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Caged cations

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Abstract - The molecular concept of caged cations is illustrated on the basis of results obtained on coordination complexes between photo-cleavable, ion-selective, macrobicyclic ligands of the cryptand type and alkali as well as alkaline earth cations. Ligand splitting due to a short UV-light pulse leads to cation liberation in the ms time range as a consequence of the resulting affinity decrease. Such cation concentration jumps can be performed in microvolumes and without any mechanical or pressure perturbances. They can be applied to shift the equilibrium position of, for example, biochemical binding or reaction systems involving the released cation. If the readjustment of the state of equilibrium is slower than the cation release and if the cation concentration jump is small, the kinetic parameters of the system can be determined by applying the chemical relaxation methodology, as shown for alkali ion binding to the membrane transport enzyme Na^+,K^+-ATPase.

INTRODUCTION

The molecular caged compound concept[1)] has been introduced for the photolytically induced release of a biologically active compound S (eg. ATP) from its photo-cleavable, covalently linked protecting group X (ref. 1),

$$X\text{-}S \xrightarrow{h\nu} P + S \qquad (1)$$

where P represents the protecting group split off (photoproduct). This technique is of interest for carrying out kinetic and thermodynamic studies on biological systems under conditions where, for example, the mechanical disturbances of fast mixing devices are not tolerable (ref. 2). The release of a compound (eg. ATP from caged ATP) leads to a concentration jump, usually occurring in the ms time range. If a subsequent reaction involving the liberated compound (eg. ATP-driven enzymatic reaction or transport) is slower than the photolytically induced release, the time course of the reaction under investigation can be studied.

Another advantage of the technique lies in the fact that reactions can be initiated in a shielded environment (eg. in biological cells or within mechanical equipment) at any time without retardation. Furthermore, it is possible to perform kinetic studies in microvolumes consisting of a few microliters which may be of importance, for example, in future physico-chemical characterization of protein mutants available in only very small amounts.

1) This notation is, however, not justified from a chemical point of view when non cage-like protecting groups are used, as in case of ATP.

In view of the fundamental importance of cations in biological systems it would be desirable to apply this concept also for inducing cation concentration jumps in solution or within specific local environments. Inorganic cations are, however, not accessible to protection in such a simple way as mentioned before for organic compounds. For these cations (M^{n+}) photo-sensitive, cage-like ligands L, exhibiting high binding affinity (apparent stability constant K_S), have to be designed. Upon photolysis, a ligand rearrangement

$$M^{n+} + L \overset{K_S}{\rightleftharpoons} L\text{-}\text{-}M^{n+} \overset{h\nu}{\longrightarrow} P\cdots M^{n+} \rightleftharpoons P + M^{n+} \qquad (2)$$

or ligand splitting can lead to a diminished affinity between the photoproduct P and the cation. This leads to the liberation of the cation. For most biochemical or biological applications cation binding and the corresponding release must be ion-selective.

In the case of protection via covalent bond formation, as indicated for organic components in eq 1, comparatively large concentration changes can be achieved upon photolysis. Prior to photolysis, the concentration of the compound to be liberated is zero. In the case of cation release from a coordination complex (L- -M^{n+} in eq 2), the magnitude of the photolytically induced concentration jump depends on the stability constant of the binding equilibrium preceding the photochemical reaction as well as on the total concentrations of ligand and metal ion, provided its interaction with the photoproduct P can be neglected. The concentration jump is thus expected to be smaller than in the case of covalent protection (eq 1). Furthermore, the cation concentration jump increases the free cation concentration already existing before photolysis.

This apparent disadvantage, due to the expected relatively small cation concentration jumps, offers, however, a substantial advantage. As a consequence of the resulting small concentration perturbance, the comparatively simple evaluation methodology of chemical relaxation spectrometry (ref. 3) can be applied to the determination of kinetic parameters and under special circumstances also of thermodynamic parameters. Thus, the realization of the caged cation concept represents not only an alternative to the stopped flow method (a fast mixing technique) but also a new variant of chemical relaxation.

A special feature needs to be considered for caged cations. If similar total concentrations of ligand and metal ion are applied, a concentration jump, as shown in Fig. 1., should be observed. If, however, photolysis is performed in the presence of excess ligand, the previously built up cation concentration increase can be decreased in a time-dependent manner due to rebinding of the released cations by the uncleaved free ligand molecules.

Depending on the given concentrations and the kinetic binding parameters, a cation concentration pulse is achieved, as illustrated in Fig. 1.

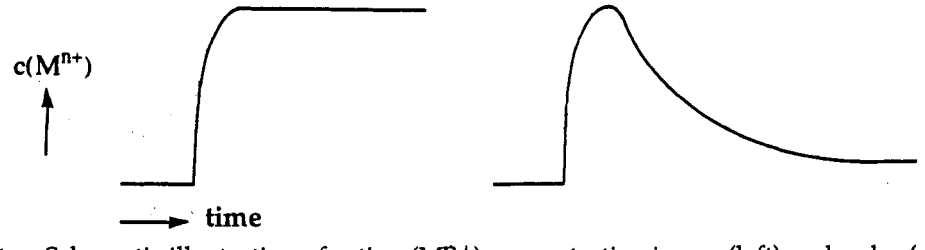

Fig. 1. Schematic illustration of cation (M^{n+}) concentration jump (left) and pulse (right).

The largest possible cation concentration jumps are to be expected when the affinity between cation and photoproduct is negligible. This is assumed to be generally the case when photolysis leads to ligand splitting rather than to ligand rearrangement. For a comprehensive analysis of the dynamics of cation release, methods have to be developed allowing the observation of the time course of the photochemical reaction as well as of the cation appearance in solution.

A REALIZATION

Ligands designed to preferentially form caged alkali ions

To selectively release a particular alkali ion in the presence of other cations of physiological interest, photo-cleavable cryptands as shown in Fig. 2. have been designed and investigated (ref. 4, 5). These cage-like polyoxadiaza macrobicycles are well soluble in water due to their polar nature and they form fairly strong alkali ion complexes. The cavity size and consequently also the cation selectivity is predetermined by the length of the chosen nitrogen bridges. Light-sensitivity is achieved by introducing the photo-cleavable 2-nitrobenzylether bond in one bridge of the macrobicycle.

The structure of NC222 and its KSCN complex has been verified in the crystalline state (ref. 4). Protonation of the bridging nitrogens affects cation binding in aqueous solution below pH 10.5. Therefore, the equilibrium constants of protolysis (K_1, K_2) as well as of cation binding (K_S) have to be determined. To achieve this aim, potentiometric titrations are carried out. For NC221 at 25°C log K_1 and log K_2 values of 7.4 and 10.5 and log K_S values of 5.35 for Na^+, 3.8 for K^+ and 6.3 for Ca^{2+} are found. The corresponding log K_1 and log K_2 values for NC222 are 7.2 and 9.7 and those for log K_S are 3.8 for Na^+, 5.45 for K^+, 6.6 for Tl^+ and 4.2 for Ca^{2+}. Similar values are obtained also for DMNC222. All these parameters are close to those published for the corresponding parent compounds (ref. 6).

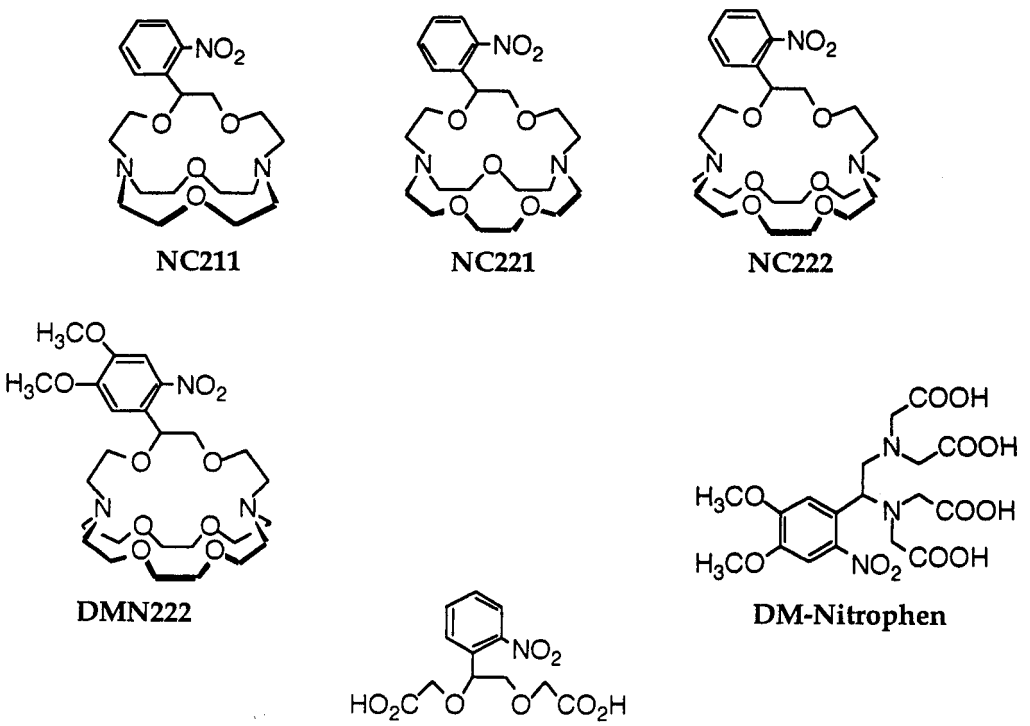

Fig. 2. Structures of caged cation ligands.

The absorption spectrum of NC221 and of NC222 shows a maximum around 270 nm, that of DMNC222 around 350 nm. Photolysis at 25°C, characterized by a quantum yield around 0.3 at 266 nm for NC221 and NC222 as well as their cation complexes and around 0.1 at 347 nm for DMNC222, leads to the predicted ether bond splitting as indicated in Fig. 3. A monocyclic structure with lower cation affinity, bearing the 2-nitrobenzoketo residue, is formed.

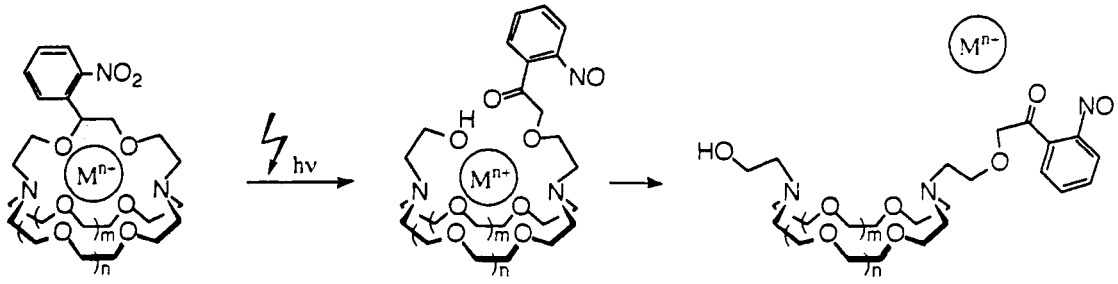

Fig. 3. Light-induced release of a cation from a photo-cleavable cryptand (n = 1, m = 0: caged Na^+; n = m = 1: caged K^+).

The mechanistic proposal illustrated in Fig. 3. corresponds to results obtained from the investigation of the main photoproduct (isolated after preparative UV-irradiation of the diacid shown in Fig. 2.) by employing different spectroscopic techniques (ref. 5). It is also consistent with the results reported in photochemical studies of small model compounds (ref. 7, 8).

Flash photolysis (2 ms Xenon lamp flash, λ-range between 250 and 400 nm; 25°C) of these cryptands and their complexes studied by fast recording absorption spectrometry at 400 nm indicates that a fast (not time resolvable) formation of an intermediate state (λ_{max} around 415 nm) occurs which decays with time constants ranging from 0.8 to several ms, representing the time-determining process of the photochemical reaction. The intermediate is assumed to be analogous to the anionic "aci-nitro" state (ref. 7, 8). The observed time course suggests, that cations are released upon photolysis in the ms time scale. To test this assumption, experiments are carried out on the NC221-Ca^{2+} complex in the presence of the Ca^{2+}-sensitive dye indicator methylthymol blue. This is done under conditions where it has been verified that Ca^{2+} binding to the indicator is considerably faster than the release of the cation after photolysis. Thus the spectrophotometric detection of Ca^{2+} release is not retarded by its binding to the indicator. The corresponding flash photolysis experiments, performed at pH 8.1, indicate that Ca^{2+} appearance in solution is characterized by a time constant of about 3 ms. This is at least 3 times slower than the detectable ligand splitting. Among other possibilities this result could imply that Ca^{2+} dissociation from the monocyclic photoproduct (second reaction step in fig. 3) represents in this particular case the rate-limiting step of cation release.

These results indicate that NC221 can also be applied as a caged Ca^{2+} ligand, which forms a twice positively charged coordination complex. Since no suitable dye indicators are available for alkali ions in aqueous solution, the time course of the photolytically induced alkali ion release cannot be measured. It is, however, assumed that it will be similar to that observed for Ca^{2+}.

Ligands designed to form caged alkaline earth cations

Prior to the introduction of NC221 as a caged Ca^{2+} ligand, BAPTA type compounds (ref. 9, 10) and DM-nitrophen (ref. 11, 12), a photo-cleavable EDTA derivative (Fig. 2.), have been synthesized. Both classes of compounds are capable of inducing Ca^{2+} and to some extent also

Mg^{2+} concentration jumps. The affinities of both ligand types are high to divalent metal ions but low to alkali ions. The DM-nitrophen ligand is split upon photolysis and yields imino-diacetic acid. The resulting cation affinity decrease is larger than the one estimated for the BAPTA type compounds, where essentially a ligand rearrangement occurs. For DM-nitrophen the log K values of protolysis and the affinities to various cations have been determined (ref. 13). The corresponding parameters are similar to those of EDTA. It can thus be concluded that the introduction of the photo-sensitive residue does not significantly change the complex formation properties of the parent ligands. In contrast to the NC222-Ca^{2+} complex, the alkaline earth cation complexes of DM-nitrophen and of the BAPTA type ligands exhibit a resulting negative charge.

In the case of DM-nitrophen, the photolytically induced Ca^{2+} release is faster than that observed for NC221 (ref. 14). Flash photolysis experiments on the DM-nitrophen-Mg^{2+} complex in the presence of the dye indicator methylthymol blue provide a Mg^{2+} release time of a few ms.

APPLICATION OF THE MOLECULAR CAGED CATION CONCEPT

Since the photolytically induced alkali ion concentration jumps are assumed to be established within a few ms, kinetic studies can be carried out with systems that equilibrate at least in the 10 ms time range or slower. Fast recording spectrometry (absorption or fluorescence) is again a suitable technique to follow the readjustment after the cation concentration jump.

In the case of the transport enzyme Na^+,K^+-ATPase (purified from pig kidney), a covalent labelling with fluorescein isothiocyanat isomer I (FITC) enables the indirect detection of alkali ion binding (ref. 15). The fluorescence emission intensity decreases markedly upon K^+ binding. Based on a 1:1 binding model, log K_S values around 4.0 are found for K^+ and around 5.0 for Tl^+ (ref. 5, 15), under the conditions applied here. Fig. 4. shows the result of a kinetic flash photolysis (2 ms UV-light pulse) experiment performed in the presence of 0.45 µM enzyme (E), 0.6 mM KCl and 1.2 mM NC222 at pH 8.35 in a volume of 40 µl, containing also low concentrations of stabilizing agents.

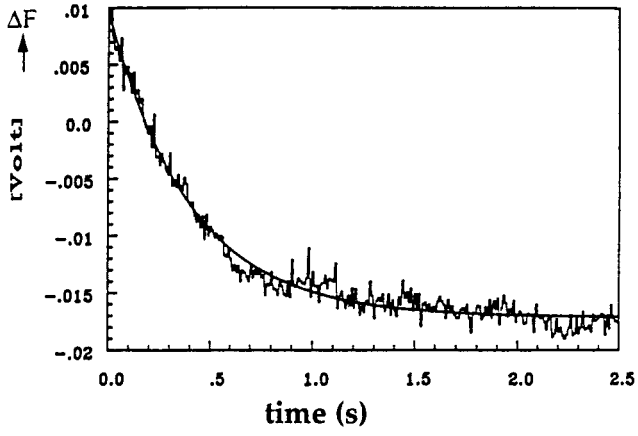

Fig. 4. Flash photolysis of caged K^+ (NC222- -K^+) inducing K^+ binding to FITC-Na^+,K^+-ATPase: measured fluorescence intensity (ΔF) as a function of time (for details see text).

After the flash, the fluorescence intensity above 520 nm is recorded as a function of time. Under the given experimental conditions only a small K^+ concentration jump can be achieved. The expected time-dependent amplitude decrease is indeed found and it is characterized by a single exponential function with a relaxation time τ of 400 ms. Similar results are obtained with Tl^+ (ref. 5).

For the simplest kinetic reaction scheme in accordance with this observation

$$K^+ + E \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} E\text{-}\text{-}K^+ \qquad (3)$$

we predict according to the chemical relaxation methodology (ref. 3) a linear dependence of $1/\tau$ versus the sum of the free concentrations (c) of K^+ and enzyme:

$$1/\tau = k_{on}\,(c_{K^+} + c_E) + k_{off} \qquad (4)$$

This dependence allows the determination of the kinetic parameters k_{on} and k_{off}. In the case of the kinetic Tl^+ binding studies, such a linear dependence is observed over a fairly wide concentration range. Values of k_{on} for K^+ and Tl^+ are 10^4 $M^{-1}s^{-1}$ and 2.5×10^4 $M^{-1}s^{-1}$ and of k_{off} are 1 s^{-1} and 0.4 s^{-1}, respectively. The rate constants for cation binding are about 1000 times smaller than those observed for the K^+ carrier valinomycin in methanol, where a ligand conformational rearrangement occurring in the 0.1 μs to 1 μs time range was shown to be rate-limiting (ref. 16). The results obtained with Na^+,K^+-ATPase suggest that even considerably slower structural rearrangements than in the case of valinomycin are likely to be relevant for alkali ion binding to this enzyme. The observed dissociation rate constants are consistent with kinetic data published for the deocclusion of Rb^+ from the enzyme in the absence of nucleotides (ref. 17, 18).

Preliminary electric studies on black lipid membranes with caged K^+ in the presence of valinomycin and with caged Na^+ in the presence of adsorbed Na^+,K^+-ATPase and ATP indicate that this technique is also applicable to transport studies on membranes (ref. 19).

In conclusion, the caged cation concept opens promising new possibilities for the investigation of cation-sensitive biochemical and biological systems.

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