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ATP selective acridone based fluorescent probes for monitoring of metabolic events†

Jatinder Kaur and Palwinder Singh*

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Acridones carrying an appropriate substituent at N-10 showed significant fluorescence changes on interacting with ATP in HEPES buffer at pH 7.2. The selectivity and sufficient binding of these probes with ATP could be useful for monitoring of metabolic processes.

Fluorescence signalling is an important phenomenon for the monitoring of biological processes through selective recognition of a crucial component of the process. In fact this is the primary step towards the development of drugs, probes, molecular machines, *etc*. Amongst various biological anions, adenosine triphosphate (ATP)[1] is the energy currency of the cell and various cellular processes are linked to the interchange between ATP and adenosine diphosphate (ADP). To name a few, the glycolytic pathway of glucose metabolism,[2] Kreb's cycle,[3] conversion of ribose sugar to deoxyribose sugar (raw material for DNA synthesis),[4] transporting activities of proteins,[5] working of the Na^+/K^+ pump[3] are the fundamental activities of life involving generation/consumption of ATP. The monitoring of these activities genuinely helps in exploring the working of natural systems and secrets of life.[6] The fluorogenic sensing and quantitative measurement of ATP could be a versatile method for studying the metabolic pathways. Irrespective of a number of chromogenic as well as fluorogenic sensors of ATP[7] there are few reports where a molecule could selectively interact with ATP and only one commercialized 'luciferin–luciferase bioluminescence assay' for ATP determination is available.[8] Here, working under aqueous conditions, the new molecules selectively interact with ATP and have successfully been demonstrated for the monitoring of two metabolic events and hence an issue of biological and medicinal importance was addressed.

The design of new molecules was based on our previous reports[9] concerned with the development of multi-drug resistance modulators.[10] Those molecules (**A**, Chart 1) inhibit the efflux of R6G from the cell and seemed to work through the inhibition of the P-glycoprotein efflux pump[11] (constituted by P-gp, ATP and Mg^{2+}). It was envisaged that increasing the length of the N-10 substituent of acridone and introducing more H-donor/acceptor sites thereon might result in their better interactions with ATP. Compounds **1–3** (Chart 1) were prepared by the reaction of acridone with epichlorohydrin followed by ring opening with benzyl-/*o*-aminobenzyl-/*p*-aminobenzylamine (Scheme S1, Fig. S1–S3, ESI†).

The UV-visible spectrum of compound **1** at 50 μM concentration in HEPES buffer (pH 7.2) exhibited absorption bands at 253, 387 and 405 nm. Addition of 0–3 equivalents of ATP to the above solution of compound **1** resulted in significant decrease in absorbance at 253 nm indicating interactions of this compound with ATP (Fig. S4, ESI†). Similar changes in the absorption spectra of compounds **2** and **3** (Fig. S5 and S6, ESI†) were observed on incremental addition of ATP. The fluorescence spectrum of a solution of compound **1** (0.1 μM, Φ = 0.67) in HEPES buffer (pH 7.2) showed emission bands at 417 nm and 440 nm when excited at 253 nm. Upon incremental addition of ATP (0–17 equiv.) (0–1.7 μM) to the solution of compound **1**, the fluorescence gets quenched (97.5%) at 417 nm as well as at 440 nm (Fig. 1). The Benesi–Hildebrand[12] plot indicated 1 : 1 stoichiometry of **1**·ATP complex (Fig. S7, ESI†). Similar emission bands and quenching of fluorescence were observed in the fluorescence spectra of compounds **2** (Φ = 0.60) and **3** (Φ = 0.66) upon incremental addition of ATP (Fig. S8 and S9; Table S1, ESI†).

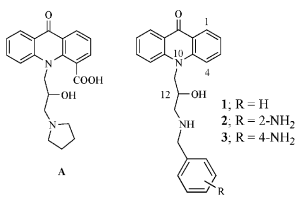


Chart 1

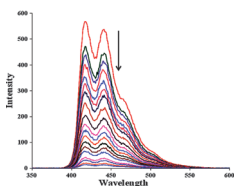


Fig. 1 Fluorescence spectra of **1** (0.1 μM) on incremental addition of ATP.

Department of Chemistry, Guru Nanak Dev University, Amritsar-143005, India. E-mail: palwinder_singh_2000@yahoo.com; Fax: +91 183 2258819; Tel: +91 183 2258802x3495

† Electronic supplementary information (ESI) available: Synthetic procedure, experimental data and NMR spectra of compound **1–3**, fluorescence spectra for selectivity and competitive binding of compounds **1–3** with ATP. See DOI: 10.1039/c1cc10253b

The change in the UV spectra as well as in the fluorescence quenching of these compounds on addition of ATP seems to be due to their π–π and electrostatic interactions with ATP.

To look into the probable mode of binding of compounds **1–3** with ATP, ^{1}H NMR titrations of these compounds upon addition of ATP were carried out. ^{1}H NMR spectra of compounds **1–3** (DMSO + D_2O) in the presence of 3 equiv. of ATP showed a downfield shift by 0.13–0.25 ppm of two CH_2 groups attached to NH, 0.05–0.13 ppm of H attached to C-12 and 0.094–0.116 ppm of the aromatic protons (Fig. S10–S12; Table S2, ESI†). It seems as ATP is overlapping (Fig. S13, ESI†) compound **1** with polar interactions between the two tails (a substituent at N-9 of adenine and N-10 of acridone) and π–π interactions between the head groups (adenine and acridone), photoinduced electron transfer occurs between adenine and acridone fragments resulting in quenching of fluorescence. No such overlapping in the energy minimized structures of **1** with ADP and AMP was observed. The binding constants of compounds **1–3** for ATP ($6–8 \times 10^7$ M^{-1}) are significantly better than the reported ones[7a,b,f] (Table S3, ESI†).

In contrast to the response of compounds **1–3** to ATP, addition of adenosine, AMP, ADP, GDP, IDP, CDP, UDP, GTP, ITP, CTP, and UTP separately to the solutions of compounds **1–3** resulted in no change in their fluorescence spectra pointing towards the selective recognition of ATP (amongst its homologues/analogues) by compounds **1–3** (Fig. 2). This indicates that the nucleobase as well as the phosphate group(s) contribute to interaction between the compound and nucleotide.

Moreover, to check the practical applicability of compounds **1–3**, their competitive binding with ATP, in the presence of homologues/analogues of ATP, was studied which showed that these compounds bind to ATP even in the presence of ADP, AMP, adenosine, GTP, GDP, ITP and IDP (Fig. 3; Fig. S14 and S15, ESI†).

Therefore, with the acridone derivatives **1–3**, working under aqueous conditions, 94–97% quenching of fluorescence was observed on addition of ATP, which along with their selectivity for ATP could be utilized for the monitoring/controlling of cellular processes involving generation/consumption of ATP. Here we demonstrated how an acridone derivative is utilized for *in vitro* real time monitoring of fluorescence in response to the generation and consumption of ATP in two enzymatic reactions.

First enzymatic reaction (last step of the payoff phase of glucose metabolism) is the phosphate transfer reaction, involving the transfer of phosphate from phosphoenolpyruvate to ADP

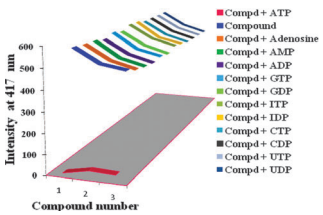


Fig. 2 Selectivity of compounds **1–3** for ATP amongst adenosine, AMP, ADP, GDP, IDP, CDP, UDP, GTP, ITP, CTP and UTP.

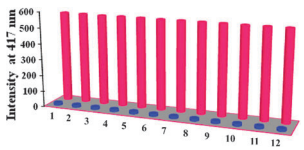
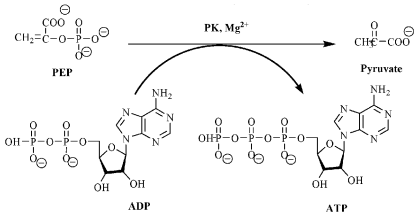


Fig. 3 Fluorescence response of compound **1** to various homologues/analogues of ATP. Pink bars: (1) **1**, (2) **1** + adenosine, (3) **1** + AMP, (4) **1** + ADP, (5) **1** + GTP, (6) **1** + GDP, (7) **1** + ITP, (8) **1** + IDP, (9) **1** + CTP, (10) **1** + CDP, (11) **1** + UTP, (12) **1** + UDP. Blue bars represent subsequent addition of 17 equivalents of ATP to the solution (1) **1** + ATP, (2) **1** + adenosine + ATP, (3) **1** + AMP + ATP, (4) **1** + ADP + ATP, (5) **1** + GTP + ATP, (6) **1** + GDP + ATP, (7) **1** + ITP + ATP, (8) **1** + IDP + ATP, (9) **1** + CTP + ATP, (10) **1** + CDP + ATP, (11) **1** + UTP + ATP, (12) **1** + UDP + ATP.

and the conversion of phosphoenolpyruvate (PEP) to pyruvate in the presence of pyruvate kinase (PK) and Mg^{2+} coupled with the transformation of ADP into ATP (Scheme 1).

A modeled reaction was carried out in HEPES buffer at pH 7.2 and compound **1** was used as fluorescent probe. ADP, PEP and PK did not exhibit fluorescence either individually or in combination with one another (overlapped green, violet, maroon traces; inset of Fig. 4). Compound **1** (0.1 μM) in combination with ADP and PEP exhibited usual fluorescence when excited at 253 nm (uppermost red trace, Fig. 4). Addition of PK and Mg^{2+} to the above solution (compound **1** + ADP + PEP) resulted in quenching of fluorescence (Fig. 4). Further increase of concentration of ADP in this solution resulted in a stepwise quenching of fluorescence in the same pattern as qthe quenching of fluorescence of compound **1** on incremental addition of ATP (Fig. 1). Therefore compound **1** was employed to monitor the transformation of PEP into pyruvate, accompanied with the efficient conversion of ADP to ATP, which resulted in quenching of fluorescence in a time-dependent manner (Fig. 5).



Scheme 1 Enzymatic reaction involving formation of ATP.

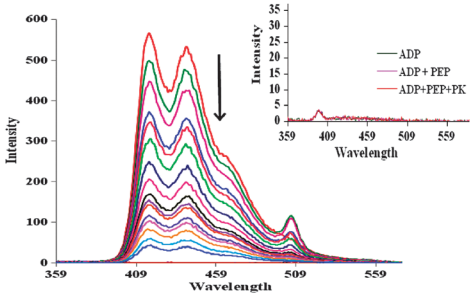


Fig. 4 Fluorescence change of compound **1** in a phosphate transfer reaction catalyzed by enzyme PK in 0–90 min. The inset shows the expanded part: ADP (green line), ADP + PEP (violet line), ADP + PEP + PK (maroon line).

The reaction rate was accelerated by increasing the concentration of ADP. The Lineweaver–Burk plot (Fig. 6) gave a Michaelis constant (K_m) of 249 μM which agrees with the reported one.[13]

The first step of the preparatory phase of glucose metabolism, involving phosphorylation of glucose in the presence of hexokinase (HK) and Mg^{2+} coupled with the conversion of ATP to ADP (Scheme 2), was also monitored using compound **1**. Here, reverse of Scheme 1 occurred, the non-fluorescent solution of ATP, glucose and compound **1** gains about 50% fluorescence intensity (w.r.t. the pure compound) upon addition of hexokinase in a time dependent manner indicating the conversion of ATP to ADP (Fig. 7 and 8).

Kinetic analysis indicated that the initial rate of reaction is proportional to the amount of HK which is based on first-order reaction kinetics (Fig. 9).

Therefore, we have developed ATP selective acridone based fluorescent probes workable under physiological conditions and through simple experiments, their use for the monitoring of enzymatic reactions was studied. This paves the way for their more applications in studying the metabolic pathways involving generation/consumption of ATP.

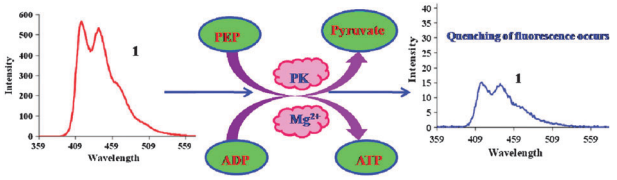


Fig. 5 Representation of fluorescence intensity change of compound **1** in a phosphate transfer enzyme reaction.

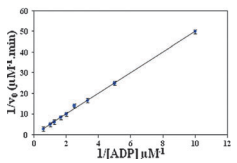
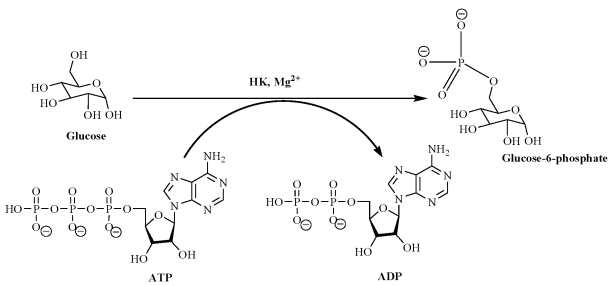


Fig. 6 Lineweaver–Burk plot of a PK mediated enzyme reaction.



Scheme 2 Enzymatic reaction involving formation of ADP.

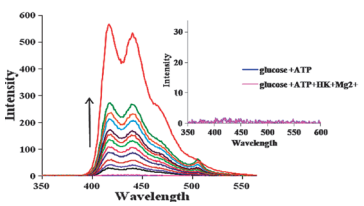


Fig. 7 Fluorescence change of compound **1** (red line) in a phosphorylation reaction catalyzed by enzyme HK in 0–50 min. The inset shows glucose + ATP (overlapped blue line), glucose + ATP + HK + Mg^{2+} (overlapped pink line).

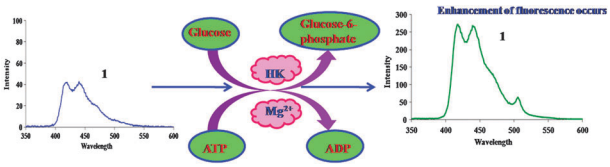


Fig. 8 Representation of fluorescence intensity change of compound **1** in a phosphorylation reaction.

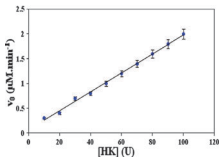


Fig. 9 Plot of the initial rate (v_0, μM min^{-1}) as a function of the amount of HK.

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Notes and references

1 H. Dugas, in *Bioorganic Chemistry*, Montreal, 1995, 3rd edn, ch. 3, pp. 116–122.

2 A. L. Lehninger, D. L. Nelson and M. M. Cox, in *Principles of Biochemistry*, Medison, 1992, 2nd edn, ch. 14, pp. 400–414, pp. 288–291.

3 D. Voet and J. G. Voet, in *Biochemistry*, Hoboken, NJ, 2004, 3rd edn, ch. 21, pp. 765–796.

4 J. Stubbe, *J. Biol. Chem.*, 1990, **265**, 5329–5332.

5 P. D. W. Eckford and F. J. Sharom, *Chem. Rev.*, 2009, **109**, 2989–3011.

6 (*a*) M. Ikeda, R. Ochi, A. Wada and I. Hamachi, *Chem. Sci.*, 2010, **1**, 491–498; (*b*) L. D. Lavis, T.-Y. Chao and R. T. Raines, *Chem. Sci.*, 2011, **2**, 521–530.

7 (*a*) P. P. Neelakandan, M. Hariharan and D. Ramaiah, *Org. Lett.*, 2005, **7**, 5765–5768; (*b*) A.-S. Delepine, R. Tripier and H. Handel, *Org. Biomol. Chem.*, 2008, **6**, 1743–1750; (*c*) Z. Xu, N. J. Singh, J. Lim, J. Pan, H. N. Kim, S. Park, K. S. Kim and J. Yoon, *J. Am. Chem. Soc.*, 2009, **131**, 15528–15533; (*d*) A. Ghosh, A. Shrivastav, D. A. Jose, S. K. Mishra, C. K. Chandrakanth, S. Mishra and A. Das, *Anal. Chem.*, 2008, **80**, 5312–5319; (*e*) F. Sancenon, A. B. Descalzo, R. Martinez-Manez, M. A. Miranda and J. Soto, *Angew. Chem., Int. Ed.*, 2001, **40**, 2640–2643; (*f*) Y. Kurishita, T. Kohira, A. Ojida and I. Hamachi, *J. Am. Chem. Soc.*, 2010, **132**, 13290–13299; (*g*) M. Schaferling and O. S. Wolfbeis, *Chem.–Eur. J.*, 2007, **13**, 4342–4349; (*h*) A. J. Moro, P. J. Cywinski, S. Korstena and G. J. Mohr, *Chem. Commun.*, 2010, **46**, 1085–1087; (*i*) H. Abe, Y. Mawatari, H. Teraoka, K. Fujimoto and M. Inouye, *J. Org. Chem.*, 2004, **69**, 495–504; (*j*) M. T. Albelda, J. C. Frias, E. Garcia-Espana and S. V. Luis, *Org. Biomol. Chem.*, 2004, **2**, 816–820.

8 Cat # A22066, Product Name: ATP Determination Kit *special packaging* *200-1000 assays*, *Molecular Probes*, Invitrogen detection technologies.

9 (*a*) P. Singh, J. Kaur, P. Kaur and S. Kaur, *Bioorg. Med. Chem.*, 2009, **17**, 2423–2427; (*b*) P. Singh, J. Kaur, B. Yadav and S. S. Komath, *Bioorg. Med. Chem.*, 2010, **18**, 4212–4223.

10 (*a*) E. Teodori, S. Dei, S. Scapecchi and F. Gualtieri, *Farmaco*, 2002, **57**, 385–415; (*b*) A. H. Dantzig, K. L. Law, J. Cao and J. J. Starling, *Curr. Med. Chem.*, 2001, **8**, 39–50.

11 P. D. W. Eckford and F. J. Sharom, *Chem. Rev.*, 2009, **109**, 2989–3011.

12 J. H. Hildebrand and H. A. Benesi, *J. Am. Chem. Soc.*, 1949, **71**, 2703–2705.

13 D. Balinsky, E. Cayanis and I. Bersohn, *Biochemistry*, 1973, **12**, 863–870.

