyanide was derived from the 1-(2-nitrophenyl)ethyl (NPE) compounds which were used for the generation of rapid H^+ release. The principle of action is presented in Scheme 1 for the sulphate **1**,[3] which has been successfully applied to study the acid unfolding of myoglobin.[3a,4] Electronic excitation leads to the formation of a nitronic acid ($pK_a \sim 3.5$),[3b,4,5] which then decays to a bicyclic intermediate by nucleophilic attack of the nitronic acid on the Cα–CN.[6] Upon release of the leaving group X^- ring opening occurs to yield the product **3**.

In the previous approach, the sulfate derivative **1** was used to achieve large concentrations of H^+ after photolysis, thanks to the very weak base character of SO_4^{2-} which assures the full availability of the H^+ as the active species.[3a] In the approach reported herein, CN^- was introduced as the leaving group X, which represents also the desired active species. The synthesis of the caged cyanide **2** was performed by nucleophilic substitution of



Scheme 1 Reaction mechanism for the photolysis of 1-(2-nitrophenyl) ethyl sulfate **1** and cyanide **2**.[3]



Scheme 2 Synthesis of caged cyanide **2**.

the bromide in 1-(1-bromo)ethyl-2-nitrobenzene **4** with CN^-, so that a 2-cyanoalkyl is obtained in *ortho* position to the nitro group (Scheme 2). The compound is obtained in good yields.‡

The concomitant appearance of H^+ expected on the basis of the reaction shown in Scheme 1 should then be neutralized by the application of a strong enough buffered solution. Photodecomposition of **2** in 100 mM sodium phosphate buffer at pH = 7.0 by near UV (355 nm) light leads to absorption changes, reported in Panel A of Fig. 1, which are very similar to those observed for the related compound **1**. The spectral changes upon irradiation are identical when **2** is irradiated in 100 mM sodium acetate buffer at pH = 5.5 (data not shown). Panel B in Fig. 1 compares the time courses for formation and decay of the *aci*-nitro reaction intermediates, observed for **1** and **2**. Experiments were performed using a previously described setup.[7] The absorbance at 400 nm for **1** rises with a biexponential kinetics, due to formation of the nitronic acid followed by its deprotonation.[3b] Decay of intermediate occurs with a pH-dependent lifetime, in keeping with acid catalysis of this reaction, which results in shifting the equilibrium towards the nitronic acid, so favoring formation of the bicyclic intermediate.[3b] The observed *aci*-nitro decay kinetics sets a lower limit for the time constant for CN^- release which, at neutral pH is 10 ms and at pH 5.5 becomes 300 μs.

[a]*Max-Planck-Institut für Bioanorganische Chemie, Stifstrasse 34-36, D-45470 Mülheim an der Ruhr, Germany. E-mail: mknipp@mpi-muelheim.mpg.de; Fax: +49 (0)208 306 3591; Tel: +49 (0)208 306 3671*
[b]*Dipartimento di Fisica, Università degli Studi di Parma, and NEST, Istituto Nanoscienze-CNR, viale delle Scienze 7A, 43124 Parma, Italy. E-mail: cristiano.viappiani@fis.unipr.it; Fax: +39 0521 905223; Tel: +39 0521 905208*

 This journal is © The Royal Society of Chemistry and Owner Societies 2012

View Article Online

Published on 12 March 2012. Downloaded by The University of Manchester Library on 30/10/2014 10:52:32.

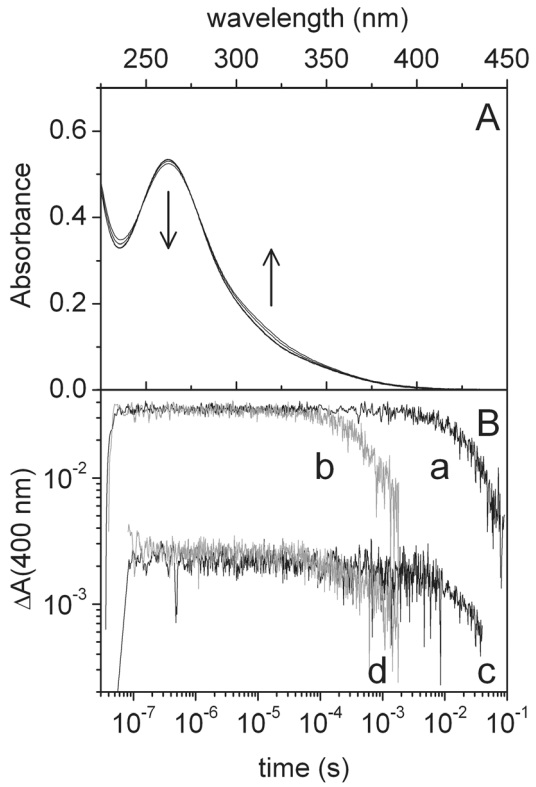


Fig. 1 A. Absorption spectra of caged cyanide **2** in buffered (100 mM sodium phosphate) aqueous solution before and after exposure to an increasing number of 355 nm, 28 mJ laser shots. B. Time-resolved absorbance changes at 400 nm following exposure to single, 355 nm, 28 mJ laser shots for NPE caged sulfate **1** (a, c) and caged cyanide **2** (b, d) in 100 mM sodium phosphate buffer at pH = 7.0 (black curves) and in 100 mM sodium acetate buffer at pH = 5.5 (gray curves). Absorbance changes are normalized for the absorbance at 355 nm. In the case of caged cyanide **2**, the very small signals prevented the monitoring of the *aci*-nitro intermediate formation occurring on the short nanosecond time scale, which is observed for caged sulfate **1** (curves a, c).

Since the chromophoric properties of **1** and of **2** are essentially identical, the former can be used as an actinometer and the data in Fig. 1 can be exploited to estimate the yield for formation of the *aci*-nitro intermediate. Using the deprotonation yield determined for **1** (0.47)[3a] as a reasonable estimate for the yield of formation of the *aci*-nitro intermediate, the yield for formation of the *aci*-nitro intermediate of **2** is ~0.03. This represents an upper limit for the quantum yield for decomposition of **2** to release CN^-.

To test the capability of **2** to release CN^- upon photoexcitation with near UV light, we have monitored formation of the [Fe^{III}–CN^-] species starting from the [Fe^{III}] complex of recombinant nitrophorin 4 (NP4).§ Nitrophorins comprise a unique class of heme proteins that originate from the saliva of the blood sucking insect *Rhodnius prolixus*. Their purpose is the storage and transport of NO from the insect saliva to the victim's blood where the iron is formally kept in the +3 oxidation state[8] with rather large affinities (K_{eq}(NO) = 7.9 ± 0.1 × 10^6 M^{-1} at pH = 7.5).[9] Like ferriheme–NO complexes, CN^- binds in a linear axial fashion to ferric hemes, thus resembling a structural model for {FeNO}[6]

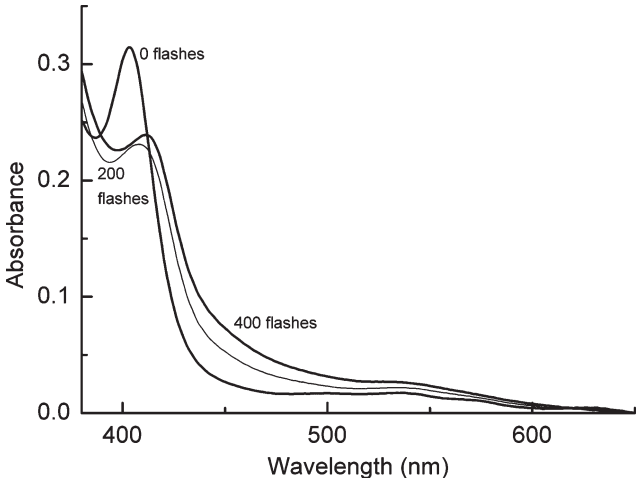


Fig. 2 Change in the absorbance spectrum of 2 μM of NP4 in 50 mM sodium phosphate buffer containing 2 mM of **2**. The sample was stirred continuously in a quartz cuvette and illuminated with 200 and 400 flashes of a nanosecond 355 nm Nd:YAG laser (32 mJ per puls). From these data, the efficiency of the photoinduced release of CN^- is estimated to be in the order of ~10^{-4}.

hemes. The affinity of CN^- to NP4 is very high (K_{eq}(CN^-) = 2.5 ± 0.1 × 10^8 M^{-1} at pH = 7.5).¶

When a solution of NP4[Fe^{III}] containing **2** is illuminated with the 355 nm output of the third harmonic of a nanosecond Nd:YAG laser, the absorbance spectrum is converted into that of NP4[Fe^{III}–CN^-] (Fig. 2). In particular, the absorbance at 403 nm, diagnostic of NP4[Fe^{III}] decreases while the absorption at 416 nm, typical of NP4[Fe^{III}–CN^-], concomitantly increases.

Our findings suggest that the photodissociation of the caged cyanide **2** occurs in low yield, and leads to decomposition of the compound on time scales comparable to those observed for the related caged sulphate **1**. Nevertheless, it is possible to estimate that, under experimental conditions where 1 mM of SO_4^{2-} release was observed,[3b,4] the concentration of released CN^- would exceed 60 μM. This concentration is large enough to allow NO displacement experiments from heme proteins, where CN^- must be added to large excess of the protein.

Conclusions

A new caged cyanide compound **2** was synthesized, using the 1-(2-nitrophenyl)ethyl chromophore as a photosensitive protecting group. This preliminary characterization demonstrates that the compound is fully functional and can lead to release of CN^- upon excitation with near UV light. However, the photorelease efficiency is rather low; thus, the development of a next generation of caged cyanide will aim at the increase of the photosensitivity by the insertion of suitable phenyl ring substituents.

Acknowledgements

The authors like to thank Leslie Currell and Dagmar Merkel for technical support. Financial support was from the Max Planck Society (MPG, to M. K.), from the German Academic Exchange Service (DAAD), Project No. 50728589 (to M.K.), and from the Vigoni program (to C.V.).

View Article Online

Published on 12 March 2012. Downloaded by The University of Manchester Library on 30/10/2014 10:52:32.

Notes and references

† According to the widely applied notation of Enemark and Feltham.[10]
‡ Synthesis of 2-(2-nitrophenyl)propionitrile (1.9 g, 57% light yellow crystals). The substrate 1-(1-bromo)ethyl-2-nitrobenzene **4** was synthesized as described.[11] 4.3 g (19 mmol) of **4** was dissolved in DMF and 4 g (61 mmol) of KCN were added. The solution was stirred over night in the dark at 35 °C. Afterwards, the solution was filtered and DMF was removed under reduced pressure. The product was dissolved in 100 mL of ethyl acetate and extracted 3 times with 300 mL of water and 3 times with 300 mL of saturated NaCl. After drying with Na_2SO_4, the product was purified by silica gel chromatography using 9 : 1 cyclohexane/ethyl acetate. mp 40 °C (from ethyl acetate); λ_{max}(100 mM phosphate buffer pH 7.5)/nm 263 (ε/dm^{-3} mol^{-1} cm^{-1} 2860); λ_{max}(MeOH)/nm 253 (ε/dm^{-3} mol^{-1} cm^{-1} 3 870), ~338sh; NMR: δ_H(400 MHz; $CDCl_3$; $(CH_3)_4Si$) 1.66 (3 H, d, CH_3), 4.69 (1 H, q, CHCN), 7.48 (1 H, t, Ph–C_5H), 7.67 (1 H, t, Ph–C_4H), 7.75 (1 H, d, Ph–C_6H), 7.98 (1 H, d, Ph–C_3H); δ_{13C}(400 MHz; $CDCl_3$; $CDCl_3$) 20.9 (CH_3), 27.9 (CHCN), 120.6 (CN), 127.2 (Ph–C_5), 129.2 (Ph–C_4), 129.5 (Ph–C_6), 132.1 (Ph–C_1), 134.1 (Ph–C_3), 147.3 (Ph–C_2).
§ Recombinant NP4 was expressed in *Escherichia coli*, purified, and reconstituted as was previously described.[12]
¶ The equilibrium constant was determined by titration of 3 μM of NP4 with KCN in 100 mM NaH_2PO_4/NaOH (pH = 7.5) at ambient temperature using absorption spectrophotometry. The titration was analysed as was previously described.[13]

1 (*a*) S. R. Adams and R. Y. Tsien, Controlling cell chemistry with caged compounds, *Annu. Rev. Physiol.*, 1993, **55**, 755–784; (*b*) J. H. Kaplan, Photochemical manipulation of divalent-cation levels, *Annu. Rev. Physiol.*, 1990, **52**, 897–914; (*c*) J. H. Kaplan, B. Forbush and J. F. Hoffman, Rapid photolytic release of ATP from a protected analogue: utilization by the Na : K pump of human red blood cell ghosts, *Biochemistry*, 1978, **17**, 1929–1935; (*d*) J. A. MacCray and D. R. Trentham, Properties and uses of photoreactive caged compounds, *Annu. Rev. Biophys. Biophys. Chem.*, 1989, **18**, 239–270; (*e*) *Caged Compounds, Methods Enzymol*, ed. G. Marriott, Academic Press, San Diego, 1998, vol. 291; (*f*) A. P. Pellicciolo and J. Wirz, Photoremovable protecting groups: reaction mechanisms and applications, *Photochem. Photobiol. Sci.*, 2002, **1**, 441–458; (*g*) G. Mayer and A. Heckel, Biologically active moleculaes with a "light switch", *Angew. Chem., Int. Ed.*, 2006, **45**, 4900–4921; (*h*) H.-M. Lee, D. R. Larson and D. S. Lawrence, Illuminating the chemistry of life: design, synthesis, and applications of "caged" and related photoresponsive compounds, *ACS Chem. Biol.*, 2009, **4**, 409–427.
2 E. M. Maes, F. A. Walker, W. R. Montfort and R. S. Czernuszewicz, Resonance Raman spectroscopic study of nitrophorin 1, a nitric oxide-binding heme protein from *Rhodnius prolixus*, and its nitrosyl and cyano adducts, *J. Am. Chem. Soc.*, 2001, **123**, 11664–11672.
3 (*a*) A. Barth and J. E. T. Corrie, Characterization of a new caged proton capable of inducing large pH jumps, *Biophys. J.*, 2002, **83**, 2864–2871; (*b*) S. Abbruzzetti, S. Sottini, C. Viappiani and J. E. T. Corrie, Kinetics of proton release after flash photolysis of 1-(2-nitrophenyl)ethyl sulfate (caged sulfate) in aqueous solution, *J. Am. Chem. Soc.*, 2005, **127**, 9865–9874.
4 S. Abbruzzetti, S. Sottini, C. Viappinani and C. Correia, Acid-induced unfolding of myoglobin triggered by a laser pH jump method, *Photochem. Photobiol. Sci.*, 2006, **5**, 621–628.
5 (*a*) M. Schwörer and J. Wirz, Photochemical reaction mechanism of 2-nitrobenzyl compounds in solution. 1. 2-nitrotoluene: thermodynamic and kinetic parameters of the *aci*-nitro tautomer, *Helv. Chim. Acta*, 2001, **84**, 551–606; (*b*) G. Wettermark, E. D. Black and L. Dogliotti, Reactions of photochemically formed transients from 2-nitrotoluene, *Photochem. Photobiol.*, 1965, **4**, 229–239.
6 J. W. Walker, G. P. Reid, J. A. McCray and D. R. Trentham, Photolabile 1-(2-nitrophenyl)ethyl phosphate esters of adenine nucleotide analogs: synthesis and mechanism of photolysis, *J. Am. Chem. Soc.*, 1988, **110**, 7170–7177.
7 S. Abbruzzetti, S. Bruno, S. Fagiano, E. Grandi, A. Mozzarelli and C. Viappiani, Monitoring haem proteins at work with nanosecond laser flash photolysis, *Photochem. Photobiol. Sci.*, 2006, **5**, 1109–1120.
8 (*a*) M. J. Lehane, *The Biology of Blood-Sucking in Insects*, Cambridge University Press, Cambridge (United Kingdom), 2nd edn, 2005; (*b*) R. H. Nussenzveig, D. L. Bentley and J. M. C. Ribeiro, Nitric oxide loading of the salivary nitric-oxide-carrying hemoproteins (nitrophorins) in the blood-sucking bug *Rhodnius prolixus*, *J. Exp. Biol.*, 1995, **198**, 1093–1098; (*c*) F. A. Walker, Nitric oxide interaction with insect nitrophorins and thoughts on the electron configuration of the {FeNO}^{6} complex, *J. Inorg. Biochem.*, 2005, **99**, 216–236; (*d*) M. Knipp, C. He and H. Ogata, Nitrite disproportionation reaction: investigations on the mechanism of the conversion of nitrite into nitric oxide at the ferriheme center of nitrophorins at blood plasma pH, *Nitric Oxide*, 2011, **24**, S33–S34; (*e*) M. Knipp and C. He, Nitrophorins: nitrite disproportionation reaction and other novel functionalities of insect heme-based nitric oxide transport proteins, *IUBMB Life*, 2011, **63**, 304–312.
9 R. E. Berry, M. N. Shokhirev, A. Y. W. Ho, F. Yang, T. K. Shokhireva, H. Zhang, A. Weichsel, W. R. Montfort and F. A. Walker, Effect of mutation of carboxyl side-chain amino acids near the heme on the midpoint potentials and ligand binding constants of nitrophorin 2 and its NO, histamine, and imidazole complexes, *J. Am. Chem. Soc.*, 2009, **131**, 2313–2327.
10 J. H. Enemark and R. D. Feltham, Principles of structure, bonding, and reactivity for metal nitrosyl complexes, *Coord. Chem. Rev.*, 1974, **13**, 339–406.
11 S. Banala, A. Arnold and K. Johnsson, Caged substrates for protein labeling and immobilization, *ChemBioChem*, 2008, **9**, 38–41.
12 (*a*) A. Weichsel, J. F. Andersen, D. E. Champagne, F. A. Walker and W. R. Montfort, Crystal structures of a nitric oxide transport protein from a blood-sucking insect, *Nat. Struct. Biol.*, 1998, **5**, 304–309; (*b*) C. He, H. Ogata and M. Knipp, Formation of the complex of nitrite with the ferriheme *b* β-barrel proteins nitrophorin 4 and nitrophorin 7, *Biochemistry*, 2010, **49**, 5841–5851; (*c*) C. He, S. Neya and M. Knipp, Breaking the proximal Fe^{II}–N_{His} bond in heme proteins through local structural tension: lessons from the heme *b* proteins nitrophorin 4, nitrophorin 7, and related site-directed mutant proteins, *Biochemistry*, 2011, **50**, 8559–8575.
13 (*a*) R. E. Berry, X. D. Ding, T. K. Shokhireva, A. Weichsel, W. R. Montfort and F. A. Walker, Axial ligand complexes of the *Rhodnius* nitrophorins: reduction potentials, binding constants, EPR spectra, and structures of the 4-iodopyrazole and imidazole complexes of NP4, *JBIC, J. Biol. Inorg. Chem.*, 2004, **9**, 135–144; (*b*) M. Knipp, F. Yang, R. E. Berry, H. Zhang, M. N. Shokhirev and F. A. Walker, Spectroscopic and functional characterization of nitrophorin 7 from the blood-feeding insect *Rhodnius prolixus* reveals an important role of its isoform-specific *N*-terminus for proper protein function, *Biochemistry*, 2007, **46**, 13254–13268.

 This journal is © The Royal Society of Chemistry and Owner Societies 2012