ETHYLATION IN VIVO OF PURINES IN RAT-LIVER tRNA BY L-ETHIONINE*

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The long-term feeding of ethionine, the ethyl analog of methionine, leads to hepatic carcinoma (Farber, 1963). The intraperitoneal injection of [ethyl-1-^{14}C-] L-ethionine into rats also leads to the rapid ethylation of both hepatic protein and nucleic acid fractions (Natori, 1963; Stekol et al., 1960; Stekol, 1965; Farber et al., 1967). Stekol (1965) reported that DNA is ethylated and 7-ethylguanine was the only labeled nucleic acid component that could be isolated following acid hydrolysis. However, others (Farber et al., 1967; Ortwerth and Novelli, 1968) demonstrated that RNA, particularly tRNA, is the most significantly labeled nucleic acid fraction and that a more complex pattern of RNA ethylation was observed (Farber et al, 1967) than indicated by Stekol (1965).

In the present paper, the major portion of the labeled ethylpurines obtained from rat liver tRNA following the injection of ethyl-labeled ethionine is identified as N^2-ethyl-, 7-ethyl- and N^2,N^2-diethylguanine, which together account for 23%, 10%, and 2% respectively of the total radioactivity found in tRNA. This accounts for two-thirds of the purine ethylation observed.

EXPERIMENTAL PROCEDURE

Methods: Two male rats, 400 g each, Charles River (caesarian derived), were starved overnight and injected i.p. with 1 mc of [ethyl-1-^{14}C] L-ethionine (New England Nuclear; 21 Ci/mole). Starvation was continued and the animals were killed 20 hours later. Hepatic tRNA was isolated by the procedure of Ortwerth and Novelli (1968). The final tRNA fraction had 173 A_{260} units and a specific activity of 3330 cpm/A_{260} unit.

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Descending chromatography was done on Whatman 3 MM paper and the following solvents were used: (A) n-butanol:water:conc. NH_4OH = 86:9:5 (V/V/V); (B) isopropanol:water: 6 N HCl = 65:2:33 (V/V/V); and (C) isopropanol:water:conc. NH_4OH = 85:15:1.3 (V/V/V). Adenine was run on all chromatograms as an external marker. Ethylpurines were also used as markers where indicated. After drying, the chromatograms were cut into 1 cm strips, immersed in a scintillation mixture, and counted in a Nuclear Chicago Scintillation Counter.

Materials: N^6-Ethyl-, N^6, N^6 -diethyladenine (Elion et al., 1952) and N^2-ethylguanine (Elion et al., 1956) were synthesized. Other ethylpurines were prepared by appropriate modifications of the methods used to prepare the corresponding methylpurines as follows: N^2, N^2-diethylguanine (Elion et al., 1956); 7-ethylguanine (Jones and Robins, 1963); 1-ethylguanine (Broom, 1965); 1-ethyladenine (Jones and Robins, 1963); and 3-ethyladenine (Jones and Robins, 1962). All compounds were purified by paper chromatography and their identities established by comparison of their UV-absorption spectra with those of authentic specimens (Details will be given in a later paper).

RESULTS

Hydrolysis of RNA at 100°C in N HCl for 30 minutes leads to the formation of free purines and pyrimidine nucleotides (Björk and Svensson, 1967). In solvent A, nucleotides remain at the origin, whereas ethylpurines migrate and partially separate (Fig. 1). Two different times of development were used to isolate fast-moving components at 21 hours (Fig. 1A) and to better separate the bulk of ethylpurines at 36 hours (Fig. 1B). The results show that pyrimidine nucleotides (I, Fig. 1) account for 46% of the radioactivity and that the remaining radioactivity is distributed among ethylated purines (II, III, IV; Fig. 1). Chromatography in solvent C confirmed these results. Electrophoresis of the ethylpurines at pH 4.0 eliminated 1-ethyladenine and 3-ethyladenine since they possess high cationic pK values, as do the corresponding methyladenines (Pal and Horton, 1964).

The slowest-moving labeled purine fraction (II; Fig. 1) was eluted from both chromatograms and rechromatographed in solvents A and B (Fig. 2). Essentially all of the radioactivity migrated with authentic 7-ethylguanine in both solvents, which accounts for 10% of the total radioactivity.

The fastest-moving ethylpurine fraction (IV; Fig. 1A) moved close to N^6-ethyladenine and N^2, N^2-diethylguanine. After elution, IV was electrophoresed and 70%

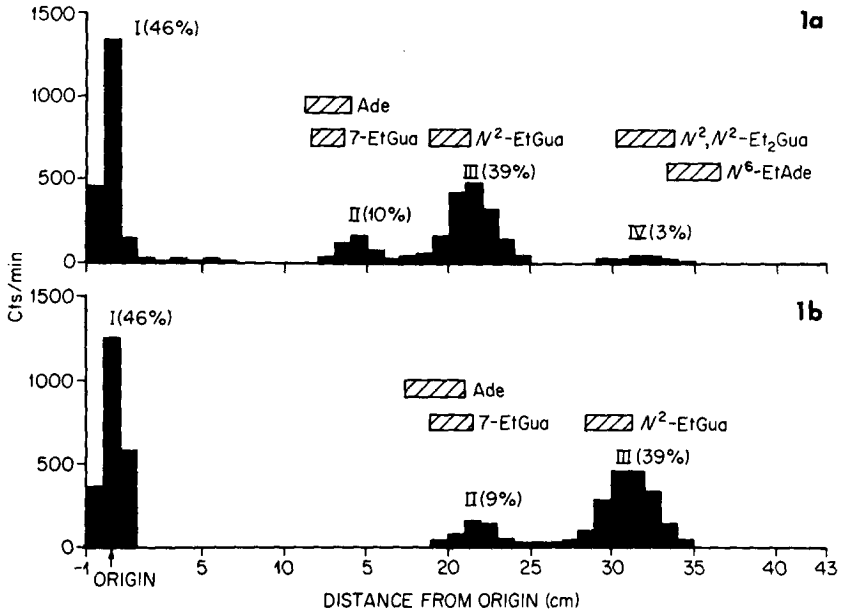

Fig. 1. Chromatographic separation of radioactive pyrimidine nucleotides and purines. Portions of the acid hydrolysate were chromatographed in solvent A for (a) 21 hours and (b) 36 hours. All markers (hatched lines) were chromatographed on the same paper external to the hydrolysate. (%) refers to % of total radioactivity found in the indicated peak. In (b) correction was made for 3% of radioactivity that ran off the paper in 36 hours.

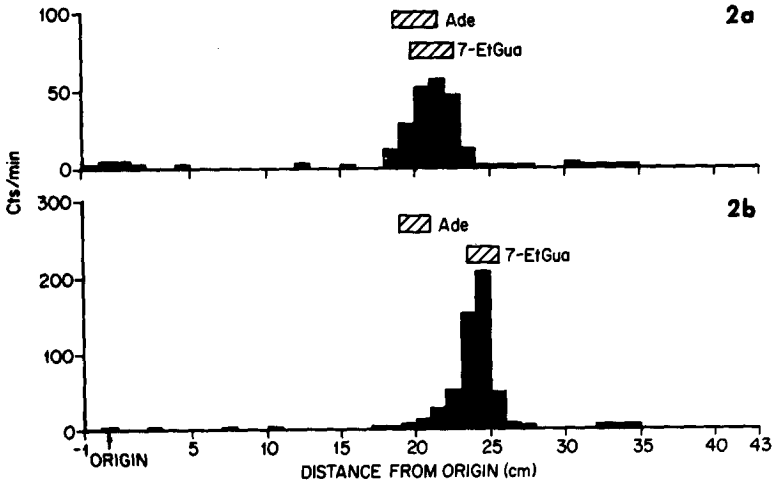

Fig. 2. Chromatographic identification of 7-ethylguanine. Peak II (Fig. 1) was eluted and taken to dryness, and adenine and 7-ethylguanine were added as internal markers. Portions of the mixture were then chromatographed in (a) solvent A for 48 hours and (b) solvent B for 40 hours.

of the radioactivity migrated with diethylguanine and none with N^6-ethyladenine; therefore N^2, N^2-diethylguanine accounts for 2% of the total radioactivity.

The major ethylpurine fraction (III; Fig. 1) was eluted and rechromatographed in solvents A and B (Fig. 3). Although apparently homogeneous in solvent A (Fig. 3A), it was resolved into 2 components in solvent B (Fig. 3B). When these two fractions were eluted from the latter chromatogram and rechromatographed separately in solvent B, no changes occurred. The faster component (III B; Fig. 3B) accounts for 57% of III (Fig. 1) and migrates with N^2-ethylguanine, which represents 23% of the total radioactivity and is the major ethylpurine component. Electrophoresis of the slower componen suggests that it is another ethylguanine, as yet unidentified; however it is apparently not 1-ethylguanine (Fig. 3A).

More than 50% of the radioactivity in the pyrimidine nucleotide fraction (I; Fig. 1) was distillable following hydrolysis in 1 N perchloric acid for 25 minutes at 100°C. This indicates that extensive 2'-O-ethylation has occurred, since this procedure preferentially hydrolyzes 2'-O-alkyl groups (Baskin and Dekker, 1967). Details will be given in a later paper.

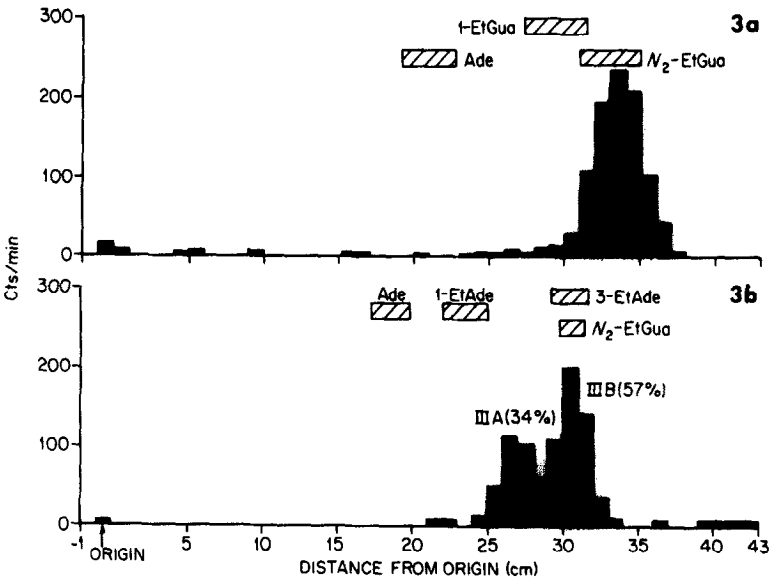

Fig. 3. Chromatographic identification of N^2-ethylguanine. Peak III (Fig. 1) was eluted, taken to dryness, and the indicated markers were added as internal markers. Portions of the mixture were then chromatographed in (a) solvent A for 38 hours and (b) solvent B for 30 hours.

DISCUSSION

Although quantitative differences exist, some of the sites of purine ethylation *in vivo* are the same as those for purine methylation by methyl-labeled L-methionine, since N^2-methyl-, N^2, N^2-dimethyl-, and 7-methylguanine have been identified in rat-liver RNA (Craddock *et al.*, 1968). However, since 1-methyladenine and 1-methylguanine were also identified in rat-liver RNA (Craddock *et al.*, 1968), two important qualitative differences do exist. These methylated purines have been identified as normal minor components in sRNA (Dunn, 1959 and 1963; Smith and Dunn, 1959).

The qualitative and quantitative differences demonstrated for purine alkylation *in vivo* by ethionine and methionine may be related to different enzyme mechanisms, since RNA methylases that utilize S-adenosylmethionine apparently do not utilize S-adenosylethionine (Borek, 1965; Peterkofsky, 1965; also see Hancock, 1968).

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