Operational stability and ATP regeneration performance of PPK-2-III

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Introduction

Biocatalysis enables sustainable synthesis of target products under mild reaction conditions, reducing energy consumption by avoiding high temperatures and pressures. [1] Operational stability depicts the ability of an enzyme to maintain its activity during the reaction. For industrial application, enzyme operational stability is a crucial parameter affecting process efficiency, productivity and overall economics. [2]

ATP is considered the universal energy carrier in living cells. It is used as a coenzyme in numerous biocatalytic reactions, but as a substrate it is costly. Therefore, an ATP regeneration step is necessary to enable cost-effective production. [3] PPK enzymes can be used for ATP regeneration during organic synthesis. The enzyme investigated in this work is polyphosphate kinase (PPK-2-III), which catalyses the conversion of AMP to ATP (Figure 1). PPK enzymes are classified into two subclasses, PPK-1 and PPK-2, based on the nucleotide they generate: ADP and ATP respectively. [4] The aim of this work is to investigate operational stability of PPK-2-III and its performance in batch reactors for ATP regeneration in biocatalytic synthesis.

Batch reactor experiments and operational stability measurements were done at two different temperatures (25 and 37 °C) and in two different buffer systems (50 mM MES and 50 mM KPi buffer, pH 7.5).

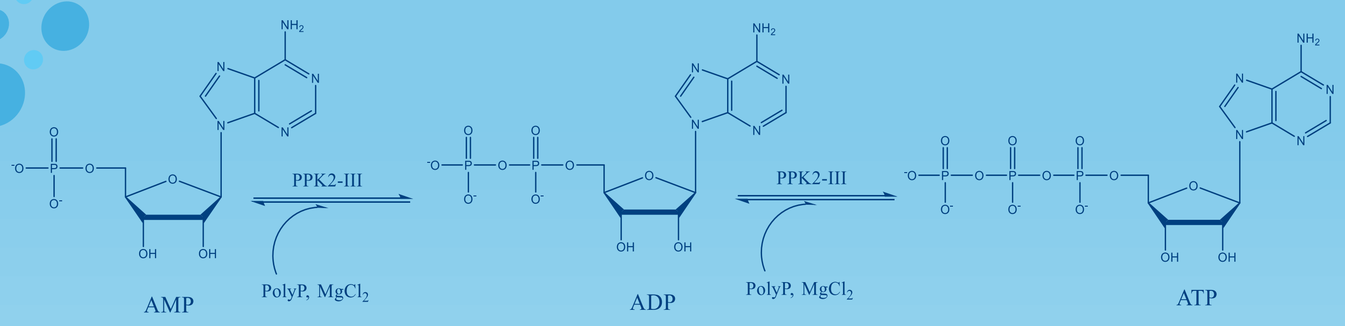
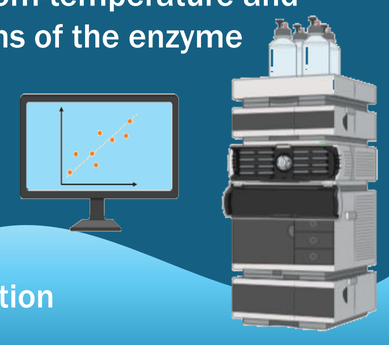


Figure 1. PPK2-III-catalyzed reaction of AMP phopshorylation scheme

Objectives

Investigating:

- PPK-2-III-catalyzed ATP synthesis in the batch reactor
- Operational stability of PPK-2-III at two conditions: 50 mM MES buffer, pH 7.5 at 25 °C and 50 mM KPi (phosphate) buffer, pH 7.5 at 37 °C to enable comparison of the stability at room-temperature and physiologically relevant conditions of the enzyme



Methodology

- HPLC for concentration determination
- MicroMath Scientist for parameter estimation

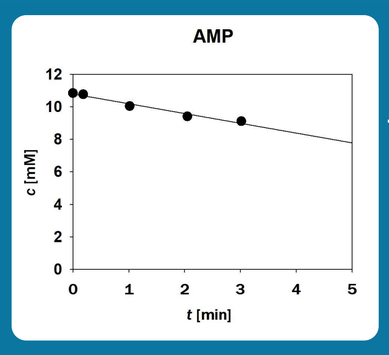
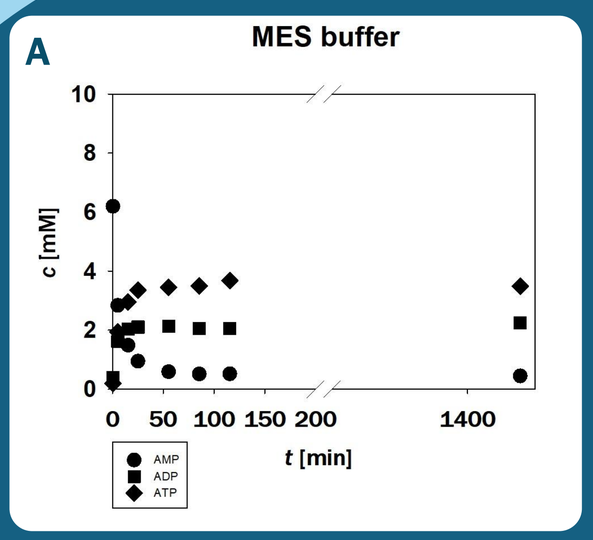


Figure 2. Example of an experiment for determining PPK-2-III activity in the reaction of ATP synthesis. Enzyme volume and specific activity was calculated according to Eqs. 1 and 2.

$$V.A. = \frac{\frac{dA}{dt} \cdot V_R}{V_E} \quad \text{Eq. 1}$$

$$S.A. = \frac{V.A.}{\gamma} \quad \text{Eq. 2}$$

Results



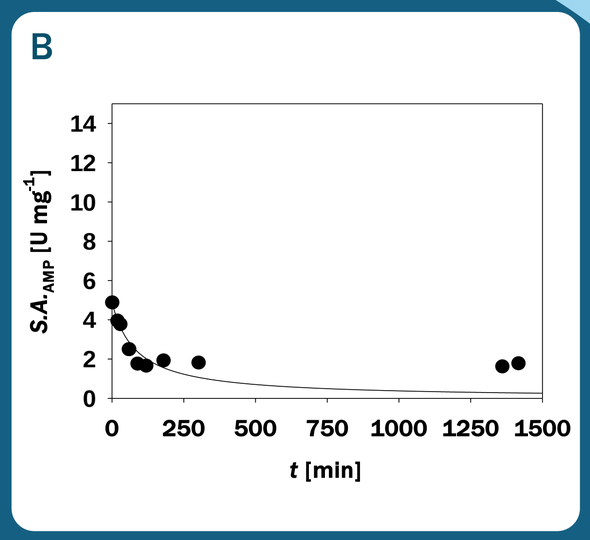
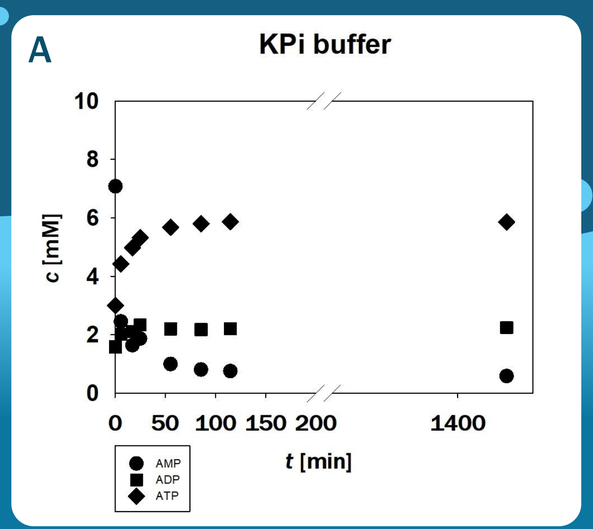


Figure 3. A) Batch reaction of ATP synthesis in the reaction catalyzed by PPK-2-III (50 mM MES buffer, pH 7.5, 25 °C, 5 mM AMP), B) PPK-2-III specific activity change during reaction



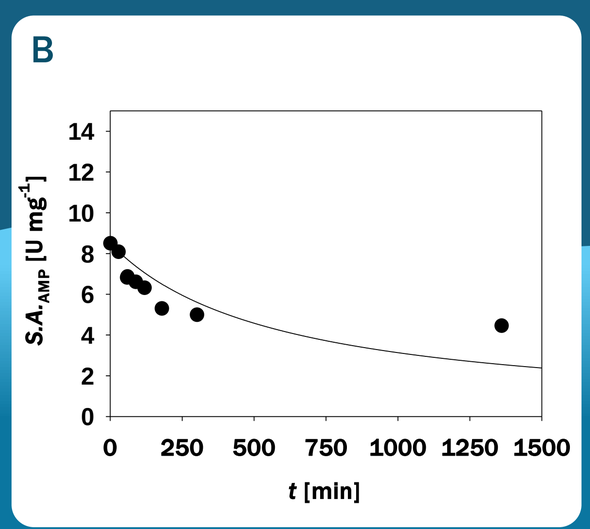


Figure 4. PPK-2-III operational stability in 50 mM KPi buffer, pH 7.5, 37 °C, 5 mM AMP A) batch reactor, B) PPK-2-III specific activity change during reaction

- Operational stability decay rate constants (k_d) were estimated from the data shown in Figures 3 and 4 by using first-order kinetics (Eq. 3) and are shown in Table 1 together with the enzyme half-life time

$$\frac{dS.A.}{dt} = -k_d \cdot S.A. \quad \text{Eq. 3}$$

Table 1. Operational stability decay rate constants and enzyme half-life times in two different buffer systems

MES buffer, 25°C	KPi buffer, 37°C
k_d = 0.505 h^{-1}	k_d = 0.044 h^{-1}
$t_{1/2}$ = 1.98 h	$t_{1/2}$ = 22.61 h

Conclusions

- Operational stability, an important enzyme property, was investigated in this work. The results (Table 1.) show that KPi buffer is a better reaction medium than the MES buffer for PPK-2-III-cataylzed synthesis of ATP since it shows a better half-life time of the enzyme at higher temperature.

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