

A Fluorimetric Assay for Real-time Monitoring of Adenylyl Cyclase Activity Based on Terbium Norfloxacin

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Abstract

Adenylyl cyclases catalyze the production of the second messenger cyclic AMP from ATP. Until now, there is no fluorescent adenylyl cyclase assay known which is applicable to high throughput screening and kinetic determinations which can directly monitor the turnover of the unmodified substrate ATP. In this study, a fluorescence based assay is described using the Ca(II)- and calmodulin-dependent adenylyl cyclase edema factor from *Bacillus anthracis* and Tb(III)-norfloxacin as probe for the enzyme activity. This assay can be utilized to study enzyme regulators, allows real-time monitoring of adenylyl cyclase activity and does not substitute ATP by fluorescent derivatives. These derivatives have to be judged critically due to their interference on the activity of enzymes and their specificity towards inhibitors. Furthermore, the new assay makes the application of radioactively labeled substrates such as [α - 32 P]ATP or fluorescently labeled antibodies like anti-cyclic AMP redundant. We determined the Michaelis-Menten-constant K_M , the v_0^{\max} -value of ATP turnover as well as IC_{50} values for three inhibitors of EF by this newly developed fluorescent method.