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Photolabile chelators for the rapid photorelease of divalent cations

(caged calcium/caged magnesium)

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ABSTRACT **The properties of a recently synthesized photolabile chelator for divalent cations are described, the affinity of which for Ca^{2+} changes by some 5 orders of magnitude on illumination. The compound 1-(2-nitro-4,5-dimethoxyphenyl)-*N*,*N*,*N*′,*N*′-tetrakis[(oxycarbonyl)methyl]-1,2-ethanediamine (DM-nitrophen) binds Ca^{2+} ($K_d \approx 5.0 \times 10^{-9}$ M) and Mg^{2+} ($K_d \approx 2.5 \times 10^{-6}$ M) with relatively high affinities. On exposure of the DM-nitrophen–Ca^{2+} complex to UV light in the 350-nm range, the chelator is cleaved yielding iminodiacetic products with a much lower affinity for Ca ($K_d \approx 3 \times 10^{-3}$ M) and the free [Ca^{2+}] increases. The quantum yield for Ca^{2+} release is 0.18. In experiments with chemically skinned skeletal muscle fibers, a fully relaxed fiber equilibrated with DM-nitrophen–Ca^{2+} complex produced maximal contraction after a single flash from a frequency-doubled ruby laser (347 nm). Half-maximal tension was achieved in ≈40 ms, some 5 times faster than that obtained after a rapid solution change from a Ca^{2+}-free to a Ca^{2+}-containing solution. In experiments with resealed human erythrocyte ghosts, irradiation of ghosts containing the DM-nitrophen–Ca^{2+} complex activates a Ca^{2+}-dependent K^+ efflux pathway, which is not observed in the absence of illumination. DM-nitrophen is sufficiently stable and photolabile to be used as a caged Ca (or caged Mg) for the rapid photoinitiation of divalent cation-dependent processes over a wide concentration range with a significant increase in temporal resolution over conventional mixing methods.**

Divalent cations, especially Ca^{2+} and Mg^{2+}, play essential roles in a wide array of physiological processes. The regulation of intracellular [Ca^{2+}] is intimately involved in excitation–contraction coupling in muscle and in excitation–secretion coupling in secretory cells, and it has been postulated to play a critical role in exocytosis and membrane fusion (1–3). In recent years, the special role of Ca^{2+} as a second messenger in many cellular processes has received greater attention in the sequence of events associated with the stimulation of inositol phospholipid metabolism (4). Mg^{2+} is an essential cofactor in many energy transducing processes in which ATP is the proximate energy source; these include ion pumps, myosin ATPase, etc. Mg^{2+} is also involved in most processes in which enzymatic substrates contain phosphate residues, including kinases, isomerases, etc. (5). To study the kinetic, regulatory, and structural mechanisms involved in many of these processes, it would be desirable to be able to alter rapidly the divalent cation concentration with a minimal perturbation in the system being studied. This report describes a photochemical approach to the problem of rapidly increasing free divalent cation concentrations in which brief pulses of light trigger an increase in free [M^{2+}].

The approach we have used is based on our earlier studies demonstrating the utility of a photolabile precursor of ATP, caged ATP, which on photolysis with UV light rapidly released free ATP (6). This work led to the application of the photorelease approach to the study of the mechanism of a wide range of ATP-dependent processes, including the ion-transporting ATPases (7–12) and muscle contraction (13, 14), and it has been used for time-resolved structural studies of the sarcoplasmic reticulum Ca^{2+} pump (15) and of time-resolved structural changes during contraction in muscle fibers (16). The application of the photorelease of "caged" compounds has subsequently been extended to include cyclic nucleotides (17), other nucleotides (18), acetylcholine agonists (19), inositol trisphosphate (20), etc. (for review, see ref. 21).

Caged ATP is a 2-nitrobenzyl ester of ATP, which on illumination at 350 nm undergoes a photocleavage, releasing ATP and a nitrosoketone (Fig. 1). Similar principles were used in the present work in which an EDTA molecule, bearing a 2-nitrophenyl moiety on the carbon skeleton, has been synthesized (22), which we anticipated would be photolabile. Our rationale was that the tetraacetic acid molecule (see Fig. 6) with a high affinity for Ca^{2+} (and/or Mg^{2+}) ions would photocleave to produce two diacetic acid molecules. Since iminodiacetic acid is known to have a much lower affinity for Ca^{2+} than does EDTA, the free [Ca^{2+}] in solution would thus be increased after photocleavage. The present paper describes the properties of such a compound, representative of a series we have synthesized, which fulfills these expectations and promises to be of considerable use in the study of divalent cation-dependent processes.

METHODS

Synthetic and Analytical. The compound on which this paper focuses was synthesized by reaction of excess iminodiacetic acid diethyl ester with 1-(2-nitro-4,5-dimethoxyphenyl)ethane-1,2-diyl 1,2-dibromide (22). The tetraethyl ester product was then hydrolyzed to yield 1-(2-nitro-4,5-dimethoxyphenyl)-*N*,*N*,*N*′,*N*′-tetrakis[(oxycarbonyl)methyl]-1,2-ethanediamine by incubation at 37°C overnight with 4 equivalents of NaOH. This product is referred to subsequently, with some relief, as DM-nitrophen. The complete hydrolysis of the tetraethyl ester of DM-nitrophen was verified by the disappearance of the ethyl resonances in the ^1H NMR spectrum (1.28, 4.20 ppm). The ^1H NMR (250 MHz, ^2H_2O, TPS internal standard) of DM-nitrophen (tetrasodium salt) is 7.60 (1H, Ar, s), 6.82 (1H, Ar, s), 4.92 (1H, CH-CH_2, dd, J = 11.4, 2.2 Hz), 3.98 (3H, OMe, s), 3.86 (3H, OMe, s), 3.18 (1H, CH-CH_2, dd, J = 13.6, 11.4 Hz), 3.39, 3.29, 3.04, 2.60 (8H, NCH_2CO_2, apparent d, J = 16.3 Hz) 2.55 (1H, CH-CH_2, dd, J = 13.6, 2.2 Hz). The detailed synthetic strategy and full characterization of chemical intermediates are described in a separate publication (22). Measurements of absorbance spectra were made in a Perkin–Elmer Gamma spectrophotometer. Measurements of the free [Ca^{2+}] in aqueous solutions were made with a Ca^{2+}-selective electrode constructed according to published methods using an ETH 1001 membrane (23). The electrode was connected to an Orion pH meter and calibrated by using standard solutions

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Abbreviation: DM-nitrophen, 1-(2-nitro-4,5-dimethoxyphenyl)-*N*,*N*,*N*′,*N*′-tetrakis[(oxycarbonyl)methyl]-1,2-ethanediamine.

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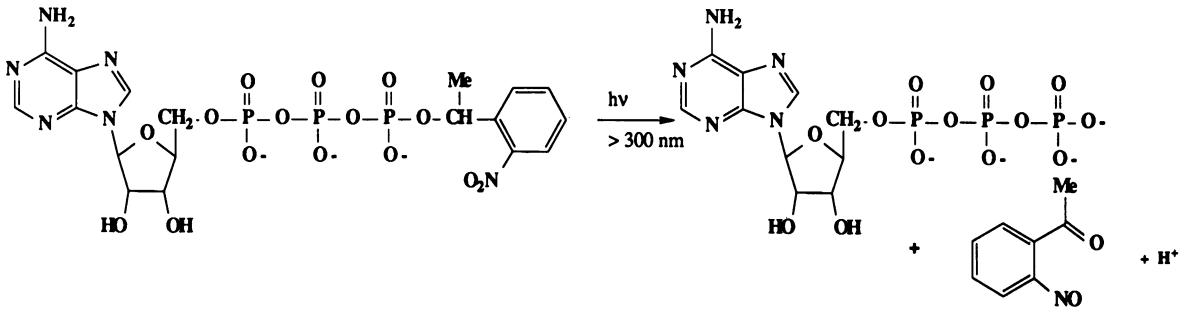

FIG. 1. Photorelease of ATP from caged ATP. The structure of caged ATP and the photoproducts ATP and a 2-nitrosoketone are shown.

of known $[Ca^{2+}]$ (24). Laser-flash photolysis of chelator solutions was performed with a frequency-doubled ruby laser (347 nm) as described (14). Laser-flash energy levels were measured, for the quantum efficiency determinations, with a disc calorimeter (Scientech, Boulder, CO) in line with the laser beam after the sample.

Muscle Fiber Studies. The experiments shown in Fig. 4 on skinned rabbit psoas fibers were carried out with the optical and mechanical system described (14).

Resealed Erythrocyte Ghosts. Resealed erythrocyte ghosts were prepared by a gel filtration technique already described (8). The contents of the resealed ghosts were established by the composition of the reversal medium. The ghosts contained 100 mM choline chloride, 30 mM $^{86}RbCl$, 0.25 mM $MgCl_2$, 1 mM $CaCl_2$, 2 mM DM-nitrophen, and 10 mM Pipes (pH 7.0). After washing three times by centrifugation and resuspension in cold efflux medium, the ghosts were pelleted and held at 0°C until efflux was initiated. The efflux medium contained 140 mM choline chloride, 2 mM $MgCl_2$, 5 mM RbCl, and 10 mM Pipes (pH 7.0). The measurement of ^{86}Rb efflux was initiated by suspending a sample of the pelleted ghosts (25 μl) in isotope-free buffer (200 μl) at room temperature and either photolyzed or not photolyzed for 15 s. The suspension was then immediately diluted in 4 ml of buffer at 37°C. At appropriate intervals (see Fig. 5), a 0.58-ml sample was taken and the ghosts were rapidly pelleted in an Eppendorf microcentrifuge. A sample (0.5 ml) of the supernatant was removed and the ^{86}Rb content was measured (via its Cerenkov radiation) in a Beckman LS 7500 scintillation counter. A sample of the flux suspension (0.5 ml) was also counted to obtain the total ^{86}Rb content. Photolysis was performed by using the filtered output of a 1000-W Hg arc lamp with the samples in 1-mm pathlength cuvettes as described (6), where the incident illumination has a peak wavelength at 342 nm (73% transmission at peak) and a half bandwidth of 60 nm.

RESULTS

Spectral Properties and Cation Affinities. DM-nitrophen has a characteristic absorbance spectrum that is altered in the 300- to 400-nm range on the addition of Ca^{2+} ions (Fig. 2*A*). The extinction coefficient for DM-nitrophen is 4.33×10^3 $M^{-1} \cdot cm^{-1}$ at 350 nm and pH 7.1, and the decrease in absorbance on the addition of excess Ca^{2+} is of the order of 7%. Similar changes are observed with Mg^{2+} but the decrease in absorbance is not as great (data not shown). The decrease in absorbance was used to estimate the affinity with which DM-nitrophen binds Ca^{2+}. DM-nitrophen in buffered KCl was placed in the reference and sample cuvettes (1 cm) of the spectrophotometer. The reference cuvette was then titrated with serial small additions of a stock $CaCl_2$ (50 mM) solution. Since additions are made in the reference cuvette, the decrease in absorbance seen on the addition of Ca^{2+} to DM-nitrophen (Fig. 2*A*) now appears as an increase (Fig. 2*B*). Following the recording of each spectrum, the free Ca^{2+} in the solution was measured with the Ca-selective electrode. The change in absorbance at a particular wavelength (for example, 350 nm) is linear with added total Ca^{2+} until very close to saturation of the DM-nitrophen (Fig. 2*C*), indicative of a high binding affinity. Indeed, estimates of the free Ca^{2+} at known fractional saturation (for example, 50% or 75%) of the absorbance change yields estimates of 5.0×10^{-9} M for the equilibrium dissociation constant at 22°C and pH 7.1. Since DM-nitrophen is based on EDTA and similar spectral effects were observed on the addition of Mg^{2+} to DM-nitrophen solutions, it was also necessary to estimate the affinity of DM-nitrophen for Mg^{2+} ions. This was done by titrating DM-nitrophen with added Ca^{2+} as described above and adding various known concentrations of $MgCl_2$. From measurements of the free $[Ca^{2+}]$ with the Ca^{2+}-sensitive (and Mg^{2+}-insensitive) electrode, the affinity of DM-nitrophen for Mg^{2+} could be estimated. This value was 2.5×10^{-6} M at 22°C and pH 7.1.

Light-Dependent Changes. If a solution of DM-nitrophen containing excess Ca^{2+} is exposed to the filtered output of a 1-kW Hg arc lamp, the absorption spectrum is altered (Fig. 2*A*). Such a change (curve b to c in Fig. 2*A*) is unaltered by extending the photolysis time. In solutions that contained equimolar mixtures of DM-nitrophen and Ca^{2+} (2 mM) so that the free $[Ca^{2+}]$ was several μM prior to photolysis, after 60 sec photolysis with the Hg arc lamp, the free $[Ca^{2+}]$ increased to $\approx$1 mM. This free $[Ca^{2+}]$ is what would be expected if the photolysis of 2 mM DM-nitrophen–Ca^{2+} complex yielded 4 mM iminodiacetic acid molecules having an affinity of $\approx 3 \times 10^{-3}$ M. Not surprisingly, DM-nitrophen in the absence of Ca^{2+} (or Mg^{2+}) is also photosensitive (Fig. 3). The absorption spectrum of the photoproducts is different from those produced by photolysis of DM-nitrophen–Ca^{2+} (compare Figs. 2*A* and 3). The spectrum of the photoproducts produced by photolysis of DM-nitrophen alone is not converted to the spectrum of the photoproducts obtained from irradiating DM-nitrophen–Ca^{2+} complexes by adding Ca^{2+} after photolysis (Fig. 3). Thus, it seems that the photochemical pathway is different in the presence or absence of Ca^{2+} during photolysis and a different chromophoric product is obtained. Having seen that the free $[Ca^{2+}]$ is increased after UV irradiation, it was of interest to obtain an estimate of the quantum efficiency of this process. The quantum yield was obtained by measuring the energy absorbed (in mJ) by a solution of the Ca^{2+}–chelator complex in a 1-cm cuvette (3 ml) in the laser beam (347 nm). This was corrected for the amount of absorption due to the buffer and cuvette alone. The free $[Ca^{2+}]$ in the solution was measured before and after a laser pulse using the Ca^{2+}-sensitive electrode. The ratio of Ca^{2+} ions released to the numbers of photons absorbed gave the quantum yield. Typical values obtained in these measurements (with excess free Ca^{2+} to avoid absorbance of light by, and excitation of, free chelator) showed that the absorption of $\approx$60 mJ of 347-nm irradiation was accompanied by an increase in free $[Ca^{2+}]$ from 6 to 15 μM. The quantum yield for Ca^{2+} release with solutions of the DM-nitrophen–Ca^{2+} complex at pH 7.1 and 20°C was 0.18.

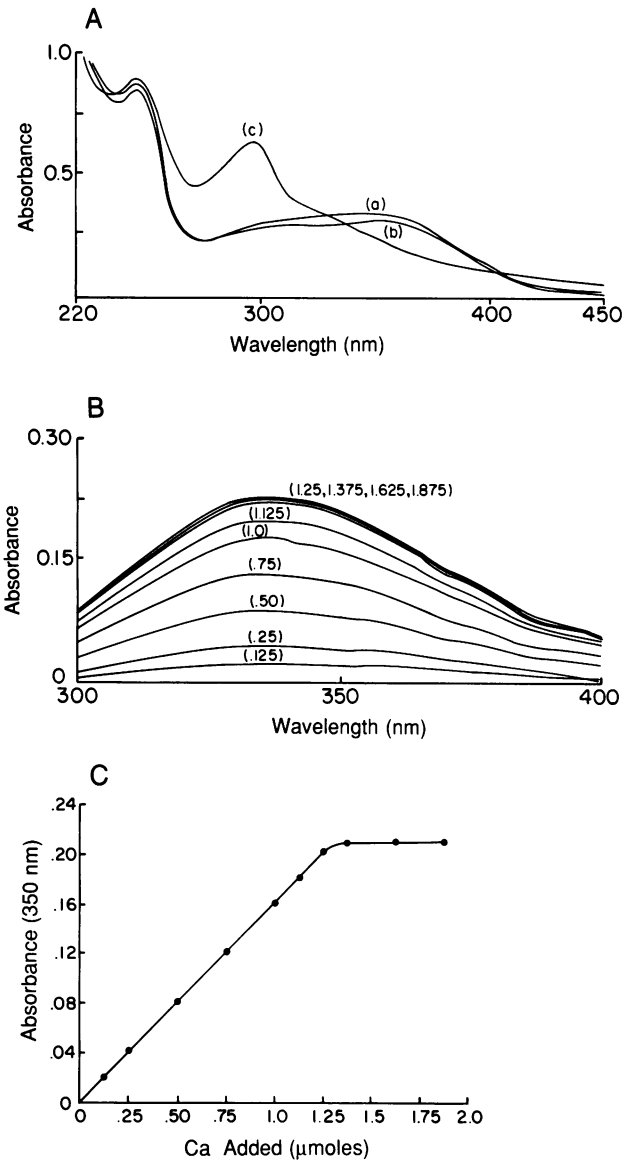

FIG. 2. Absorbance spectrum of DM-nitrophen and complexes. (*A*) Changes in absorbance of DM-nitrophen on addition of excess Ca^{2+} and subsequent photolysis. The absorbance spectrum of a solution of DM-nitrophen (0.08 mM) in buffer (130 mM KCl/10 mM Hepes, pH 7.1) is shown (spectrum a). This spectrum is altered (spectrum b) on the addition of $CaCl_2$ (final concentration, 0.16 mM). Photolysis of this sample for 60 s using the filtered output of a 1-kW Hg arc lamp yielded photoproducts having the absorbance spectrum shown in spectrum (c). (*B*) Titration of DM-nitrophen by Ca^{2+}. The difference spectrum obtained by titration of a solution of DM-nitrophen (0.38 μM) in 130 mM KCl/10 mM Hepes, pH 7.1. Numbers in parentheses indicate the total [Ca^{2+}] added to the reference cuvette (see text). (*C*) Titration of DM-nitrophen by Ca^{2+}. The absorbance values at 350 nm from *B* are plotted vs. the total added Ca^{2+}.

Experiments in Physiological Systems. If DM-nitrophen is to be useful in physiological studies, it is important that the DM-nitrophen–Ca^{2+} complex be stable prior to illumination to allow experimental manipulations and that sufficient Ca^{2+} is released sufficiently rapidly to activate the systems of interest. These two properties were examined in exploratory experiments with resealed human erythrocyte ghosts and skinned psoas fibers from rabbit skeletal muscle.

If a chemically skinned muscle fiber is transferred rapidly from a solution containing all the necessary substrates for contraction, but lacking Ca^{2+}, to the same solution containing Ca^{2+}, tension is developed (Fig. 4*B*). The typical rise time

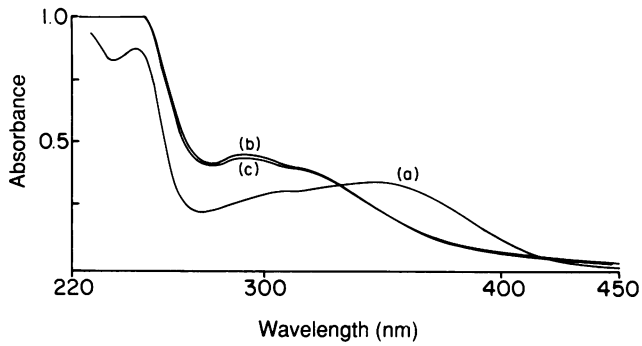

FIG. 3. Changes in absorbance of DM-nitrophen on photolysis. The spectrum of a solution of DM-nitrophen (0.08 mM) in buffer is shown in spectrum (a). Photolysis of this solution for 60 s using the filtered output of a 1-kW Hg arc lamp yielded photoproducts having the absorbance spectra shown in spectrum (b). Addition of excess $CaCl_2$ (0.16 mM) had little effect on this spectrum (c).

(time to achieve half-maximal tension) is ≈200 ms. This period presumably includes the diffusional delay involved in Ca^{2+} entering the skinned fiber and reaching the contractile apparatus as well as the response time of the contractile system to the increase in [Ca^{2+}]. The response time of the muscle contractile system and the molecular events involved in the process of activation are obviously of greater interest. In another experiment with the same fiber, performed with similar solutions but with DM-nitrophen used to buffer free Ca^{2+} to below contractile threshold levels, a single light flash from the laser causes a rapid increase to maximal tension. Half-maximal tension in this situation was attained in ≈40 ms (Fig. 4*A*). Thus, the photolysis of DM-nitrophen results in a significantly faster (5-fold) production of tension providing the possibility of studying the Ca^{2+}-dependent events involved in muscle contraction with significantly enhanced temporal resolution. By focusing the laser pulse onto the fiber (i.e., reducing the illuminated area and thus increasing the light intensity at the fiber), it is possible to decrease the half-time of increase in tension to as low as 17 ms under otherwise similar conditions (25). The use of DM-nitrophen as a "caged-Ca" in the skinned muscle fiber system has evidently circumvented a slow step in the solution change experiment, presumably largely associated with diffusional delays in Ca^{2+} gaining access to the relevant sites of action.

Human erythrocytes have a K^+ transport system that is activated if intracellular [Ca^{2+}] increases to the μM range (26). We prepared resealed human erythrocyte ghosts and examined the effect of photolysis of entrapped DM-nitrophen–Ca^{2+} complex (caged Ca) on the rate of $^{86}Rb^+$ (as a K^+ congener) efflux. The results of such an experiment are shown in Fig. 5. If the resealed ghost suspension was not irradiated, there was a very slow rate of efflux (maximally, ≈0.005 min^{-1}) of intracellular ^{86}Rb to the extracellular medium. However, after a 15-s photolysis of the ghost suspension, a rapid efflux ensued with an initial rate constant of ≈0.15 min^{-1}. Irradiation of ghosts not containing caged Ca (but containing caged Mg) had little effect on the ^{86}Rb efflux rate (data not shown) and in experiments in which Rb_o was <100 μM, the light-induced increase in Rb efflux was not observed. Thus, the caged Ca was stable throughout the resealed ghost preparative procedure (centrifugation, incubation, resuspension, etc.) and, after irradiation, activation of a Ca^{2+}-dependent, Rb_o-activated K^+ (Rb^+) transport process occurred. The photolytic release of intracellular Ca^{2+} caused an ≈30-fold increase in the rate constant for Rb^+ efflux.

DISCUSSION

In the present work, we have described the properties of DM-nitrophen (for chemical structure, see Fig. 6), repre-

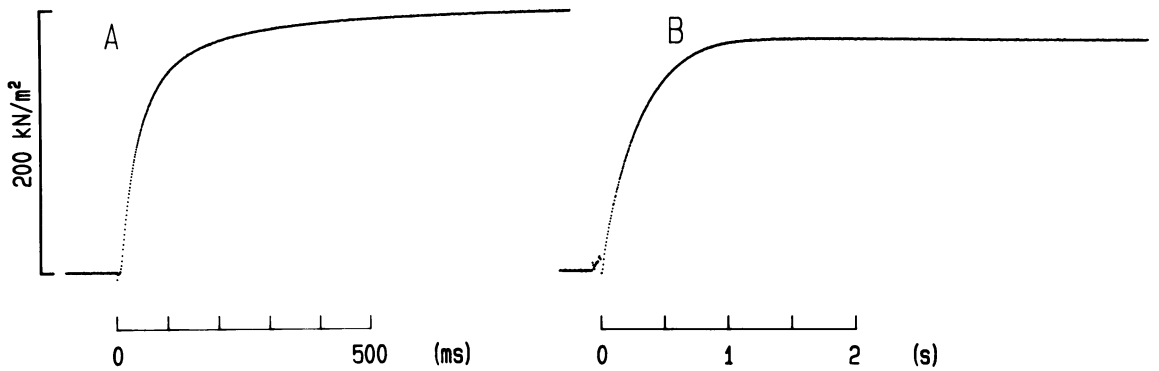

FIG. 4. Ca-induced contraction in skinned skeletal fibers. (*A*) A skinned psoas fiber was equilibrated in a solution composed of 100 mM TES/1.37 mM Na_2ATP/20 mM creatine phosphate/29.4 mM HDTA/10 mM glutathione/1.2 mM $MgCl_2$/1 mM $CaCl_2$/2 mM DM-nitrophen. At $t = 0$, the fiber was irradiated with a single flash from the frequency-doubled ruby laser (347 nm). Tension development was measured and recorded as described (14). (*B*) The skinned psoas fiber was equilibrated in a preactivating solution composed of 100 mM TES/6.93 mM $MgCl_2$/5.45 mM Na_2ATP/0.1 mM EGTA/24.9 mM HDTA/19.49 mM creatine phosphate/10 mM glutathione, pH 7.1, at 19°C. The free Mg^{2+} was 1 mM and MgATP was 5 mM. At $t = 0$, the fiber was transferred into a similar solution composed of 100 mM TES/6.76 mM $MgCl_2$/5.49 mM Na_2ATP/25 mM CaEGTA/19.49 μM creatine phosphate/10 mM glutathione, pH 7.1, at 19°C. Note the change in time scale between *A* and *B*.

sentative of a class of photolabile chelators for divalent cations. Illumination of the DM-nitrophen–Ca^{2+} complex (caged Ca) with UV light in the 350-nm range results in a rapid increase in the free [Ca^{2+}] as the complex is photocleaved.

Prior to illumination, the properties of DM-nitrophen are similar to EDTA, the chelator from which it is derived. DM-nitrophen selects Ca^{2+} over Mg^{2+} by ≈3 orders of magnitude in the respective equilibrium dissociation constants at neutral pH. The K_d is $\approx 5.0 \times 10^{-9}$ M for DM-nitrophen–Ca^{2+} and $\approx 2.5 \times 10^{-6}$ M for DM-nitrophen–Mg^{2+}. These estimates for the dissociation constants should be regarded as provisional since such high binding affinities are difficult to determine accurately by using electrodes to measure extremely low concentrations of free Ca^{2+} ions. It is very clear, however, that the affinity is greatly altered after exposure to UV light.

The quantum yield for Ca^{2+} release from the DM-nitrophen–Ca^{2+} complex is ≈0.18; this is not as high as for ATP release from caged ATP (0.54) (6) but is substantially higher than for cAMP from caged cAMP (17) and for Ca released from an alternative class of caged Ca compounds (27, 28) discussed below. The scheme we suggest for the photochemical transformation is by no means yet established. However, in initial studies of the proton NMR of DM-nitrophen–Ca^{2+} complexes in ^2H_2O, photolysis yields a

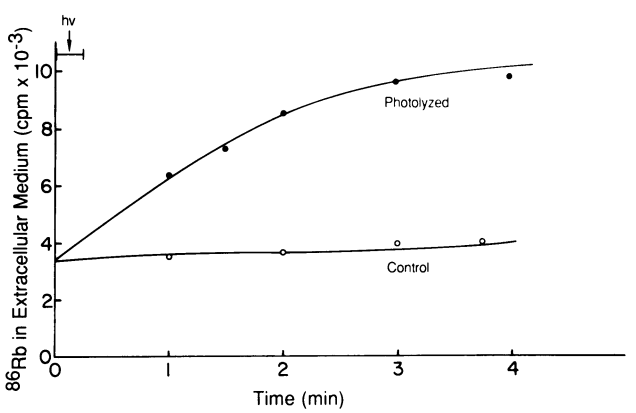

FIG. 5. ^{86}Rb efflux from resealed erythrocyte ghosts. Resealed erythrocyte ghosts containing the DM-nitrophen–Ca^{2+} complex and ^{86}Rb were suspended in isotope-free buffer for the measurement of ^{86}Rb efflux into the extracellular medium. Upper curve, rate of appearance of ^{86}Rb in the extracellular medium after photolysis; lower curve, rate of efflux when no photolysis was performed.

major product, the NMR spectrum of which is identical with that of iminodiacetic acid (unpublished data).

The usefulness of caged Ca compounds such as DM-nitrophen will depend to some extent on the rate at which Ca^{2+} is released after illumination. The rate of release of ATP from caged ATP appears to be coincident with the decay of a spectral intermediate absorbing at 410 nm (29). In the case of caged ATP, the intermediate decays with a half-time of ≈3 ms at pH 7 (29). In preliminary experiments of this type, in which 430-nm absorbance is monitored, the absorbance decrease of this intermediate with the DM-nitrophen–Ca^{2+} complex has a half-time of decay of 60–200 μs at pH 6.9 (30). Measurements of the Ca^{2+} release rate using antipyrylazo III to monitor the increase in free Ca^{2+} place this rate in the range 100–150 μs (30).

Recently, an alternative approach to caged Ca^{2+} compounds has been described that involves chemical modifications in the bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetate (BAPTA) series of Ca^{2+} indicators (27, 28). In this group, the most readily available member of which is nitr-5, photocleavage of a small residue (H_2O) from the alcohol junction with an attached 2-nitroaromatic ring produces a greater electron-withdrawing effect (as a ketone is produced at the junction), which is transmitted to an anilino nitrogen involved in chelation. This results in an increase in the K_d for Ca^{2+} and subsequent Ca^{2+} release. This approach thus also uses the intramolecular redox photochemistry of 2-nitroaromatic compounds used with caged ATP. It is useful to compare the properties of DM-nitrophen and nitr-5 as representatives of the two different approaches. In the nitr series, a small residue is photocleaved from the chelator and the effect of this is transmitted to the chelation center; in the nitrophen series, the chelation center itself is photocleaved to two hemichelator residues.

A significant advantage of nitr-5 is the extremely high selectivity for Ca^{2+} over Mg^{2+}. This series of chelators binds Ca^{2+} essentially exclusively. DM-nitrophen has less selectivity; however, with judicious use of ATP, Mg^{2+}, Ca^{2+}, and DM-nitrophen some of the selectivity problems can be overcome (as was the case, for example, in the muscle experiment of Fig. 4). The major reason for this is that as long as comparable concentrations of Mg^{2+} and Ca^{2+} compete for DM-nitrophen, Ca^{2+} will be preferred. On the other hand, acetoxymethyl esters of nitr-5 might be suitable membrane-permeant molecules to act as caged Ca^{2+} in intact systems. If such an approach were used with DM-nitrophen, where in most cells free [Mg^{2+}] is in the millimolar range and free

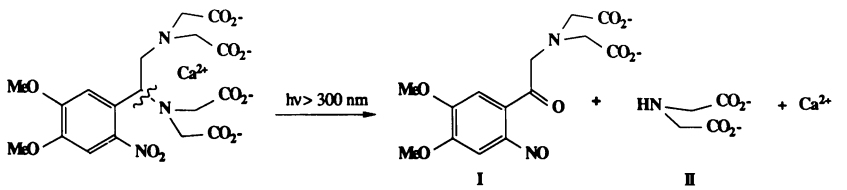

FIG. 6. Photorelease of Ca^{2+} from caged Ca^{2+}, the DM-nitrophen–Ca complex. The structure of the caged Ca^{2+}, DM-nitrophen–Ca^{2+} complex is shown. Wavy line indicates the C–N bond, which is probably photocleaved. The photoproducts obtained consist of iminodiacetic acid (II), a nitrosoacetophenone-substituted iminodiacetic acid (I), and free Ca^{2+} ions.

$[Ca^{2+}]$ is submicromolar, without additional increase in total Ca^{2+}, DM-nitrophen would function as a caged Mg^{2+} compound. Indeed, in the study of Mg^{2+}-dependent processes, DM-nitrophen might be used as a caged Mg^{2+} compound, a property not shared with the nitr series. Most ATP-dependent processes require Mg^{2+} as well as ATP for activation. At pH 7, it is likely that divalent cation release from DM-nitrophen is ≈1 order of magnitude faster than the release of ATP from caged ATP (as discussed above). Thus, DM-nitrophen can be used as a caged Mg^{2+} and may be a faster alternative way of photoinitiating MgATP-dependent processes. Similarly, we anticipate that DM-nitrophen will complex other polyvalent cations as does EDTA so that it may find application as a caged Ba^{2+} or caged Cd^{2+}, for example.

The affinity with which nitr-5 binds Ca^{2+} ($K_d \approx 0.15 \times 10^{-6}$ M) is somewhat poorer than DM-nitrophen ($K_d \approx 5 \times 10^{-9}$ M) but the most dramatic difference lies in the respective changes in affinity after illumination. Photolysis of nitr-5 produces a compound with an affinity of ≈6.3 μM (a 40-fold change); photolysis of DM-nitrophen yields an affinity of ≈3 × 10^{-3} M (a 10^5-fold change). The quantum yield for Ca release is ≈0.04 (32) from the nitr-5–Ca^{2+} complex and 0.18 for DM-nitrophen. The ideal photosensitive chelator should have high affinity for Ca^{2+} prior to photolysis and a very low (preferably zero) affinity after photolysis to avoid reequilibration problems associated with rebinding of released Ca^{2+} by the photoproducts and to enhance the effective conversion of bound to free Ca^{2+}. Clearly, DM-nitrophen and molecules of this type will be superior to nitr-5 in this regard. In practical terms, this difference translates into a greater release of Ca^{2+} from the DM-nitrophen–Ca^{2+} complex than from the nitr-5–Ca^{2+} complex at comparable illumination levels. In studies on the effects on frog heart of rapid release of extracellular Ca^{2+} from the DM-nitrophen complex, single light flashes from a 200-W arc lamp (duration, 100 μs) increase the free $[Ca^{2+}]$ by 50–100 μM (31).

Detailed studies with this caged Ca^{2+} in skeletal muscle, smooth muscle, cardiac muscle, and neurons should greatly extend our appreciation of the usefulness and limitations of DM-nitrophen in physiological systems. It is also anticipated that the time-resolved structural studies that have been initiated with caged ATP photolysis may now be extended to the photorelease of Ca^{2+} and Mg^{2+}.

In summary, we have characterized the properties of a divalent cation chelator, DM-nitrophen, which has a high affinity for Ca^{2+} and on photolysis releases the complexed Ca^{2+} at a high rate with an adequate quantum yield. We have demonstrated that Ca^{2+}-dependent cellular processes initiated by brief light pulses can be studied with a significant increase in temporal resolution over conventional mixing methods.

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