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Hypothesis

How is rat liver *S*-adenosylmethionine synthetase regulated?

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The in vivo regulation of *S*-adenosylmethionine synthetase, a key enzyme in methionine metabolism, is so far unknown. The enzyme activity has been shown to be modulated by glutathione and the oxidation state of its sulfhydryl groups. Analysis of the protein sequence has revealed the presence of putative phosphorylation sites. A mixed regulatory mechanism combining phosphorylation and the oxido/reduction of sulfhydryl groups is proposed. The role of glutathione in this mechanism is also discussed.

S-Adenosylmethionine; Glutathione; Sulfhydryl group; Phosphorylation

1. INTRODUCTION

S-Adenosylmethionine (AdoMet) synthetase is the enzyme responsible for the synthesis of AdoMet using methionine and ATP as substrates [1]. Its importance is due to the fact that AdoMet is the main methyl donor group for most of the transmethylation reactions in the cell. As mentioned by Cantoni [2], no other biological compound, including ATP, participates in as many reactions as AdoMet. Moreover, AdoMet synthesis is the main pathway for methionine metabolism in the liver [3], where 6–8 g of AdoMet are produced daily in an adult individual. It is also known that the AdoMet synthetase activity is reduced in hepatic pathologies [4]. Therefore, the study of AdoMet synthesis is subject of great importance, specially in the liver.

For a long time it has been known that there are several AdoMet synthetase forms, which were called α (M_r 210 kDa), β (M_r 110 kDa) and γ (190 kDa) [5]. However, it was not clearly established if these forms were different proteins or different oligomeric forms of the same subunit. Recently, it has been shown that only the α and β forms are present in the normal adult rat liver [6]. Both forms on SDS-PAGE show a single band with an estimated M_r of around 48 kDa. Tryptic peptide mapping of both forms present the same profile on HPLC and antibodies raised against the 110 kDa form (low-M_r form) are able to recognize both AdoMet synthetase [7]. Moreover, the incubation of the 210 kDa form (high-M_r form) in the presence of 1.8 M LiBr converts this form into the low-M_r form of the enzyme [8]. All these data led Cabrero et al. [8] to suggest that the high-M_r form is a tetramer and the low-M_r form a dimer of the same subunit.

2. ROLE OF SULFHYDRYL GROUPS ON AdoMet SYNTHETASE

2.1. *In vitro effect of* N-*ethylmaleimide modification on AdoMet synthetase*

AdoMet synthetase can be modified with *N*-ethylmaleimide (NEM), showing an incorporation of 2 mol NEM/mol of subunit in both, the high- and the low-M_r forms. This modification causes a loss of the enzyme activity in both enzyme forms. Also, when two of the ten sulfhydryl groups present in the protein are modified per subunit, its analysis by gel filtration chromatography shows a change in M_r for the 210 kDa form. This form now appears with an estimated M_r of 110 kDa, while the NEM-modified low-M_r form does not change its elution position [9].

When the pattern of the NEM-modification reaction and the reduction of the enzyme activity are compared for both enzyme forms, it can be observed that a loss of about 80% of the activity is accomplished while only one NEM group is incorporated per AdoMet synthetase subunit. This result has been interpreted as indicative that one of the sulfhydryl groups modified by this procedure is primarily responsible for the reduction in the enzyme activity. This group has been identified as

Abbreviations: AdoMet, *S*-adenosyl-L-methionine; AdoHcy, *S*-adenosyl-L-homocysteine; AdoMet synthetase, *S*-adenosyl-L-methionine synthetase; GSH, glutathione reduced form; GSSG, glutathione oxidized form; PK-A; protein kinase cAMP dependent; PK-C, protein kinase Ca^{2+} phospholipid dependent; CK-II, casein kinase II.

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the sulfhydryl group of Cys-150 of the protein sequence [10]. This finding differs from what was expected from the data published on the *Escherichia coli* enzyme, where Cys-90 of the sequence was modified [11]. This residue corresponds to Cys-105 in the rat liver AdoMet synthetase. Cys-150 of the rat liver enzyme also corresponds to another *E. coli* Cys, number 135 of the sequence [10]. These cysteine residues, rat liver Cys-150 and *E. coli* Cys-135, are located close to the consensus sequence for the ATP-binding site. In fact, NEM reaction can be partially protected, by increasing the time needed to obtain 50% of the modification, by including the enzyme substrates (methionine) or analogues of the substrates (AMP-PNP) in the incubation mixture [10,11].

2.2. *Glutathione and AdoMet synthetase relationship*

Related to these findings, in vivo experiments carried out by the injection of buthionine sulfoximine (BSO, a compound that inhibits glutathione synthesis) [12] have shown that a 30% reduction in the glutathione (GSH) concentration goes parallel with a 60% inhibition in the AdoMet synthetase activity in rat liver [13]. The alteration of these parameters is accompanied by the appearance of variations in the hepatocyte mitochondrial shape and also affects the rough endoplasmic reticulum. These changes can be overcome by the injection of a monoethylester of glutathione, a permeable derivative of glutathione [13]. Similarly, rats treated with CCl_4 present a decreased AdoMet synthetase activity and reduced GSH levels, effects that can be prevented by intramuscular injection of AdoMet [14].

All the above results indicate the importance of sulfhydryl groups in AdoMet synthetase activity. The role of these groups may be related to the maintenance of the enzyme oligomeric state. It should also be considered that different groups may be involved in the events produced in each enzyme form. In addition, these data suggest a possible role for glutathione in the protection of these groups against oxidation.

Therefore, the effect of glutathione on AdoMet synthetase activity and behaviour has been studied, showing that the presence of GSSG (glutathione oxidized form) can inhibit the enzyme activity in vitro and, that this effect can be modulated by GSH (glutathione reduced form) [15]. Therefore, a regulation by the ratio GSH/GSSG is expected. Changes in the kinetic behaviour of the high-M_r form of the enzyme when it is subjected to chromatography on thiopropyl Sepharose 6B (using sulfhydryl groups as the attachment site) and eluted with GSH have also been observed [15]. These changes can be summarized as a disappearance of a high K_{mMet} component of its kinetics and the appearance of a non-sigmoidal (hyperbolic) kinetic shape. This alteration can be due to the occurrence of some sulfhydryl groups of the protein in the reduced state. The former presence of two kinetic components could be due to the

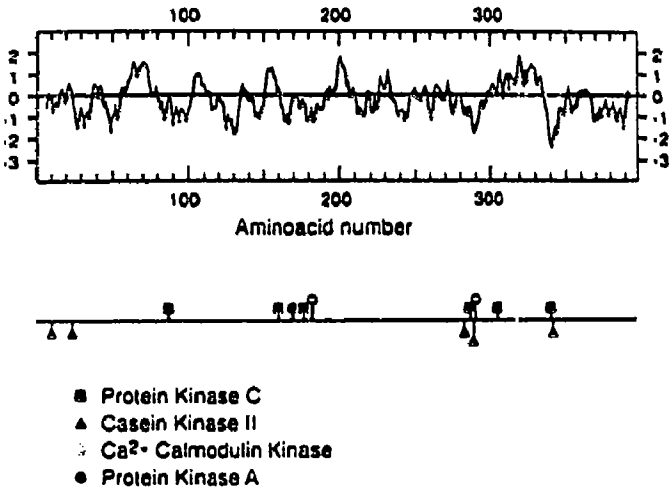

Fig. 1. AdoMet synthetase structural features. The hydrophobicity of the deduced sequence of AdoMet synthetase was analyzed by the Kyte-Doolittle algorithm. The relative location of putative phosphorylation sites are shown.

coexistence of an oxidized and a reduced form of this 210 kDa enzyme. No change in the kinetic behaviour for methionine was observed with the low-M_r form, maybe due to a different configuration of the enzyme or to the fact that different sulfhydryl groups are involved in the binding, leading to a reduced dimer that has different characteristics than the reduced tetramer [15,16].

The kinetic changes observed for the high-M_r form correlate with a variation in its behaviour on SDS-PAGE, where a reduction in the subunit M_r for this enzyme is observed [16]. These results and the former about its kinetic behaviour make us to suggest that the low-M_r form is probably an oxidized-dimer configuration that may change to a reduced dimer under special conditions (e.g. thiopropyl Sepharose 6B chromatography), but that may or may not be able to become a tetramer and, therefore, does not show the same behaviour as the high-M_r form. Its capacity to form a tetramer could be modulated by a modification such as phosphorylation, that can interfere with the association of two dimers.

3. PROPOSAL

At the moment there is no description of any phosphorylation reaction involving AdoMet synthetase, even when it could be expected. The analysis of the deduced sequence shows several putative phosphorylation sites, 6 for protein kinase C, 1 for protein kinase cAMP-dependent, 2 for Ca^{2+}-calmodulin-dependent protein kinase and 5 for casein kinase II (Fig. 1). Based on these data and the hydrophobicity profile it could be expected that at least one PK-C and one CK-II site are

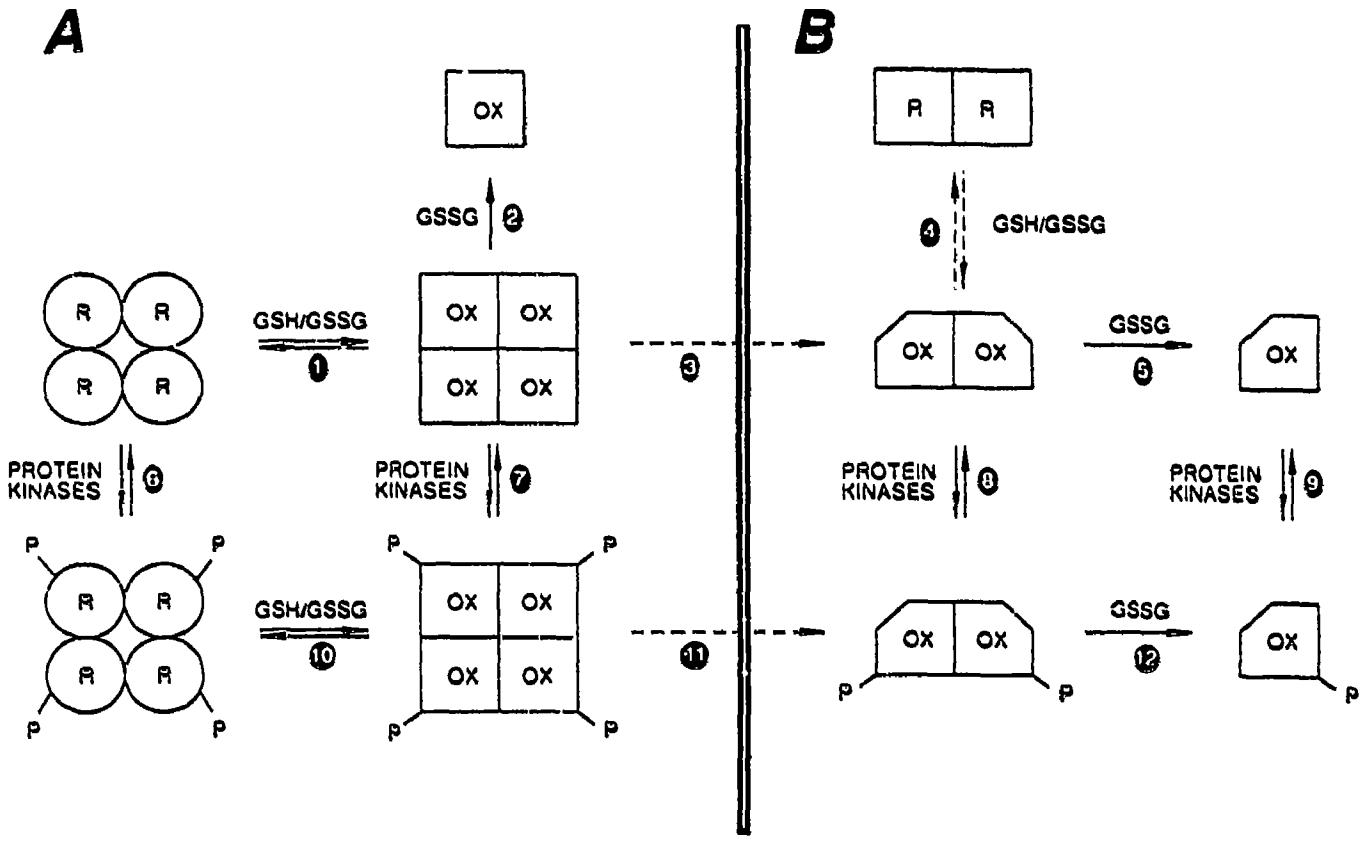

Fig. 2. Scheme of the proposed mixed regulatory mechanism of AdoMet synthetase. Panel A represents the changes in the tetramer form and panel B shows the reactions involving the dimer form. Dashed arrows show reactions carried out under special conditions, in the presence of LiBr or *N*-ethylmaleimide (reactions 3 and 11), and by binding to a thiopropyl Sepharose 6B column (reaction 4). Filled arrows indicate reactions shown in vitro or proposed and that could occur in vivo under similar conditions. AdoMet synthetase subunits that differ in the oxidized/reduced sulfhydryl groups have been drawn as distinct polygonal forms, as discussed in the text.

phosphorylated in vivo. This deduction is based on the presence of some of these putative phosphorylation sites on the most hydrophilic area of the protein (Fig. 1). There are other results that also suggest a possible regulation by a phosphorylation mechanism. These are based on the increase of the activity of several transmethylation reactions due to phosphorylation of the enzymes involved, as is the case for glycine methyltransferase [17] and phospholipid methyltransferase in the liver [18,19]. All these methyltransferases use AdoMet as the methyl donor group for their reactions, therefore, an increase in their activities will need AdoMet higher production, which can only be done by an increase in AdoMet synthetase activity, suggesting that there should be a coordination between the regulation of these enzymes and AdoMet synthetase. At the same time the increase in AdoMet synthetase activity should not be very dramatic because changes in the ratio AdoMet/AdoHcy regulate methyltransferase activities [20].

Other possible regulatory mechanisms, involving a modulation of the AdoMet synthetase mRNA levels have been also analyzed under conditions where the enzyme activity is reduced (CCl_4 treatment). The results obtained seem to exclude this type of regulation, since there are no changes observed in these levels [14].

Based on all the results described we propose a regulatory mechanism combining oxidation/reduction states of the enzyme and phosphorylation (Fig. 2). The tetramer could appear in either a reduced or an oxidized state (reactions 1,10) as suggested by the results obtained after chromatography on a thiopropyl Sepharose 6B column [15,16]. As mentioned above, AdoMet synthetase presents consensus sequences for phosphorylation by several protein kinases (Fig. 1). Therefore, it could be expected that both the reduced tetramer and the oxidized tetramer are phosphorylated by any of these protein kinases (reactions 6, 7). These phosphorylated forms could be dephosphorylated by phosphatases reverting these reactions. In addition, the purified tetramer incubated in the presence of GSSG leads to the appearance of an inactive oxidized monomer [15] (reaction 2).

Similarly, the dimer could appear in a reduced or an

oxidized form (reaction 4). Since the K_{mMet} value for the dimer and the oxidized tetramer are identical [15], it is suggested that the dimer appears as an oxidized form. A reduced dimer has been obtained only after chromatography on a thiopropyl Sepharose 6B column [15]. In contrast to the results obtained with the reduced tetramer, the reduced dimer does not show any kinetic change or molecular weight variation after this chromatography. Therefore, we suggest that the sulfhydryl group(s) reduced in the dimer are different than the one(s) involved in binding of the tetramer, and are drawn in a different form.

The incubation of the dimer in the presence of GSSG leads to the formation of an inactive monomer [15], that should be an oxidized form (reactions 5, 12). Like for the tetramer, the dimer could be phosphorylated in either of its forms, reduced or oxidized (reaction 8). A possible phosphorylation of the reduced dimer cannot be excluded, but this form only seems to be formed by thiopropyl Sepharose 6B chromatography, and therefore of no physiological significance. The monomer originated by oxidation could also be formed by phosphorylation (reaction 9). The phosphorylation reactions involving dimer and monomer could also be reverted by the action of phosphatases (reactions 8, 9).

Finally, since our hypothesis is that the dimer is an oxidized form we suggest that the conversion of the tetramer to the dimer is produced from the oxidized tetramer by reaction 3, or from the oxidized-phosphorylated tetramer by reaction 11. A possible aggregation reaction of the dimer to form a tetramer appears to be excluded based on the data available [8,9].

This mixed mechanism combining phosphorylation and oxido/reduction fits with the structural features of AdoMet synthetase and could explain the complexity of its regulation. Moreover, this proposal indicates that to fully establish this regulatory mechanism, phosphorylation experiments need to be carried out.

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