Biochemical and Biophysical Research Communications 400 (2010) 447–451

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Characterization of caged compounds binding to proteins by NMR spectroscopy

Joanna Bandorowicz-Pikula [a], René Buchet [b], F. Javier Cañada [c], Martin Clémancey [d], Patrick Groves [c,e,*], Jesus Jiménez-Barbero [c], Jean-Marc Lancelin [d], Olivier Marcillat [b], Slawomir Pikula [a], Anna Sekrecka-Belniak [a,f], Agnieszka Strzelecka-Kiliszek [a]

[a] Department of Biochemistry, Nencki Institute of Experimental Biology, 3 Pasteur Street, 02093 Warsaw, Poland
[b] ICBMS UMR CNRS 5246, Université Lyon I, 69622 Villeurbanne Cedex, France
[c] Centro de Investigaciones Biológicas, Ramiro de Maeztu 9, 28040 Madrid, Spain
[d] Anabio, Université Lyon 1, CNRS UMR 5180, Sciences Analytiques, 69622 Villeurbanne Cedex, France
[e] Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. Da República, 2780-157 Oeiras, Portugal
[f] Warsaw University of Life Sciences, SGGW, Nowoursynowska 166, 02787 Warsaw, Poland

ARTICLE INFO

Article history:
Received 17 August 2010
Available online 4 September 2010

Keywords:
Annexin A6
Caged ligands
Fourier transform infrared spectroscopy
NMR spectroscopy
Creatine kinase

ABSTRACT

Photolysable caged ligands are used to investigate protein function and activity. Here, we investigate the binding properties of caged nucleotides and their photo released products to well established but evolutionary and structurally unrelated nucleotide-binding proteins, rabbit muscle creatine kinase (RMCK) and human annexin A6 (hAnxA6), using saturation transfer difference NMR spectroscopy. We detect the binding of the caged nucleotides and discuss the general implications on interpreting data collected with photolysable caged ligands using different techniques. Strategies to avoid non-specific binding of caged compound to certain proteins are also suggested.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

Photolysable caged ligands have been developed as tools for various biochemical, biophysical and physiology experiments to investigate protein function and activity [1,2]. Several rules define the optimal design of caged compounds for equilibrium (biochemical) experiments. The caged ligand should have a high solubility in aqueous solutions and be chemically stable. Another requirement which may be necessary is that the ligand should be released from its cage rapidly by light. For example, calcium should be released from its cage within a millisecond [3] as calcium activates many processes in as little as a few milliseconds [4]. Neither the caged ligand, nor the released cage molecule, should affect the biological activity of the protein. The cage moiety itself should also not bind to the protein.

The design rules for caged compounds are more constricted for intracellular physiological experiments [1,2] as the caged compound should be transportable across the cell membrane. If the caged compound is to be used in conjunction with fluorescent probes, inner filter effects and other wavelength interfering factors should be avoided. In addition, caged ligands have been proposed as vehicles for targeted drug delivery in recent years [5–9]. Such an application adds new problems related to their efficient delivery and targeting to the right cells and in the proper site, the toxicity of the released cage moiety and its efficient excretion or degradation to non-toxic products.

Here we use NMR methods to investigate the mechanism of binding of caged nucleotides and released products to two unrelated nucleotide-binding proteins *in vitro*, rabbit muscle creatine kinase [10] and human annexin A6 [11]. We applied Saturation Transfer Difference spectroscopy (STD) to protein–ligand samples. This method proceeds by specifically labeling the protein with magnetization that can be transferred to parts of the ligands that contact the protein [12–14]. Ideally, we would expect to record STD signals for released nucleotide and no STD signals for the caged nucleotide and released cage moiety. In this report, we show that caged nucleotides bind to the proteins and this could affect the

Abbreviations: Anx, annexin; CK, creatine kinase; DMNPE-caged GTP-γ-S, guanosine 5′-O-(3-thiotriphosphate), P 3(S)-(1-(4,5-dimethoxy-2-nitrophenyl) ethyl) ester, triammonium salt; NPE-ADP, adenosine 5′-diphosphate, P^2-(1-(2-nitrophenyl)ethyl) ester, disodium salt; RIDS, reaction-induced infrared difference spectroscopy; RMCK, rabbit muscle creatine kinase; STD, saturation transfer difference spectroscopy.

* Corresponding author at: Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. Da República, 2780-157 Oeiras, Portugal.

E-mail addresses: j.bandorowicz-pikula@nencki.gov.pl (J. Bandorowicz-Pikula), rbuchet@univ-lyon1.fr (R. Buchet), jcanada@cib.csic.es (F.J. Cañada), pgroves@itqb.unl.pt (P. Groves), jjbarbero@cib.csic.es (J. Jiménez-Barbero), jean-marc.lancelin@univ-lyon1.fr (J.-M. Lancelin), olivier.marcillat@univ-lyon1.fr (O. Marcillat), s.pikula@nencki.gov.pl (S. Pikula), s.strzelecka-kiliszek@nencki.gov.pl (A. Strzelecka-Kiliszek).

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.08.104

interpretation of data collected by other methods. We discuss which new designs of caged compounds could be adopted to provide improved products for biochemical studies.

2. Materials and Methods

2.1. Materials

Anx A6, isoform-1 was prepared according to previously published procedures [34] and NMR samples were dissolved in 10 mM d_{11}-Tris (Cambridge Isotopes Limited), 100 mM NaCl, pD 7.3 ^2H_2O buffer with 1 mM EDTA or 0.1 mM $CaCl_2$. RMCK was dissolved in 1 mM $MgCl_2$, 5 mM deuterated phosphate buffer, pD 7.3. DMNPE-caged GTP-γ-S (guanosine 5′-O-(3-thiotriphosphate), P 3(S)-(1-(4,5-dimethoxy-2-nitrophenyl)ethyl) ester, triammonium salt) and NPE-caged ADP (adenosine 5′-diphosphate, P^2-(1-(2-nitrophenyl)ethyl) ester, disodium salt) were purchased from Molecular Probes and ADP from Sigma.

2.2. NMR Spectroscopy

The RMCK data was collected on a Varian 600 MHz instrument equipped with a triple resonance (HCN) probe. The Anx A6 data was collected on a Bruker Avance 500 MHz instrument equipped with a triple resonance probe. Data were collected at 281 K (Anx A6) and 293 K (RMCK) according to the standard STD experimental protocol with on- and off-resonances of 0.75 and 100 ppm for Anx A6 and 0 and 27 ppm for RMCK.[12]

3. Results and Discussion

3.1. Binding of ADP to creatine kinase

Phosphagen kinases are a family of phosphoryl transfer enzymes comprising of arginine kinase, found in invertebrates, and creatine kinase, expressed in vertebrates [15–17]. Creatine kinase (CK, EC 2.7.3.2) catalyzes reversibly the phosphoryl transfer from phosphocreatine to ADP by forming creatine and ATP [18–20]. There are two mitochondrial forms of CK (mt-CK) encoded by different genes [21,22]. Cytosolic CKs are obligate dimers composed of M (muscle) and/or B (brain) subunits leading to MM, BB and MB isoenzymes expressed in a tissue-specific manner whereas two cytosolic isoenzyme CK (MM-CK being the muscle isoform and BB-CK being the brain isoform [23,24]. The secondary structure changes caused by nucleotide binding to CK are difficult to observe by infrared spectroscopy (absorbance changes are 0.1–0.2%) due to sample to sample errors (buffer and nucleotide contribute to infrared spectra of enzymes). However, the binding of photoreleased nucleotide from caged nucleotide to arginine kinase [25], muscle creatine kinase [26,27], recombinant mitochondrial creatine kinase [28] could be monitored by reaction-induced infrared difference spectroscopy (RIDS). The principle of RIDS has been reviewed elsewhere [29]. Here, we investigated the binding of ADP and caged NPE-ADP (Fig. 1) to rabbit muscle creatine kinase (RMCK) by STD competition (Fig. 2).

We first collected a STD spectrum of ATP and RMCK (Fig. 2A). Then, we titrated NPE-ADP into the sample. ATP and NPE-ADP have different chemical shifts and can be distinguished on this basis (Figs. 2B and 2C). The STD signal for the cage moiety of NPE-ADP indicates that the cage group interacts with RMCK (Fig. 2C). The STD signal for ATP decreases as a function of increased concentration of NPE-ADP Fig. 2D, consistent with NPE-ADP displacing ATP from its binding site. The competitive titration indicates that the NPE-ADP has a similar binding affinity (0.7 mM) as ATP (1.2 mM), under the measured conditions (Fig. 2D).

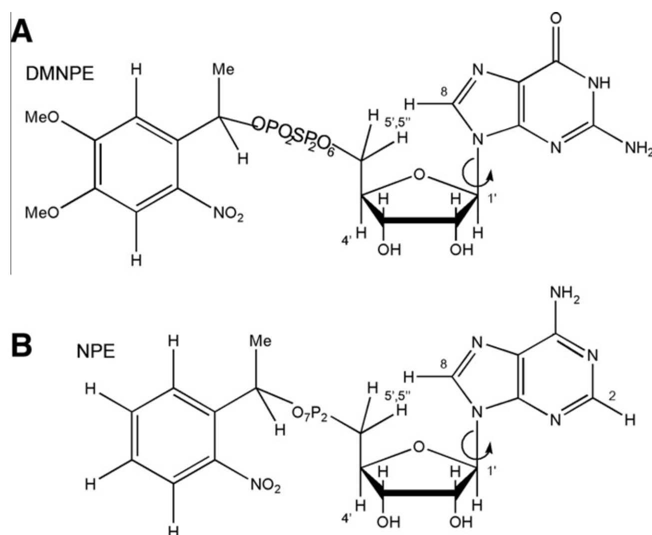

Fig. 1. Chemical structures of (A) DMNPE-GTP-γ-S and (B) NPE-ADP.

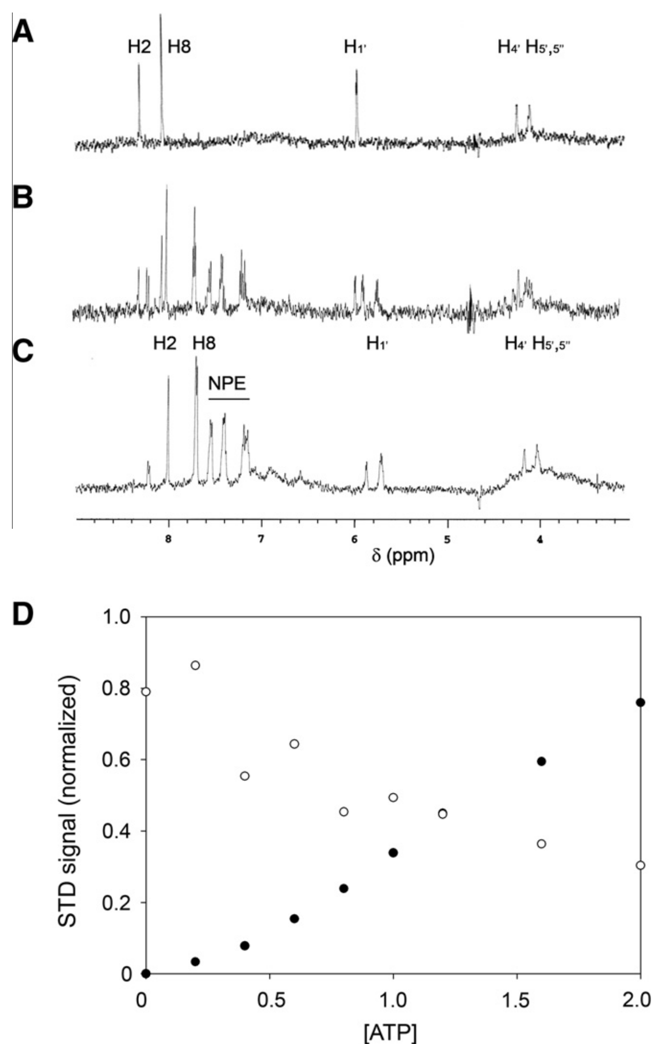

Fig. 2. STD competition of ATP and NPE-ADP for RMCK. (A) STD spectrum for 2 mM ATP, (B) STD spectrum of 2 mM ATP plus 2 mM NPE-ADP, (C) STD spectrum of 2 mM NPE-ADP. All STD samples contain 25 μM RMCK. (D) Plot of relative intensities of STD peaks of ATP and NPE-ADP as a function of added NPE-ADP to a 25 μM RMCK/2 mM ATP complex. Black circles represent the STD signal of NPE-ADP while white circles correspond to STD signal of ATP.

3.2. Binding of GTP/ATP by human annexin A6

Several biochemical and biophysical methods have been used to characterize the binding of nucleotides to annexins [30–36], including the binding of photoreleased GTP, ATP and P_i to porcine and human recombinant annexin A6 and its mutants by reaction-induced infrared difference spectroscopy (RIDS) [33–35]. The putative nucleotide-binding site of annexin A6 has been identified and its validity was confirmed by point mutations of W343 and several lysine residues [35,36]. The functions of annexin A6 are not fully understood but nucleotide-binding is known to cause changes in its function (ability to perform ion channel activity), conformation, oligomerization and the lipid- and membrane-binding properties of annexin A6. These properties may support the suggested activity of annexin A6 as a protein participating in vesicular transport.[37,38]

Fig. 1 shows the structures of the nucleotides that were tested with annexin A6. In a first approach, the sample of protein and caged nucleotide was submitted to photolysis in the NMR tube. Photolysis proved to be inefficient under these conditions (due to a large volume of sample) and renders roughly a 1:1:1 mixture of free nucleotide and caged nucleotide diastereomers (both epimers at the benzylic carbon). Control 1D ^{1}H and STD spectra clearly indicated that all three compounds, the released ADP and unphotolysed caged nucleotides, interact with annexin A6. Furthermore, the STD indicates contacts of the cage moieties with annexin A6 and that the majority of low-field signals are resolved and could be unambiguously assigned.

In a second step the interaction of DMNPE-GTP-γ-S with annexin A6 was studied. Fig. 3A shows a 1D ^{1}H NMR spectra of the mixture after partial photorelease. The signals indicate an approximately 1:1:1 mixture of species based on the DMNPE-GTP-γ-S, H8 and H1′ signals for the two caged epimers and released GTP-γ-S (Fig. 3A). Fig. 3B shows the STD spectrum of the same sample as in Fig. 3A. Although the STD signals in Fig. 3B cannot be accurately quantitated, their values range between 20% and 100% with an approximately 1:1:1 ratio for the GTP-γ-S moiety. STD scores of 50%–100% for the cage moiety indicate strong interactions between the cage moiety of the DMNPE-GTP-γ-S epimers and Anx A6. STD signals attributable to the released cage indicate that it is also able to bind to the protein without being coupled to GTP.

A similar set of STD experiments was carried out for NPE-ADP and released ADP binding to human annexin A6 (Fig. 3C and D). NPE-ADP behaves similarly to DMNPE-GTP-γ-S. The main difference relates to the decreased solubility of the free NPE cage, evident from the 1D ^{1}H NMR spectrum (Fig. 3C). No STD peaks are discerned for the released cage (Fig. 3D).

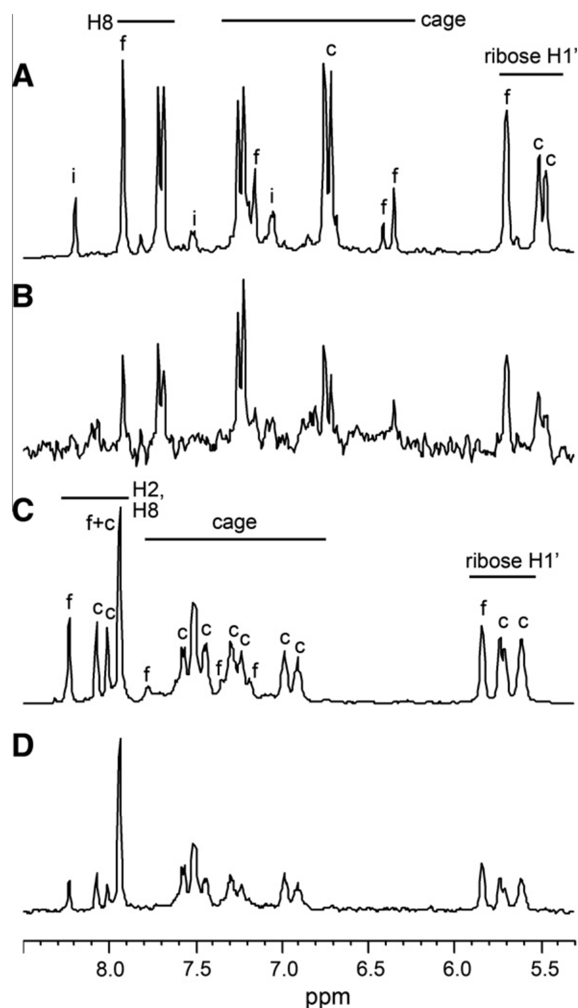

Fig. 3. 1D ^{1}H and STD spectra of Anx A6 and caged nucleotides. (A) 1D ^{1}H NMR spectrum of 17 μM annexin A6 and 2 mM DMNPE-GTP-γ-S, which has been approximately 50% uncaged. (B) STD of same sample shown in panel (A). (C) 1D ^{1}H NMR spectrum of 17 μM annexin A6 and 2 mM NPE-ADP, which has been approximately 50% uncaged. (D) STD spectrum of sample shown in panel (C). Peaks are labeled c, caged; f, free; i, impurity; together with their assignments.

3.3. NMR can validate RIDS data

We observe the binding of caged compounds to two proteins by STD NMR. The NMR data indicate that caged nucleotides competitively bind to the same sites as their related free nucleotides and apparently with similar binding properties. The similar STD intensities for caged nucleotide diastereomers suggests both have similar binding kinetics and no selectivity is observed for one isomer over the other. Caged compounds might appear to be inert but one should not assume that they do not bind to the proteins of interest. The effect of bound caged compounds on the interpretation of data is poorly understood.

One important question to ask is whether infrared data obtained on creatine kinase [26–28] and on annexins [33–35] by using caged nucleotides are still valid or need to be reevaluated. To answer this question, one should realize that secondary structure changes (as viewed by infrared spectroscopy) induced by nucleotide binding to creatine kinase or annexins are extremely small (below 1% of absorbance changes and below experimental errors induced by sample to sample variations), therefore alternate methods such as RIDS were employed. The principle of RIDS consists of measuring a difference infrared spectrum that could be triggered by using a flash to photolytically induce a concentration jump in the same infrared cell, which could cancel the sample to sample errors [29]. Ideally, the caged compound should not interact with the protein, reflecting the infrared spectrum of the protein in unbound state. Once, photolyzed, the released nucleotide could bind to the protein reflecting the infrared spectrum in bound state. Our RIDS data indicated that around 3–6 amino acid residues in RMCK [25–28], or around 3–9 amino acid residues in Anx A6 [34,35], are implicated in nucleotide binding and in the view of NMR data, this should be considered as the lower limit.

The difference spectrum (unbound state minus bound state) reports global changes in protein structure that are induced by ligand-binding. RIDS can detect small changes in a protein's hydrogen-bond network that cannot be detected by other methods and provides an improvement over infrared spectroscopy by providing an exactly matched sample thickness and concentrations for samples of protein and protein with ligand. RIDS utilizes high

concentrations of protein (0.05–0.1 mM protein and 1–2 mM caged ligand). The affinity of RMCK for ATP is 1.2 mM, while the affinity of RMCK for ADP is 0.05 mM [18]. According to Fig. 2D, the affinity of NPE-ADP is ~0.7 mM. The affinity of nucleotides to porcine and human annexin A6 by using azido-ATP is in the range of micromolar concentrations [30,39]. Thus, non-specific binding, which is difficult to characterize, can be present and STD may detect such problems. RIDS utilizes higher protein and ligand concentrations than those used here for STD, although RIDS samples have much smaller volumes (10 μl). In principal, a 200 μl sample can be subjected first to STD in a small volume NMR tube before recovery for RIDS experiments. In this case, we would obtain data similar to that obtained for annexin A6. While the experiments in Fig. 3 unequivocally show the binding of caged nucleotides to annexin A6, we cannot conclude that the caged nucleotide and free nucleotide bind to the same, or different, ligand binding sites on the protein. To be able to make this conclusion, the competition experiments illustrated in Fig. 2 are preferred although this requires larger amounts of protein and caged compounds. Another question is whether the ligand binding, as observed by STD or by RIDS corresponds to the same binding site (i.e. active site and not secondary binding site). This could be checked by using inhibitors or mutants to prevent ligand binding to the active site. We have reexamined the RIDS data for RMCK. The structural changes caused by NPE-ATP, NPE-caged ADP and NPE-caged Pi, revealed that the main structural changes to RMCK are associated with P_i interacting with the active site of RMCK [25] as checked with inactive mutants [40]. The largest changes were observed with NPE-ADP consistent with its larger affinity for RMCK as compared with NPE-ATP. Comparison of the RIDS on Anx A6 with NPE-ATP, DMNPE-GTP-γ-S and NPE-caged P_i indicated that the main structural changes are associated to the interactions of phosphate moiety with the peptide backbone of Anx A6 [35]. RIDS either on RMCK or on AnxA6 did not reveal specific changes associated to the ribose or purine moieties of nucleotides in the amide-I and amide-II regions (sensitive to secondary structures). There were only very minor changes that could be assigned to the purine moiety in the case of RMCK [25] which could not be detected by Raman spectroscopy [26]. Reexamination of spectral changes of the cage revealed that the infrared spectrum of the photoreleased cage in the presence or absence of protein was unaltered suggesting that polar groups of the cage did not interact with RMCK by forming hydrogen bonds, while addition of DTT altered its infrared spectrum due to its reaction with the cage [41]. RIDS of Anx A6 induced either by NPE-ATP or DMNPE-GTP-γ-S were almost similar [35] suggesting that NPE and DMNPE cages interacted in the same manner or that their interactions were small. In contrast, NMR experiments illustrate that both the cage compound (in the cases of RMCK and Annexin A6) and free cage (only unambiguously shown for annexin A6) may bind to the protein. To reconciliate both RIDS and NMR data, we have to consider that the purine, ribose and cage moieties may bind to the proteins (within the active site or elsewhere) as revealed by STD NMR, while RIDS did not indicate any changes implicating purine, ribose and cage moieties since the changes were too small to be detected (only a fraction of them were bound, while mostly were unbound) or/and the ligands remain bound before and after photorelease. Concerning the RIDS data, they still reflect the interactions between phosphate moiety and the proteins or to a rearrangements of phosphate groups toward the targeted sites. Therefore most of the interpretations of RIDS remain unchanged [25–28,34,35] except that one may cast doubt on the magnitude of structural changes induced by nucleotide binding. Indeed, if the caged nucleotide is mostly bound before its photolytic reaction, RIDS will underestimate structural changes caused by phosphate binding to proteins. Our RIDS data indicated that around 3–6 amino acid residues in RMCK [25–28] or around 3–9 amino acid residues in Anx A6 [34,35] are implicated in nucleotide bindings and in the view of NMR data, this should be considered as the lower limit.

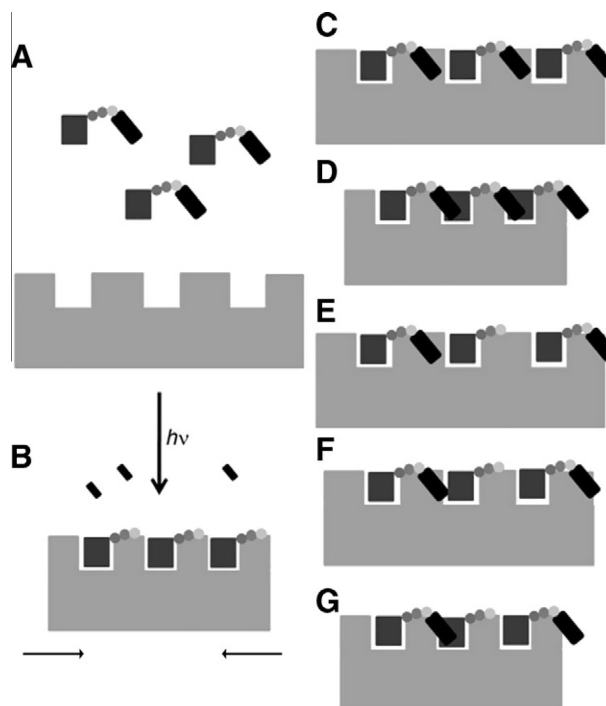

Fig. 4. Use of caged compounds in physiological experiments. The mechanism is assumed to involve a transition from (A) to (B) for releasing caged nucleotide, its binding to a muscle protein and protein activation (muscle contraction). Other species are possible that may not affect the observed result, i.e. transition (C) to (B or G). Several species can lead to inconsistent results depending on the perceived inactivity of the caged compound (D) or non-linear response to the degree of photorelease (E and F).

3.4. Other applications of caged compounds and improved design strategies

Caged compounds have a wider use than RIDS. In particular, caged nucleotides can be used at millimolar concentrations for cell physiology and biomedical applications [1]. Considering our NMR data, how does the effect of partially released ligand affect experiments involving highly cooperative, multiple-binding systems? Fig. 4 highlights one potential problem involving a biomolecular target with multiple binding sites, such as actin filaments. Physiological experiments tend to assume a highly efficient photorelease of the ligand from an inert caged compound. However, the competitive binding of caged ligand with released ligand can affect the observed results. Subtle differences in laser/UV power or sample thickness could influence the results from different labs. A further complication to Fig. 4 is that while the protein of interest might not bind the caged ligand, other proteins may do so – effectively reducing the concentration of caged ligand and its released products. The development of selectively labeled in-cell NMR methods [42] may lead to the future use of STD experiments [43] to detect the unwanted binding of caged ligands to proteins and extracellular targets of cell cultures.

4. Conclusion

The binding specificity of caged compounds was tested by NMR spectroscopy for two protein–ligand complexes. We found that the caged compound binds to the protein at concentrations lower than

utilized for RIDS experiments (and at similar concentrations used in physiological experiments). The binding of caged compounds, free ligands and free cage moieties has implications on the interpretation of data involving photolysable ligands. Our conclusion is that a new generation of photolysable ligands, based on functionalized containers, nanoparticles, dendrimers etc., is required. The new generation of caged compounds should have properties to prevent, or at least to control, the potential binding of the unphotolysed caged compound and released cage moiety byproducts to the protein of interest.

Acknowledgments

This work was supported in part by a Spain–France bilateral grant to R.B./J.C. funded by the Spanish Ministry of Science (HF2005-0276) and Égide (Picasso N 10715SA), by an exchange program sponsored by the Polish Academy of Sciences (2009-2010/5) and Fundação para a Ciência e a Tecnologia to S.P./P.G, and by grant N401 049 32/1143 to J.B-P. from the Polish Ministry of Science and Higher Education.

References

[1] G.C.R. Ellis-Davies, Caged compounds: photorelease technology for control of cellular chemistry and physiology, Nat. Methods 4 (2007) 619–628.
[2] H.A. Lester, J.M. Nerbonne, Physiological and pharmacological manipulations with light flashes, Annu. Rev. Biophys. Bioeng. 11 (1982) 151–175.
[3] J.H. Kaplan, Photochemical manipulation of divalent cation levels, Annu. Rev. Physiol. 52 (1990) 897–914.
[4] J.H. Kaplan, B. Forbush, J.F. Hoffman, Rapid photolytic release of adenosine 5′-triphosphate from a protected analogue: utilization by the Na:K pump of human red blood cell ghosts, Biochemistry 17 (1978) 1929–1935.
[5] M.S. Kim, S.L. Diamond, Controlled release of DNA/polyamine complex by photoirradiation of a solid phase presenting o-nitrobenzyl ether tethered spermine or polyethyleneimine, Bioorg. Med. Chem. Lett. 16 (2006) 5572–5575.
[6] M.A. Kostiainen, D.K. Smith, O. Ikkala, Optically triggered release of DNA from multivalent dendrons by degrading and charge-switching multivalency, Angew. Chem. Int. Ed. Engl. 46 (2007) 7600–7604.
[7] B. Chandra, R. Subramaniam, S. Mallik, D.K. Srivastava, Formulation of photocleavable liposomes and the mechanism of their content release, Org. Biomol. Chem. 4 (2006) 1730–1740.
[8] Z. Zhang, H. Hatta, T. Ito, S. Nishimoto, Synthesis and photochemical properties of photoactivated antitumor prodrugs releasing 5-fluorouracil, Org. Biomol. Chem. 3 (2005) 592–596.
[9] P.D. Kehayova, C.D. Woodrell, P.J. Dostal, et al., Phototriggered delivery of hydrophobic carbonic anhydrase inhibitors, Photochem. Photobiol. Sci. 1 (2002) 774–779.
[10] M. Wyss, O. Braissant, I. Pischel, et al., Creatine and creatine kinase in health and disease–a bright future ahead?, Subcell Biochem 46 (2007) 309–334.
[11] V. Gerke, C.E. Creutz, S.E. Moss, Annexins: linking Ca2+ signalling to membrane dynamics, Nat. Rev. Mol. Cell Biol. 6 (2005) 449–461.
[12] M. Mayer, B. Meyer, Group epitope mapping by saturation transfer difference NMR to identify segments of a ligand in direct contact with a protein receptor, J. Am. Chem. Soc. 123 (2001) 6108–6117.
[13] P. Groves, A. Strzelecka-Kiliszek, A. Canales, et al., NMR Spectroscopy as a tool in annexin research, Calcium Binding Proteins 1 (2006) 20–25.
[14] P. Groves, K.E. Kövér, S. André, et al., Effect of temperature in Saturation Transfer Difference NMR experiments, Magn. Reson. Chem. 45 (2007) 745–748.
[15] V.A. Saks, L.V. Rosenshtraukh, V.N. Smirnov, E.I. Chazov, Role of creatine phosphokinase in cellular function and metabolism, Can. J. Physiol. Pharmacol. 56 (1978) 691–706.
[16] T. Suzuki, T. Furukohri, Evolution of phosphagen kinase. Primary structure of glycocyamine kinase and arginine kinase from invertebrates, J. Mol. Biol. 237 (1994) 353–357.
[17] W.R. Ellington, Evolution and physiological roles of phosphagen systems, Annu. Rev. Physiol. 63 (2001) 289–325.
[18] D.C. Watts, Creatine kinase (Adenosine 5'-triphosphate-creatine phosphotransferase), in: P.D. Boyer (Ed.), The Enzymes, vol. 8, Academic Press, New York, 1973, pp. 383–455.
[19] S.P. Bessman, C.L. Carpenter, The creatine–creatine phosphate energy shuttle, Ann. Rev. Biochem. 54 (1985) 831–862.
[20] T. Wallimann, M. Wyss, D. Brdiczka, et al., Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostasis, Biochem. J. 281 (1992) 21–40.
[21] P.C. Babbitt, G.L. Kenyon, I.D. Kuntz, et al., Comparisons of creatine kinase primary structures, J. Prot. Chem. 5 (1986) 1–14.
[22] J.P. Hossle, J. Schlegel, G. Wegmann, et al., Distinct tissue specific mitochondrial creatine kinases from chicken brain and striated muscle with a conserved CK framework, Biochem. Biophys. Res. Commun. 151 (1998) 408–416.
[23] D.C. Turner, T. Wallimann, H.M. Eppenberger, A protein that binds specifically to the M-line of skeletal muscle is identified as the muscle form of creatine kinase, Proc. Natl. Acad. Sci. USA 70 (1973) 702–705.
[24] L. Pickering, H. Pang, K. Biemann, et al., Two tissue-specific isozymes of creatine kinase have closely matched amino acid sequences, Proc. Natl. Acad. Sci. USA 82 (1985) 2310–2314.
[25] C. Raimbault, R. Buchet, C. Vial, Changes of creatine kinase secondary structure induced by the release of nucleotides from caged compounds. An infrared difference-spectroscopy study, Eur. J. Biochem. 240 (1996) 134–142.
[26] H. Hagemann, O. Marcillat, R. Buchet, C. Vial, Magnesium-adenosine diphosphate binding sites in wild-type creatine kinase and in mutants: role of aromatic residues probed by Raman and infrared spectroscopies, Biochemistry 39 (2000) 9251–9256.
[27] C. Raimbault, F. Besson, R. Buchet, Conformational changes of arginine kinase induced by photochemical release of nucleotides from caged nucleotides–an infrared difference-spectroscopy investigation, Eur. J. Biochem. 244 (1997) 343–351.
[28] T. Granjon, M.-J. Vacheron, C. Vial, R. Buchet, Structural changes of mitochondrial creatine kinase upon binding of ADP, ATP, or Pi, observed by reaction-induced infrared difference spectra, Biochemistry 40 (2001) 2988–2994.
[29] C. Zscherp, A. Barth, Reaction-induced infrared difference spectroscopy for the study of protein reaction mechanisms, Biochemistry 40 (2001) 1875–1883.
[30] J. Bandorowicz-Pikula, Y.C. Awasthi, Interaction of annexins IV and VI with ATP. An alternative mechanism by which a cellular function of these calcium- and membrane-binding proteins is regulated, FEBS Lett. 409 (1997) 300–306.
[31] J. Bandorowicz-Pikula, A. Wrzosek, P. Makowski, S. Pikula, The relationship between the binding of ATP and calcium to annexin IV. Effect of nucleotide on the calcium-dependent interaction of annexin with phosphatidylserine, Mol. Membr. Biol. 14 (1997) 179–186.
[32] M. Danieluk, S. Pikula, J. Bandorowicz-Pikula, Annexin VI interacts with adenine nucleotides and their analogs, Biochimie 81 (1999) 717–726.
[33] J. Bandorowicz-Pikula, A. Wrzosek, M. Danieluk, et al., ATP-Binding site of annexin VI characterized by photochemical release of nucleotide and infrared difference spectroscopy, Biochem. Biophys. Res. Commun. 263 (1999) 775–779.
[34] A. Kirilenko, M. Golczak, S. Pikula, et al., GTP-induced membrane binding and ion channel activity of annexin VI: is annexin VI a GTP biosensor?, Biophys J. 82 (2002) 2737–2745.
[35] J. Bandorowicz-Pikula, A. Kirilenko, R. van Deursen, et al., A putative consensus sequence for the nucleotide-binding site of annexin A6, Biochemistry 42 (2003) 9137–9146.
[36] A. Kirilenko, S. Pikula, J. Bandorowicz-Pikula, Effects of mutagenesis of W343 in human annexin A6 isoform 1 on its interaction with GTP: nucleotide-induced oligomer formation and ion channel activity, Biochemistry 45 (2006) 4965–4973.
[37] P. Podszywalow-Bartnicka, A. Strzelecka-Kiliszek, J. Bandorowicz-Pikula, S. Pikula, Calcium- and proton-dependent relocation of annexin A6 in Jurkat T cells stimulated for interleukin-2 secretion, Acta Biochim. Pol. 54 (2007) 261–271.
[38] A. Strzelecka-Kiliszek, M. Buszewska, P. Podszywalow-Bartnicka, et al., Calcium- and pH-dependent localization of annexin A6 isoforms in Balb/3T3 fibroblasts reflecting their potential participation in vesicular transport, J. Cell. Biochem. 104 (2008) 418–434.
[39] J. Bandorowicz-Pikuła, A. Wrzosek, S. Pikuła, Y.C. Awasthi, Fluorescence spectroscopic studies on interactions between liver annexin VI and nucleotides–a possible role for a tryptophan residue, Eur. J. Biochem. 248 (1997) 238–244.
[40] C. Raimbault, C. Perraut, C. Vial, R. Buchet, Nucleotide binding sites in wild-type creatine kinase and in W227Y mutant probed by photochemical release of nucleotides and infrared difference spectroscopy, Eur. J. Biochem. 250 (1997) 773–782.
[41] C. Raimbault, C. Leydier, C. Vial, R. Buchet, ADP-binding and ATP-binding sites in native and proteinase-K-digested creatine kinase, probed by reaction-induced difference infrared spectroscopy, Eur. J. Biochem. 247 (1997) 1197–1208.
[42] D.S. Burz, K. Dutta, D. Cowburn, A. Shekhtman, Mapping structural interactions using in-cell NMR spectroscopy (STINT-NMR), Nat. Methods 3 (2006) 91–93.
[43] K.E. Kövér, P. Groves, J. Jiménez-Barbero, G. Batta, Molecular recognition and screening using STD NMR: ^{15}N-group selective STD NMR experiment to study intermolecular interactions in heavily overlapped spectra, J. Am. Chem. Soc. 129 (2007) 11579–11582.