Caged FEDA-ATP: a new tool in the measurement of ATP turnover during the in vitro motility assay.

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In an attempt to distinguish between the various models of muscle contraction, attention has been focused upon calculation of the myosin crossbridge step-size (the distance moved during active interaction of a myosin head with actin per ATP molecule hydrolysed) using in vitro motility assays. We have previously used a fluorescein derivative of ATP, FEDA-ATP as a potential probe for nucleotide turnover under in vitro assay conditions [1]. Although an array of myosin heads can be visualised in the presence of FEDA-ATP by fluorescence microscopy, only low concentrations (50 nM) of the latter can be used, so as to minimise the background signal from the free fluorophore. Total internal reflectance fluorescence microscopy offers one possibility around this problem [2]. An alternative strategy is to cage the fluorescein [3] to make a non-fluorescent derivative which can be photoactivated in localised regions containing the array of myosin heads. Any free fluorophores activated in this process are expected to diffuse from the site of interest within several milliseconds.

We have prepared a non-fluorescent bis-caged derivative of FEDA-ATP by reacting the succinimidyl ester of carboxyfluorescin-bis-(4,5-dimethoxy-2-nitrobenzyl) ether (Molecular Probes Inc., Oregon, U.S.A.) with the 2',(3) ethylenediamine derivative of ATP (Fig. 1). The latter (EDA-ATP) was prepared as described previously [1]. Such a caged FEDA-ATP is a substrate for rabbit skeletal myosin subfragment 1.

Caged FEDA-ATP quenches tryptophan fluorescence allowing the binding and turnover steps to be monitored by stopped-flow methods (Fig. 2). As expected, fluorescence emission at the fluorescein wavelengths is close to background levels. Irradiation of caged FEDA-ATP with 360 nm light releases a product with comparable properties to FEDA-ATP (note because of the use of a

Fig. 1 The structure of caged FEDA-ATP

succinimidyl ester rather than isothiocyanate to link to the amine of EDA-ATP, a ureido rather than thioureido group is present in the photoactivated product). The irradiated preparation shows a quench in fluorescent emission on binding to myosin subfragment 1, followed by a recovery corresponding to the product release phase.

This is similar to FEDA-ATP itself [1].

As a preliminary to the photoactivation of caged FEDA-ATP when bound to myosin molecular arrays, it is desirable to produce a long-lived myosin products complex to allow optimisation of the microbeam optics. We have therefore explored the formation of the M.FEDA-ADP.PiF2 complex using PiF2 as a phosphate analogue [4]. Addition of 0.1 mM AlCl3 and 5 mM NaF to stoichiometric amounts of rabbit myosin subfragment 1 and FEDA-ADP produced a 60% quench in fluorescein fluorescence.