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COMMUNICATION

Chemical communication of enzymatic ATP hydrolysis using a fluorescent chemosensor

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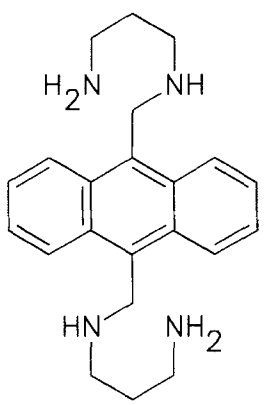
An anthrylpolyamine chemosensor, whose fluorescence is partially quenced upon electrostatic complexation to ATP, can be used to monitor the action of an enzyme (apyrase) that hydrolyses ATP to AMP and inorganic phosphate.

INTRODUCTION

The hydrolysis of ATP can be monitored in several different ways. The use of radiolabelling,[1] coupled enzyme assays,[2] spectrophotometry (UV absorption of a colour complex with molybdenum blue),[3] and chromatographic methods[4] have been described. Of course, because of the enormous variety of processes that are energetically coupled to the hydrolysis of ATP, there is continuing interest in finding convenient methods for the monitoring of this important reaction. Lehn *et al.*[5] have described an acridine derivative whose fluorescence is modulated upon binding to ATP. Our previous findings of chelation-enhanced and chelation-quenced fluorescence upon the interactions of anthrylmethylpolyamines with monomeric[6a] and polymeric[6b] anions led us to consider the use of such compounds for monitoring the hydrolysis of a monomeric polyanion. We now report that the enzymatic hydrolysis of ATP to AMP can be chemically communicated using a fluorescent chemosensor.



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Anthrylpolyamine **1**, which shows a large chelation enhanced fluorescence quenching upon binding to ATP, was used to follow the hydrolysis reaction. Solutions were prepared containing 1 μM chemosensor **1** and 750 μM ATP in 0.1 M NaOAc buffer (pH 5) with 0.05 mM EDTA. At this pH, both benzylic amines are almost completely protonated so that fluorescence is not quenched[7] (Fig 1).

Anthrylmethylpolyamine **1** was prepared as follows: A solution of 9,10-bis(chloromethyl)anthracene (600 mg, 2.2 mmol) and 1,3-diaminopropane (3.0 ml, 40 mmol) in toluene (60 ml) was heated at reflux for 3 days. The cooled mixture was washed with 6M NAOH (2 × 50 ml) then extracted with 1M HCl (50 ml). The acidic layer was made basic with NaOH and extracted with $CHCl_3$ (100 ml). The organic layer was dried over Na_2CO_3, evaporated, and the residue was dissolved in ethanol (20 ml); treatment with conc. HCl (1.0 ml) gave a yellow precipitate (742 mg, yield 68%). ^{1}H-NMR (D_2O) δ 1.94–2.06 (m, 4, aliph. CH_2), 2.98 (t, 4, aliph. CH_2), 3.23 (t, 4, aliph. CH_2), 7.68 (dd, 4, Ar-H), 8.03 (dd, 4, Ar-H): ^{13}C-NMR (D_2O) δ 23.623 (t), 36.515 (t), 42.572 (t), 45.089 (t), 123.864 (d), 124.332 (s), 127.727 (d), 129.425 (s). FAB mass spectrum, *m/e* 351 (M^+). High resolution mass spectrum on free amine, *m/e* calculated for $C_{22}H_{31}N_4$ $(M+H)^+$, 351.2549; measured, 351.2540.

ATP and apyrase type VII were purchased from Sigma Chemical Company and used as received. A stock solution of the enzyme was prepared in 0.1 M

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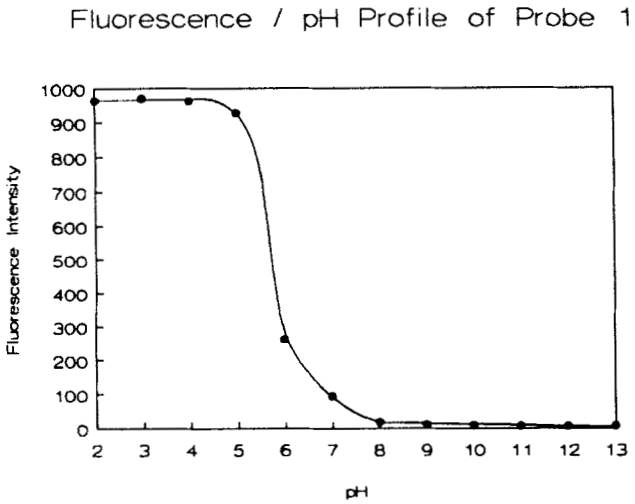

Figure 1 Fluorescence/pH profile of probe 1.

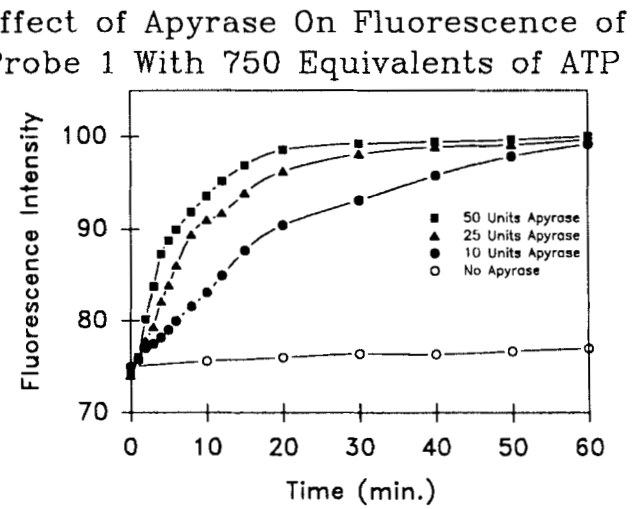

Figure 2 Effect of apyrase on fluorescence of probe 1 with 750 equivalents of ATP.

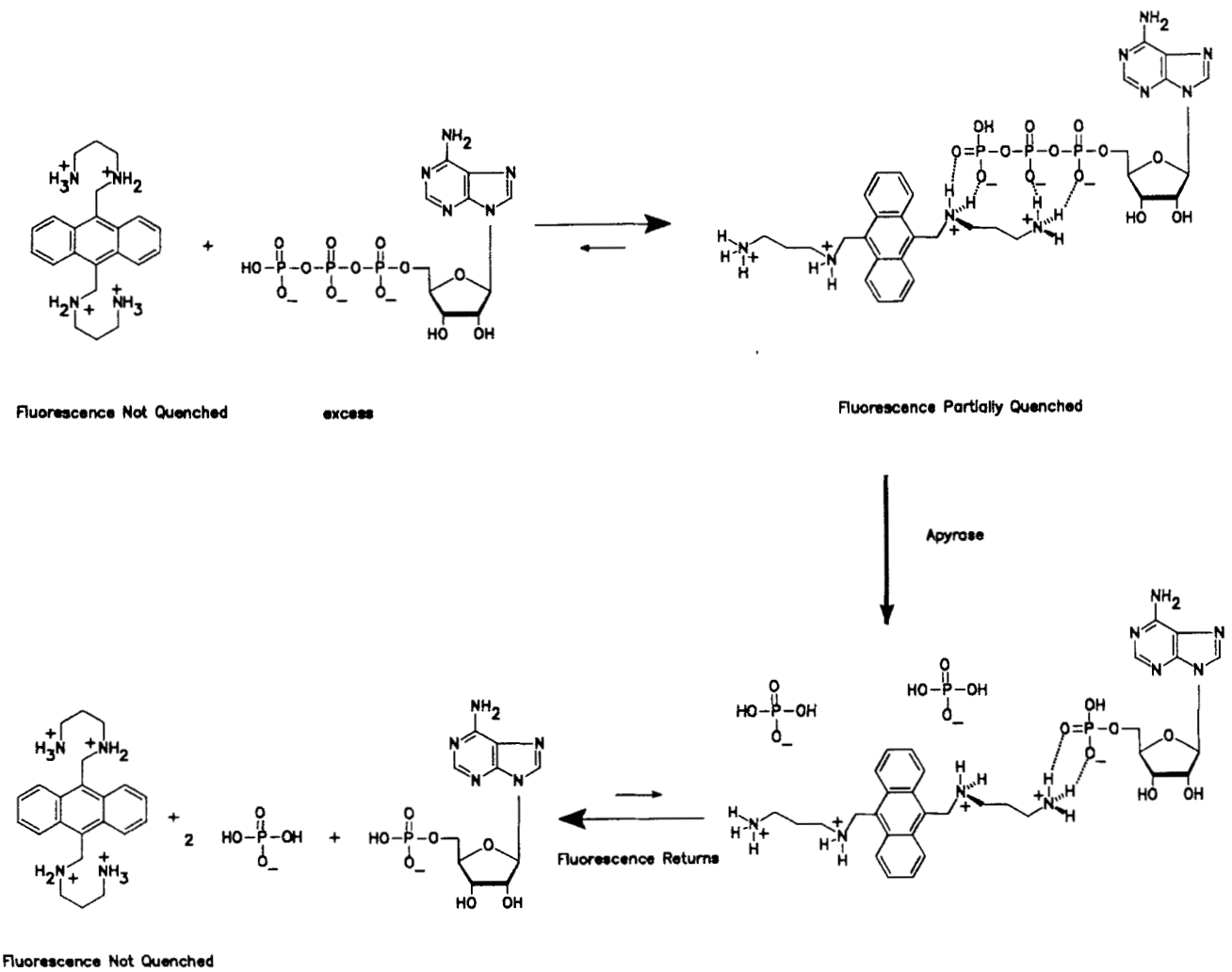

Figure 3 Proposed mechanism of action of chemosensor.

NaOAc buffer (pH 5) with 0.05 mM EDTA for delivery to the fluorophore/substrate sample.

Upon the introduction of ATP, the fluorescence of chemosensor **1** is partially quenched as a result of electrostatic association between the ATP and the protonated benzylic amine of the chemosensor, which serves to increase electron density (and therefore the intramolecular quenching ability) of nitrogen. Other chemosensors we have synthesized are not as highly protonated at the benzylic position at pH 5 and consequently do not show a large fluorescence change upon complexation with ATP.[6b] Addition of apyrase, a commercially available enzyme that sequentially hydrolyses ATP to AMP and 2 equivalents of orthophosphate,[8] resulted in a time dependent fluorescence enhancement as shown in Figure 2.

That the observed fluorescence change corresponds to dephosphorylation was corroborated by co-monitoring the hydrolysis using thin-layer chromatography. A modified TLC system of Bieleski and Young[4] showed the fluorescence increase to coincide with the formation of ADP and AMP. A reaction with 10 units of apyrase monitored by TLC showed that only AMP remained 90 min after introduction of the enzyme.

One rationale for the physical basis of this assay is shown in Figure 3. At the outset of the experiment a population of the chemosensor is bound electrostatically (via hydrogen bonding) to the nucleotide; such interaction of ATP with protonated polyamines has been reported previously.[9] Hydrogen bonding fractionally frees up the lone pair on the benzylic nitrogen, resulting in partial quenching of the chemosensor fluorescence. Addition of apyrase to the system results in cleavage of the ATP to AMP and orthophosphate. These anions have lower charge densities than ATP and therefore do not bind polycations as tightly.[9] Consequently, the higher concentration of unbound chemosensor results in enhanced fluorescence intensity.

In summary, we report that a synthetic chemosensor with polyanion binding characteristics communicates ATP hydrolysis with fluorescence signalling. Because the analytical tool is not consumed during the reaction, this approach to real-time assay is distinguished from reagent-based signal transduction schemes.

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