S-Adenosyl-L-homocysteine hydrolase from *Dictyostelium discoideum* is inactivated by cAMP and reactivated by NAD^+

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Purified *S*-adenosyl-L-homocysteine hydrolase from *Dictyostelium discoideum* is inactivated when incubated at 25°C with cAMP. Half maximal velocity of the inactivation process occurs at 10 μM cAMP. Catalytic activity is fully restored by further incubation with NAD^+, but not with $NADP^+$ or NADH. The enzyme must be preincubated with cAMP or NAD^+ to induce inactivation or reactivation, respectively, since neither of these ligands has an effect on the active or inactive enzyme when added directly to the assay. These results suggest a role for cAMP and NAD^+ in the regulation of cellular methylation reactions by altering the level of *S*-adenosyl-L-homocysteine via *S*-adenosyl-L-homocysteine hydrolase.

S-Adenosyl-L-homocysteine hydrolase *Cyclic AMP*
Methylation *NAD^+* *Differentiation* *Enzyme inactivation*

1. INTRODUCTION

Upon nutrient starvation, the ameobae *Dictyostelium discoideum* enter a developmental cycle in which vegetative cells differentiate into either stalk or spore cells, and form a fruiting body. cAMP is an important regulatory component in this process [1]. In searching for an intracellular target(s) of cAMP regulation, a number of cAMP binding proteins have been characterized in cytoplasmic extracts of *D. discoideum* at various stages of development [2–6]. One of these proteins has been identified as SAH hydrolase [7], an enzyme which catalyzes the reversible cleavage of SAH to adenosine and homocysteine [8].

SAH is a product, and potent inhibitor of transmethylases that utilize *S*-adenosyl-L-methionine as a methyl donor. In eukaryotes, the major metabolic pathway for SAH is via SAH hydrolase [9]. Therefore, by regulating the activity of SAH hydrolase, the cell can control the activity of various transmethylases. While SAH hydrolase from various species binds cAMP [10–12], no effect of cAMP on the enzyme has ever been reported. We describe here a cAMP-induced inactivation of SAH hydrolase which is reversed by NAD^+.

Abbreviations: SAH, *S*-adenosyl-L-homocysteine; SAH hydrolase, *S*-adenosyl-L-homocysteine hydrolase (EC 3.3.1.1); Mops, 3-(*N*-morpholino)propanesulfonic acid

2. MATERIALS AND METHODS

2.1. *Source of enzyme*

D. discoideum cells were grown at 22°C in HL-5 broth to a density of 5×10^6 cells/ml, and starved for 4 h as in [3]. SAH hydrolase was purified from 80 g (wet wt) of cells by a method that will appear elsewhere.

2.2. *Buffer and assay conditions*

Unless otherwise stated, the buffer for all experiments and SAH hydrolase activity assays was 25 mM Mops (pH 7.5) containing 20 mM NaCl.

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The enzyme was assayed at 25°C in the hydrolysis direction with slight modifications of the spectrophotometric method in [13]. The assay mixture contained: 975 μl buffer, 182 μl of 4.7 mM SAH, and 2 μl adenosine deaminase (Sigma A-9626, 2250 units/ml). To assay the enzyme, 50 μl assay mixture was added to 850 μl buffer, and the reaction was started by adding 5–10 μl enzyme. The conversion of SAH to inosine was followed at 265 nm, where the change in the extinction coefficient is -7.6×10^3 $M^{-1}\cdot cm^{-1}$. The assay was linear with respect to time and enzyme concentration. One unit of SAH hydrolase activity will hydrolyze 1 nmol SAH/min at 25°C.

Protein concentration was measured by the Coomassie blue method in [14] using bovine γ-globulin as a standard.

3. RESULTS AND DISCUSSION

Incubation of purified SAH hydrolase at 25°C in the presence of 30 μM cAMP results in inactivation of the enzyme (fig.1, ●). Inactivation is a first order process (until the enzyme is at least 50% inactivated) with a half life of 80 min. If incubated under the same conditions in the absence of cAMP (○), the enzyme is slightly inactivated and retains more than 90% of activity after 2 h. SAH hydrolase is stable at 0°C, in the presence or absence of cAMP (□,▲) and the inactivations observed at 25°C may be arrested at any time by placing the samples on ice.

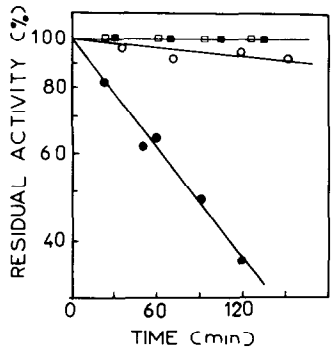

Fig.1. cAMP-dependent inactivation of SAH hydrolase. SAH hydrolase (22 units) wa incubated in a total volume of 0.1 ml in 25 mM sodium phosphate buffer (pH 6.5) in the absence (○,□) or presence (●,■) of 30 μM cAMP at either 0°C (□,■) or 25°C (○,●). At the times indicated, 5-μl aliquots were assayed as in section 2.

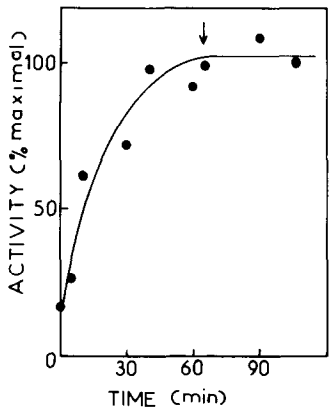

Fig.2. Reactivation of SAH hydrolase by NAD^+. SAH hydrolase (22 units) was incubated at 25°C in 0.1 ml of 25 mM sodium phosphate buffer (pH 6.5) containing 20 mM NaCl and 50 μM cAMP, until it was 85% inactivated. At $t=0$, the enzyme was diluted 2-fold into the same buffer where 25 μM NAD^+ had replaced cAMP. After incubation at 250°C for various times, 10-μl aliquots were assayed for SAH hydrolase activity.

Fig.2 shows that the addition of 25 μM NAD^+ to cAMP-inactivated enzyme leads to reactivation. Further addition of NAD^+ to reactivated enzyme does not result in additional increase in activity (arrow). The enzyme inactivated at 25°C in the absence of cAMP (fig.1,○) is not reactivated by NAD^+ (not shown) and therefore the two inactivation processes are different. Moreover, the presence of cAMP protects the enzyme from this instability at 25°C since the cAMP-inactivated enzyme can be completely reactivated by NAD^+.

Fig.3 shows the rate of inactivation of SAH hydrolase as a function of cAMP concentration.

The cAMP-dependent inactivation is not due to a hydrolytic product of cAMP since at the end of the incubation, analysis by thin-layer chromatography shows that none of the cAMP is converted to 5′-AMP or adenosine.

Inactivation of SAH hydrolase is specific for cAMP when compared with cGMP: whereas 50% of the activity is lost after 1.8 h in the presence of 100 μM cAMP, there is no loss of activity in the presence of 100 μM cGMP, even after 4 h at 25°C (not shown). Reactivation is specific for NAD^+. As shown in table 1, only NAD^+ can restore the activity of a previously cAMP-inactivated enzyme. NADH, $NADP^+$ or AMP are without effect.

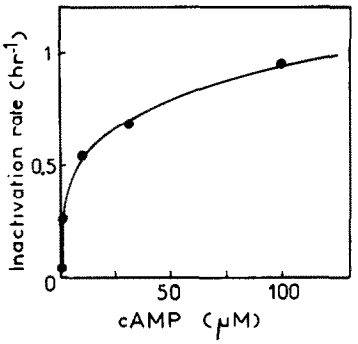

Fig.3. Rate of inactivation as a function of cAMP concentration. SAH hydrolase (220 units/ml) was incubated with various cAMP concentrations as in the experiment described in fig.1. Inactivation vs time was followed for each cAMP concentration, and the time required to achieve 50% inactivation was determined from a least squares analysis of the first order plots.

Both the loss of activity in the presence of cAMP and reactivation by NAD^+ require preincubation of SAH hydrolase with these ligands. Neither the K_m for SAH nor the V_{max} are affected by the presence of cAMP in the assay mixture. Moreover, addition of NAD^+ directly to the assay without preincubation, has no effect on either the native or cAMP-inactivated enzyme.

Reactivation of SAH hydrolase by nAD^+ is especially interesting in light of the proposed reaction mechanism for the enzyme [13], and the recent data that suggest that 2 of the 4 NAD^+ molecules per tetramer play a regulatory, rather than a catalytic role [15]. We are currently conducting experiments to determine if the enzyme-bound NAD^+ is reduced to NADH during inactivation, and if reactivation involves the NAD^+ at the active site or at a second regulatory site.

Table 1

Specificity of reactivation

Additions	Activity (units/ml)		
	$t=0$ h	$t=1.0$ h	$t=1.5$ h
NAD^+	13	219	201
NADH	13	14	11
$NADP^+$	13	16	20
5′-AMP	13	14	13

SAH hydrolase was incubated at 25°C with 0.1 mM cAMP until the residual activity was 10% of initial. At $t=0$, 5 μl of the cAMP-inactivated enzyme was added to tubes containing 850 μl of buffer and 5.8 μM of either NAD^+, NADH, $NADP^+$ or 5′-AMP, and incubated at 25°C. At the times indicates, 50 μl of assay mixture was added and SAH hydrolase activity was determined

The demonstration of a potential regulatory mechanism for SAH hydrolase suggests intriguing possibilities for regulating the various methylation reactions necessary for proper functioning of the cell. The K_i's of different transmethylases for SAH vary over a 1000-fold range [16], and it has been suggested that the ratio of K_m for *S*-adenosyl-L-methionine to the K_i for SAH sets the specific activity of a particular transmethylase [9]. Furthermore, the physiological effects of an increase in intracellular concentration of SAH are well documented and inhibition of SAH hydrolase may be involved in the regulation of *S*-adenosyl-L-methionine-dependent transmethylases [16–18].

cAMP has been shown to be intimately involved in post-aggregative gene expression in *D. discoideum* [19–21]. Furthermore, it has been recently demonstrated [22] that exogeneous cAMP can induce prestalk-specific gene expression in *D. discoideum*. A developmentally regulated cAMP-dependent protein kinase has been described in *Dictyostelium* [23,24] and proposed to be a target of cAMP regulation. Notwithstanding the fact that cAMP controlled phosphorylation is likely to be important for gene expression, it is tempting to speculate a role for cAMP-mediated control of SAH hydrolase in methylation-dependent cellular processes which may be involved in the developmental process.

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