Inactivation of S-adenosylhomocysteine hydrolase by 9-β-D-arabinofuranosyladenine (ara-A) in intact cells

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INTRODUCTION

The antibiotic, 9-β-D-arabinofuranosyladenine (ara-A), among other adenosine analogs, irreversibly inactivates S-adenosylhomocysteine (AdoHcy) hydrolase (EC 3.3.1.1.) (Chiang *et al.*,1981), the enzyme responsible for the metabolic degradation of the endogenous transmethylase inhibitor, AdoHcy. The inactivation of the enzyme in the presence of 2'-deoxyadenosine and ara-A obeys first-order kinetics, shows saturability and is associated with irreversible reduction of enzyme-bound NAD^+ (Hershfield, 1979; Helland & Ueland, 1981 a). ara-A and 2'-deoxyadenosine, as well as other adenosine analogs, have been shown to elevate the amount of AdoHcy in intact cells (Zimmerman *et al.*, 1980). The present report summarizes recent observations in our laboratory on the inactivation of AdoHcy hydrolase and disposition of AdoHcy in cells exposed to ara-A.

RESULTS AND DISCUSSION

Isolated rat hepatocytes and some cultured cell lines (mouse plasmacytoma MPC-11 cells, mouse fibroblasts L-929 cells and human chronic myelogenic leukemia K-562 cells) were used in these experiments. The kinetic characteristics of the inactivation of intracellular AdoHcy hydrolase show striking similarities to those previously reported for homogeneous enzyme (Helland & Ueland, 1981 b). Half-maximal rate of inactivation was observed at 12 μM of ara-A, and maximal rate of inactivation was 0.7 min^{-1}. Whereas the inactivation of purified enzyme proceeds to completion, a fraction of the intracellular enzyme (2-3% in hepatocytes, 0.6-2% in the cultured cells) is not available for inactivation, even after repetitive additions of ara-A to the cell suspension. Similar observations have been made by Hershfield and Kredich (1980) studying the inactivation of AdoHcy hydrolase by 2'-deoxy-

adenosine in human lymphoblasts. In a cellular medium devoid of adenosine deaminase, 2'-deoxycoformycin does not or only slightly enhances the inactivation of intracellular AdoHcy hydrolase, suggesting that intracellular deamination of ara-A is not a major factor limiting the short term effects of ara-A on AdoHcy hydrolase.

Inactivation of AdoHcy hydrolase by ara-A is associated with a massive buildup of intracellular AdoHcy which is particularly pronounced in the hepatocytes (from 50 to 3500 pmol/10^6 cells after 3 hours of exposure to ara-A). The cellular content of AdoMet increases slightly in liver cells, but remains unchanged in the cultured cells. Accumulation of intracellular AdoHcy leads to a marked egress of AdoHcy from the cells. After about 3 hours of exposure to ara-A, the amount of AdoHcy secreted into the extracellular medium is of the same order of magnitude as the amount retained within the cells. No secretion of AdoMet is observed under conditions of elevated cellular levels of this metabolite (induced by exposure of hepatocytes to repetitive additions of homocysteine). The relevance of these findings for the cellular handling of AdoHcy in vivo is underscored by the observation that high levels of AdoHcy are present in serum of mice injected with ara-A (Helland & Ueland, unpublished).

Both adenosine and high concentrations of homocysteine protect intracellular AdoHcy hydrolase in hepatocytes against inactivation by ara-A, and the effect of homocysteine is reflected by a parallel increase in AdoHcy content. Accumulation of AdoHcy induced by homocysteine also inhibits the slow phase of the uptake of ara-A, which may be explained by an effect on the metabolic component of the uptake process through inhibition of adenosine kinase by AdoHcy (Palella et al., 1980).

A progressive reactivation of intracellular AdoHcy hydrolase in hepatocytes could be demonstrated by supplementing the cellular medium with high levels of adenosine deaminase. Inosine and inhibitors of protein synthesis are without effect. The reappearance of the enzyme activity is not an artifact related to the consumption of inhibitors of the enzyme, as judged by the recovery of purified AdoHcy hydrolase added to the extraction buffer at various times during the reactivation process. Furthermore, the reactivation is associated with a marked reduction in cellular content of AdoHcy. Reactivation of intracellular AdoHcy hydrolase is inhibited by the adenosine deaminase inhibitor, 2'-deoxycoformycin.

Treatment of hepatocytes with adenosine deaminase reduces the cellular content of adenosine (from 20 to 7 pmol/10^6 cells), but also reduces the level of ara-A in the cells exposed to this agent. These effects are counteracted by the addition of 2'-deoxycoformycin. Thus, adenosine deaminase may uncover reactivation of AdoHcy hydrolase by trapping ara-A continously formed from ara-AMP. However, the following observations are not in agreement with this hypothesis. The half-life of the intracellular AdoHcy hydro-

lase-ara-A complex (90 minutes) is not affected by factors inhibiting the inactivation of the enzyme, suggesting that no rebinding of ara-A takes place following prolonged incubation of the hepatocytes in a medium devoid of ara-A. Furthermore reactivation is not induced by the addition of homocysteine to the cell suspension in spite of the fact that this compound protects the AdoHcy hydrolase against inactivation, and does not itself inactivate the enzyme. Alternatively, low cellular level of adenosine may create conditions favoring the reactivation process. This suggestion is supported by the observation that a synthetic substrate of the enzyme, 3-deazaadenosine (50 μM) nearly completely inhibits the reactivation of intracellular AdoHcy hydrolase. 3-Deazaadenosine alone does not inactivate the enzyme.

The existence of an intracellular mechanism reactivating AdoHcy hydrolase exposed to ara-A is not surprising in the light of the fact that naturally occurring purines (2'-deoxyadenosine and adenosine, Chiang et al., 1981) are inactivators of AdoHcy hydrolase. A mechanism reactivating AdoHcy hydrolase may be widely distributed in mammalian tissues. We have recently found that AdoHcy hydrolase in most tissues of mice injected with ara-A (without 2'-deoxycoformycin) shows nearly complete recovery 8-10 hours after a single injection of this compound (Helland & Ueland, unpublished).

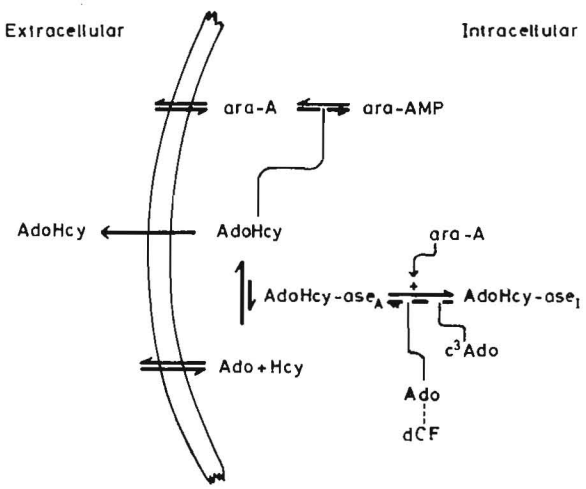

Fig. 1. ara-A and cellular disposition of AdoHcy

AdoHcy-ase, AdoHcy hydrolase$_3$(A, active; I, inactive); Ado, adenosine; Hcy, L-homocysteine; c^{3}Ado, 3-deazaadenosine; dCF, 2'-deoxycoformycin.

CONCLUSION

The inactivation of AdoHcy hydrolase in intact cells shows kinetic characteristics which resemble those observed with the isolated enzyme. However, the following features of the cellular response to ara-A cannot be derived from data with isolated enzyme.

1) A fraction of the intracellular enzyme is not available for inactivation, probably because of protection of the enzyme by metabolites.
2) Inactivation of AdoHcy hydrolase leads to a massive buildup of AdoHcy, which is exported into the extracellular medium.
3) High levels of intracellular AdoHcy may affect the cellular disposition of ara-A.
4) Intact cells possess a mechanism reactivating intracellular AdoHcy hydrolase.

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