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METHININ: A PEPTIDE INHIBITOR OF METHYLATION

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Five enzymes, active in the transfer of a methyl group to either oxygen, nitrogen or sulfur atoms, were inhibited competitively by a peptide isolated from rabbit liver. The peptide, named methinin, appears to have a chromophore that resembles pyridinoline, a cross linking amino acid found in elastin and collagen.

INTRODUCTION

The failure to observe significant methylation of arylamines in buffer extracts of rabbit or rat livers without prior overnight dialysis (1), an effect similar to that described for N-methylation of serotonin by extracts of rabbit lung (2), is due to the presence of an inhibitor that we have named methinin. On the basis of its effect on homogeneous arylamine N-methyltransferase (1), methinin has been purified to homogeneity and shown to be a peptide that contains a pyridinium ring similar to that of pyridinoline which is found in collagen (3, 4) and elastin (5). Our interest in methinin is in its inhibition of a variety of methylation reactions including methylation at oxygen, nitrogen and sulfur.

MATERIALS AND METHODS

Arylamine N-methyltransferase was a homogeneous preparation from rabbit liver (1). Phenylethylamine N-methyltransferase and catachol O-methyltransferase were purified preparations purchased from Sigma Chemical Co. (St. Louis, MO.). Hydroxyindole N-methyltransferase was an extract of rat pineal in 50 mM potassium phosphate at pH 7.0, that had been centrifuged at 100,000 g for 1 h. Thiol S-methyltransferase was prepared as a microsomal suspension as described (6).

Methinin assay. Methinin was quantified in an assay system containing the following in a total volume of 200 µl: 0.1 M potassium phosphate at pH 7.8; 35 µM (^{35}S)-adenosyl-L-methionine (10 µCi/µmol); 0.8 mM tryptamine; 0.1 unit (1) of arylamine N-methyltransferase; and varying amounts of methinin. After incubation for 30 min at 37°C the reaction was stopped with 1 ml of 0.1 M potassium borate at pH 9.5 and the methylated product was extracted with 1 ml of toluene containing 3% isoamyl alcohol. Radioactive product was measured by scintillation spectroscopy. One unit of methinin is taken as that amount yielding 50% inhibition in this system and is equal to 2 nmol of free amino group of the homogeneous inhibitor; amino group concentrations were measured fluorometrically with fluorescamine (7).

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Enzyme assays. Arylamine methyltransferase was assayed exactly as described for the determination of methinin except for variation of the concentration of tryptamine that was used to assess competitiveness. For each of the following enzymes, incubation conditions were identical except as noted: Phenylethanolamine methyltransferase with phenylethanolamine as acceptor in 0.1 M Tris HCl at pH 8.6; hydroxyindole methyltransferase with N-acetylserotonin in 0.1 M potassium phosphate at pH 7.5; thiol S-methyltransferase with 5 mM 2-mercaptoethanol and 2 mM EDTA; catechol O-methyltransferase with protocatechuiate and 50 μM $MgCl_2$ in 0.1 M potassium phosphate at pH 7.5, with the reaction terminated by addition of 1 N HCl prior to extraction. Methylation of histones and of DNA from Escherichia coli was measured by modifications of the appropriate published methods (8, 9) using an extract of rabbit thymus as enzyme source.

Purification of methinin. The wash fluid, obtained from a column of DEAE-cellulose that had been charged with an extract of 500 g of rabbit liver, as described for the preparation of arylamine N-methyltransferase (1), was used as the source of methinin. The transferase can be eluted subsequently by applying a salt gradient (1). The wash fluid, about one liter, was heated to boiling for 5 min. The suspension was filtered and concentrated to 60 ml by flash evaporation at 40°C. The concentrate was charged, in aliquots of 30 ml, onto columns (2.5 x 80 cm) of Sephadex G-10 that were equilibrated and eluted with 1 mM acetic acid. Active fractions from two such applications were combined, concentrated to 20 ml by flash evaporation, and charged in four applications of 5 ml each onto Sephadex G-25 Superfine (2 x 80 cm) that had been equilibrated with 1 mM acetic acid. Active fractions, eluted with the same solution, were concentrated to 2 ml and subjected to HPLC with water as the developing solvent on a Zorbax TMS column (4.6 x 240 mm Dupont) using 250 μl portions of the concentrate. Two dimensional separation with chromatography of the product on cellulose-TLC plates (Analtech) (10) in 1-butanol: pyridine: water: acetic acid: (65:50:40:10), and electrophoresis in the other direction in water: acetic acid: pyridine (200:20:1), yielded a single, ninhydrin-positive spot.

Results

Inhibition. Methinin is an inhibitor in each of the systems involving S-adenosylmethionine as methyl donor that have been tried, including the N-, O- and S-methyltransferases shown in Table 1. In each of the five systems tested, methinin was competitive with the acceptor for the methyl group. In the case of thiol S-methyltransferase, microsomes were used because of the aberrant kinetic properties of the soluble form of the enzyme (11), resulting in a relatively low

Table 1

Competitive Inhibition by Methinin of the Methyltransferases

Methyltransferase	Atom	Acceptor	K_i (μM)
Catechol	O	3, 4-Dihydroxybenzoate	0.5
Hydroxyindole	O	N-Acetylserotonin	1
Phenylethanolamine	N	Phenylethanolamine	2
Arylamine	N	Tryptamine	5
Thiol	S	2-Mercaptoethanol	150

affinity for the peptide inhibitor. In the methylation systems described for histones and DNA, 10 μg of methinin resulted in essentially complete inhibition. The inhibitory effect is destroyed by incubation overnight with carboxypeptidase Y (Sigma).

Sources. Buffer extracts (1) of New Zealand rabbit livers and those of Sprague-Dawley rats had a specific activity of 10 and 6 units per mg of extracted proteins, respectively. With rat tissue, extracted with 10 mM potassium phosphate at pH 7.0, the following concentrations of methinin equivalents were found in units per gram of tissue (wet weight): spleen, 36; kidney, 22; lung, 12; and brain, 6.

Characterization of methinin. Amino acid analysis of the purified peptide suggested the following composition: 14 alanine; 4 glycine; 3 lysine; 2 threonine; 1 glutamate; and 1 serine. However, attempts at establishing the qualitative presence of lysine by forming its ε-amino derivative, were repeatedly negative. By manual Edman degradation, alanine, followed by threonine, were at the amino terminus; by digestion with carboxypeptidase Y (12), both alanine and glycine were identified as carboxy termini.

The ultraviolet absorption spectrum of the peptide at pH 3.0 displayed a shoulder at 300 nm and a series of peaks at 263, 256, and 251 nm. At pH 12, a new peak emerged at 312 nm. The peptide was fluorescent at pH 7.0 with maxima at 320 nm (excitation) and 380 nm (emission). A freshly prepared sample of pyridinoline from bovine Achilles tendon had absorbance peaks at 282, 262, 253, and 250 nm at pH 3.0 with a new peak appearing at 320 nm at pH 12; the fluorescent maxima were at 330 and 395 nm, respectively. In general, the spectral properties of pyridinoline gathered from the existing literature are varied. Deyl, et al. (5) reported an absorption maximum at 315 nm (pH 7) with fluorescent maxima at 320 nm (excitation) and 405 nm (emission); Fujimota, et al. (3) note absorbance peaks at 295 nm (acid pH) and 325 nm (neutral and high pH), with fluorescence maxima at 325 nm and 410 nm, respectively. It should be noted that pyridinoline is an unstable compound (13). Indeed, subjecting methinin to light at 320 nm for 60 min in a Farrand fluorimeter resulted in loss of 90% of the inhibitory activity; pyridinoline itself was not an inhibitor either before or after irradiation.

Using the method of Starcher (14), a 24 h acid hydrolysate of methinin was separated by paper chromatography; as expected for pyridinoline, the fluorescent material remained at the origin. Amino acid analysis of the isolated fluorescent compound, and of pyridinoline, revealed that both eluted with the basic amino acids at about the same time as lysine. The biologically related analogues, desmosine and isodesmosine, were not fluorescent.

The chromophore isolated from methinin had an ^{1}H-NMR spectrum with an aromatic singlet at 8.44 ppm, a triplet at 1.25 ppm, and a number of small peaks near a strong band at 3.7 ppm, relative to sodium 3-trimethylsialyl proprionate 2, 2, 3, 3, D4 as an internal standard. The spectrum for pyridinoline isolated from collagen had an aromatic singlet at 8.37, a triplet at 1.25 ppm, and peaks at 3.6 ppm and 3.7 ppm. NMR spectroscopy was kindly performed by Dr. William Egan, Bureau of Biologics, Food and Drug Administration, on a Bruker WM 300 NMR Spectrometer.

DISCUSSION

Methinin is a potent inhibitor of each of the soluble enzymes examined in which S-adenosyl methionine participates as a methyl donor. In each instance evaluated, methinin is a competitive inhibitor, not with the methyl group donor that is common to all of the reactions, but rather with the methyl group acceptor which differs for each enzyme.

Although the structure of methinin has not been elucidated completely, it is clear that it is a peptide of known amino acid composition with a fluorescent chromophore. Based on the similarities of the properties of the chromophore from methinin and of the somewhat variable, recorded values of pyridinoline (3-5), we tentatively identify the unknown as a peptide containing a 3-hydroxypyridinium derivative. These normally occur as cross links in collagen (3, 4) and elastin fibers (5). The absence of proline from methinin indicates that it is not related to collagen; the high content of alanine suggests that it might be derived from elastin (15).

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