Inhibition of phospholipid methylation by a cytosolic factor

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Rat liver cytosol contains a heat-stable factor which inhibits phospholipid methylation by rat liver microsomes. The effect of this factor on lipid methylation was dose- and pH-dependent. This factor has an M_r of approx. 3200 as estimated by gel filtration. It could not be extracted by chloroform/methanol (2:1, v/v), and its action was inhibited by incubation with subtilisin.

Interest in phospholipid methylation has grown over the last years since it was observed that several signals acting on cell-surface receptors modulate the methylation of phosphatidylethanolamine (for a review see Mato & Alemany, 1983). In isolated rat hepatocytes several hormones activate phospholipid methyltransferase (Castaño *et al.*, 1980; Alemany *et al.*, 1981; Alvarez Chiva *et al.*, 1983; Marin Cao *et al.*, 1983). The mechanism by which different signals regulate phospholipid methylation remains to be determined, although cyclic AMP and Ca^{2+} have been implicated in this process (Castaño *et al.*, 1980; Alemany *et al.*, 1982; Marin Cao *et al.*, 1983). In this study we show the existence in rat liver of a heat-stable cytosolic factor that inhibits phospholipid methylation.

Materials and methods

Materials

S-Adenosyl-L-[methyl- 3H]methionine (15 Ci/mmol) was purchased from New England Nuclear. Bio-Gel P-6 was from Bio-Rad. Subtilisin, trypsin and papain were from Sigma.

Methods

Rat liver microsomes from normally fed Wistar rats (250–300 g) were isolated by differential centrifugation as previously described (Alemany *et al.*, 1982). Phospholipid methylation was assayed as described (Castaño *et al.*, 1980). Proteins were determined according to the method of Bradford (1976).

Results and discussion

Normally fed Wistar rats (250–300 g) were killed by decapitation, the liver was removed and homogenized in a Potter–Elvehjem apparatus in 4 vol. of ice-cold 10 mM-phosphate buffer, pH 7.0. The homogenate was centrifuged at 12500g for 20 min at 4°C and the supernatant again at 105000g for 60 min at 4°C. The supernatant of this last centrifugation was boiled for 5 min, cooled at 4°C and centrifuged at 48000g for 10 min at 4°C to remove the insoluble material. The supernatant was then lyophilized and dissolved in about 1/10 the original volume of water. The concentration of protein varied from preparation to preparation, and was between 6 and 10 mg/ml. About 4 ml of extract was obtained from a rat liver weighing approx. 10 g.

The addition of this concentrated preparation to rat liver microsomes inhibited phospholipid methylation in a dose-dependent manner (Fig. 1). Increasing concentrations of this preparation gradually increased the magnitude of inhibition of phospholipid methylation to reach a plateau. Maximal inhibition of phospholipid methylation varied from preparation to preparation, and was between 60% and 70%. The inhibition of phospholipid methylation by this factor is also dependent on the pH. As shown in Fig. 2, by increasing the pH from 6 to 9 the inhibition of phospholipid methylation was gradually lost. After chromatography through a Bio-Gel P-6 column the inhibitor was eluted to give a main peak of activity with an apparent M_r of about 3200 and a smaller peak of apparent M_r 1600 (Fig. 3). Incubation of the M_r 3200 peak with subtilisin (1 mg/ml) for 2 h at 37°C destroyed its activity (results not shown). The inhibitory activity of this factor on phospholipid methylation was however resistant to treatment

Fig. 1. Effect of different doses of concentrated rat liver cytosol on phospholipid methylation by rat liver microsomes

Microsomes were preincubated for 5 min at room temperature with different volumes of the factor in a final volume of 500 μ l before the addition of S-adenosyl-L-[methyl- 3 H]methionine. Phospholipid methyltransferase activity was assayed as described in the Materials and methods section. Results are the average $\pm$ S.E.M. of three independent experiments in duplicate; 100% activity corresponds to 340 ± 50 pmol/min per mg of protein.

Fig. 2. Effect of different pH values on the inhibition by the cytosolic factor of phospholipid methylation

Phospholipid methyltransferase was assayed as described in the Materials and methods section at various pH values in the presence or absence of 100 μ l of inhibitor and the magnitude of the effect was calculated. pH 6.0 was obtained with a phosphate buffer (25 mM) and pH values 7.0–9.0 were obtained with a Tris/HCl buffer (25 mM). Results are the average $\pm$ S.E.M. of three independent experiments in duplicate. The activities at pH values 6, 7, 8 and 9 were respectively 280, 340, 670 and 1100 pmol/min per mg of protein.

with trypsin or papain under the same conditions as those described for subtilisin. In these experiments the mixture containing the proteinase was boiled for 5 min before testing its activity on phospholipid methylation. These results suggest that this inhibitor has a peptide moiety. The inhibitor could not be extracted by chloroform/methanol (2:1, v/v), which suggests that it is not a lipid. Finally, a similar factor was present in rat kidney and lung, indicating its wide tissue distribution.

A peptide with similar characteristics to the present phospholipid methyltransferase inhibitor has been previously purified from rabbit liver (Sue Lyon *et al.*, 1982). This peptide inhibits the transfer of methyl groups by methyltransferases to 3,4-dihydroxybenzoate, *N*-acetylserotonin, phenylethanolamine, tryptamine and 2-mercaptoethanol and has been named methinin. The present results indicate the existence in rat liver of a cytosolic heat-stable factor which inhibits phospholipid methylation and which has similarities with the peptide methinin. Whether this factor plays an important role in regulating phospholipid methylation *in vivo* and how its activity might be modulated remains to be determined.

Fig. 3. Chromatography of the inhibitor of phospholipid methylation through a Bio-Gel P-6 column

The Bio-Gel P-6 column (1.1 cm $\times$ 60 cm) was equilibrated in Tris/HCl (25 mM, pH 6.5) containing 0.02% NaN₃ at a rate of 3.6 ml/h. After application of 1.2 ml of concentrated inhibitor the column was eluted with the same buffer. Fractions (1.8 ml) were collected and phospholipid methyltransferase activity was measured in 200 μ l of each fraction. This Figure shows the results from a single representative experiment. The same chromatographic profile was obtained in more than 10 independent experiments.

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