ENZYMATIC METHYLATIONS, I
ISONICOTINIC ACID HYDRAZIDE: AN INHIBITOR OF tRNA AND PROTEIN METHYLATION

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Recently, nicotinamide has been found as a powerful natural inhibitor of tRNA methylation in vitro (1).
This report shows that nicotinamide inhibits protein methylation as well as tRNA methylation and describes the action of isonicotinic acid hydrazide (INH) on tRNA and protein methylation by partially purified rat liver methylase (2).

MATERIALS AND METHODS: S-Adenosylmethionine (methyl-^{14}C), spec. act. 54.6 ci/M was obtained from NEN chemicals GmbH., and diluted to a spec. act. of 13.9 ci/M. Unlabeled S-Adenosylmethionine (SAM) was bought from Boehringer, Mannheim. Isonicotinic acid hydrazide was obtained from Fluka AG, nicotinamide from Merck AG.- tRNA from E.coli was prepared as described by Zubay (3); tRNA from rabbit and rat liver was made according to Rogg et al. (4). Methylases from rat liver were fractionated on hydroxylapatite and by ammonium sulfate precipitations as described by Kuchino and Nishimura (2); the precipitated fractions were dissolved in, and dialyzed against, Tris·HCl pH 7.8 - 0.01 m; EDTA - 0.001 m; mercaptoethanol - 0.001 m in 50 % glycerol and stored at -20°C. Highly purified E.coli tRNA^{fMet} was prepared as mentioned recently (5) or was a kind gift of Dr. S. Nishimura, Tokyo.

Assay for methylase activity was performed as described by Kuchino et al. (2) with a few differences: 120 µl contained Tris·HCl pH 7.8 : 10 µmoles, $MgCl_2$: 1 µmole, reduced glutathione : 1 µmole, (methyl-^{14}C)SAM : 1.44 µmoles (= 20 mµci), tRNA : 0.05 $O.D._{260}$ units, and methylase. From this mixture, either 100 µl or, for kinetic studies, 25 µl aliquots were removed and placed onto filter paper disks which were processed as described by Mans and Novelli (6). Radioactivity was measured in toluene-PPO (1000:6 - v:w), using a Packard Tricarb Model 3380).

RESULTS AND DISCUSSION: A natural inhibitor of tRNA methylase has been isolated from rat liver and identified as nicotinamide (1). Recently, a number of nicotinamide analogs have been tested for inhibition of KB, Walker-256, human seminoma and rat liver tRNA methylase (7). For most of these investigations unfractionated methylase preparations (105 000g supernatants) have been used, and the mechanism of this tRNA methylase inhibition has been regarded as competitive or "mixed type" by the authors cited above (1, 7).

Its structural similarity to nicotinamide, its importance as a widely used and efficient tuberculostatic agent and the fact that the molecular mechanism of isonicotinic acid hydrazide (8) action is still unknown, induced us to study the influence of this drug on tRNA and protein methylation. Our results presented here were obtained with methylase I (2). Figures 1, 2 and 3 demonstrate the following facts:

a.) Nicotinamide inhibits not only tRNA methylation, but also protein methylation (fig. 1).- Protein methylation was measured by omitting E.coli tRNA from the incubation mixture; this allows one to measure incorporation of methyl groups into proteins from the methylase fraction.

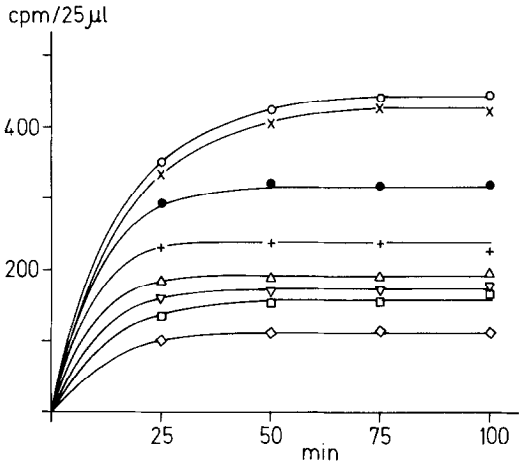

Fig. 1: Protein methylation (determined in the absence of E.coli tRNA as described in "materials and methods"): o——o without inhibitor; in the presence of inhibitors (µmoles in 120 µl reaction volume): x——x ethidium bromide (0.1); ●——● INH (0.5); +——+ INH (1.0); Δ——Δ INH (2.0); ▽——▽ INH (3.0); □——□ INH (5.0); ◇——◇ nicotinamide (1.0).- The addition of 0.05 $O.D._{260}$ units of rat liver or rabbit liver tRNA to the reaction mixture without inhibitor (o——o) did not increase the incorporation of ^{14}C-methyl groups into TCA insoluble material, hence these tRNAs are not methylated.

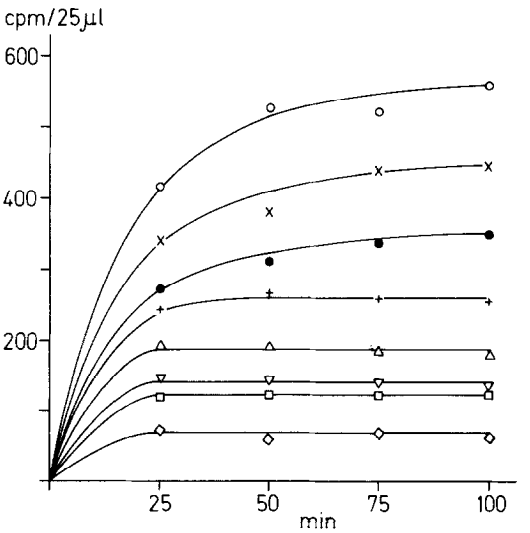

Fig. 2: Methylation of unfractionated E.coli tRNA (including protein methylation) as described in "materials and methods"; symbols and concentrations as in legend to fig. 1.

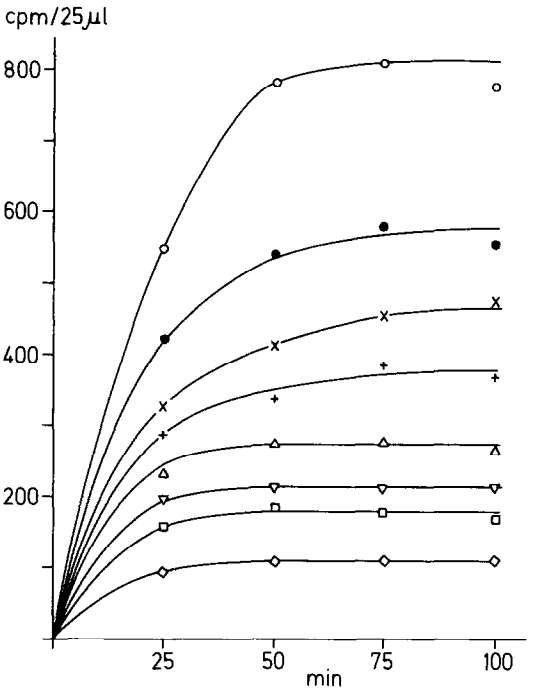

Fig. 3: Methylation of $tRNA^{fMet}_{E.coli}$ (including protein methylation) as described in "materials and methods"; symbols and concentrations as in legend to fig. 1.

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b.) Ethidium bromide has no effect on protein methylation (fig. 1), but inhibits tRNA methylation quantitatively (fig. 2 and 3): the difference between methylation without inhibitor (o——o, tRNA plus protein methylation) and with ethidium bromide (x——x, protein methylation) is due to tRNA methylation.

c.) INH is a less efficient inhibitor of protein and tRNA methylation than nicotinamide (fig. 1, 2, 3). Both nicotinamide (not shown here in detail) and INH alter the kinetics of methylation in such a way, that new plateaus of methylation occur, the altitude of which depends on the nicotinamide or INH concentration. This excludes the possibility of a competitive type of inhibition.

d.) In contrast to E.coli tRNA, rabbit and rat liver tRNA are

not methylated at all by rat liver methylase I, since these tRNAs are already fully methylated by their homologous methylases (legend to fig. 1).

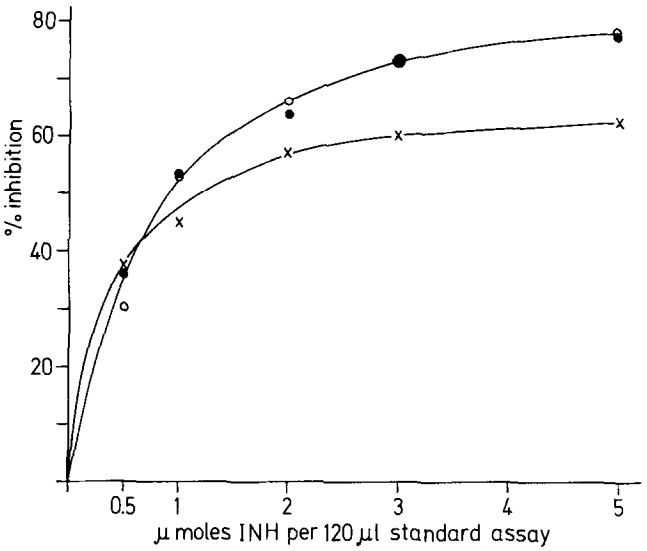

Fig. 4: Inhibition of E.coli tRNA (+ protein) o——o, $tRNA^{fMet}_{E.coli}$ (+ protein) o——o, and protein x——x methylation by increasing amounts of INH (µmoles per 120 µl assay vol.).

Finally, fig. 4 summarizes the results obtained from fig. 1, 2 and 3 concerning INH action. It is shown that inhibition of E.coli tRNA and $tRNA^{fMet}_{E.coli}$ methylation by increasing INH concentrations is identical and that protein methylation is inhibited to a similar degree. It is further obvious that inhibition of protein, unfractionated E.coli tRNA and $tRNA^{fMet}_{E.coli}$ methylation becomes independent of the inhibitor (INH) concentration above certain INH concentrations. Studies on the mechanism of protein and tRNA methylase inhibition by nicotinamide and INH are in progress, and the detection in rat liver methylase fractions of a nicotinamide-dependent, SAM-splitting enzyme will be reported (9).

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