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INTRODUCTION OF METHYL GROUPS FROM ε-N-METHYLLYSINE INTO THE ONE-CARBON POOL*

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Summary: Radioactivity from ingested ε-N-[^{3}H] methyl- and ε-N-[^{3}H]-dimethyllysine residues of reductively methylated casein are rapidly and efficiently absorbed and incorporated into tissues of the chicken. Introduction of label into choline, carnitine and methionine suggests that these amino acids contribute methyl groups to the one-carbon pool.

ε-N-Methyl, dimethyl and trimethyllysine residues have been detected in small amounts in a wide variety of proteins (1-4) and appear to be fairly common in biological systems including many foods and animal feeds.

Kakimoto and Akazawa (5) have also detected small amounts of these amino acids in "normal" human urine and suggested that they arose from the catabolism of tissue proteins. Protein methylase III, an enzyme which can catalyze the methylation of lysine residues of proteins successively to mono-, di- and trimethyllysine residues, has been demonstrated in several rat tissues (6).

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ϵ-N-Trimethyllysine has been shown to be an efficient biosynthetic precursor of the important metabolite, carnitine in both Neurospora crassa and in rats (7, 8). In rats, however, free lysine is not directly methylated and thus Paik et al. (9) have suggested that the catabolism of tissue proteins may be required for carnitine biosynthesis. LaBadie et al. (10) have, for example, observed a rapid formation of carnitine in rat liver following lysosomal degradation of an ϵ-N-[^{3}H]-trimethyllysine containing, asialo derivative of the protein, fetuin. Under the same conditions, the corresponding ϵ-N-[^{3}H]-methyl- and dimethyllysine derivative of asialo-fetuin gave many unidentified labeled products but no detectable carnitine. Methyl- and dimethyllysine under those conditions do not appear to undergo further methylation to give trimethyllysine but do apparently undergo a substantial number of metabolic transformations. An enzyme which can catalyze the conversion of both ϵ-N-methyl- and dimethyllysine to lysine and "formaldehyde" has been characterized in rat tissues (11, 12) and these compounds have also been shown to reduce the normal dietary requirements of rats for lysine (13, 14).

Little attention has been directed to the possible nutritional significance of many minor dietary amino acids like the ϵ-N-methyl substituted lysines. The metabolic evidence just summarized, however, suggests that ϵ-N-trimethyllysine in the diet might be utilized for carnitine biosynthesis particularly when dietary lysine and/or methionine are limiting. Similarly ϵ-N-methyl and dimethyllysine might be utilized as a source of lysine in the absence of adequate dietary lysine. The results described in this report suggest a further nutritional importance for ϵ-N-methyl- and ϵ-N-dimethyllysine in the diets of chickens. Thus methyl groups from these amino acids appear to be incorporated into several important one-carbon pool compounds. Their presence in the diet may therefore have

important nutritional consequences especially in the absence of other one-carbon donors. Relatively simple chemical procedures to convert some lysine residues of dietary proteins to ε-N-methyl- and dimethyllysine residues (15) may, in some cases, afford an economically feasible way to supplement their contents of nutritionally available one-carbon units.

Materials and Methods

Labeling of Protein. Radioactive (methyl - [^{3}H]) mono-and dimethyllysine residues were introduced into 100 mg of vitamin-free casein (Nutritional Biochemicals) by a previously described procedure (15, 16) using 0.58 mg of ~310 mCi/mmol NaB^3H_4 (New England Nuclear). Amino acid analysis, using the short column of a Beckman Model 116 analyzer, indicated approximately 20% conversion of lysine to ε-N-methyllysine and approximately 30% conversion to ε-N-dimethyllysine.

Introduction and Uptake of Labeled Protein. A 460 gm, 10 week old broiler type chicken was deprived of food for 12 hrs. A 30 mg sample of the described methyllysine containing casein (~12 μ Ci) in 3 ml of 0.9% NaCl was introduced directly into the crop using a 150 x 2 mm length of stiff polypropylene tubing attached to a hypodermic syringe. A second hypodermic syringe with a needle was used to take 0.5 ml samples of blood from the wing vein at 30 min intervals. After clotting, 100 μl samples of the clear amber serum were subjected to liquid scintillation counting in 0.5 ml of NCS (Amersham) plus 10 ml omnifluor (New England Nuclear) the bird was sacrificed by decapitation after 5½ hrs and tissue samples were taken and placed on ice or frozen for later characterization.

Fractionation of Liver. Samples of fresh or frozen liver of approximately 6.5 g were homogenized in 10 ml of cold double distilled, deionized water with an ice-cold, motor driven teflon pestle. The crude homogenate was diluted to 30 ml with more water, combined with an equal volume of cold 10% trichloroacetic acid (TCA) and centrifuged for 10 min at ~12,000 x g. The solid residue was recovered and reextracted with 20 ml of cold 5% TCA, recentrifuged and the two supernatant fluids were combined and evaporated. The resulting residue was suspended in 4 ml of 2 M HCl, heated vigorously for 2 hrs on a steam bath, cooled and then extracted three times with approximately equal volumes of ether. The remaining sample was again evaporated in vacuo and the oily residue was dissolved in 50 ml of 0.11 M trisodium citrate buffer, pH 5.25.

The TCA insoluble material obtained above was extracted three times with ether and the residue was freeze dried and subjected to hydrolysis in 6 M HCl at 110° for 22 hrs. After evaporation to dryness in vacuo, the sample was dissolved in 0.11 M trisodium citrate buffer, pH 5.25.

The ether soluble fractions were combined and evaporated on a steam bath. The oily residue was dissolved in 6 ml of 95% ethanol, brought to boil on a steam bath and 6 ml of 2 M HCl were added. After 1 hr of gentle boiling, the sample was cooled, extracted with an equal volume of ether and evaporated to dryness in vacuo. The residue was redissolved in 1 ml of water, extracted twice with 10 ml of ether and again evaporated to dryness. The residue was dissolved in 5 ml of pH 5.25 citrate buffer.

Chromatographic Analyses. Hydrolyzed liver fractions in 0.5 ml of pH 5.25 citrate buffer were chromatographed on a 0.9 x 5 cm column of Beckman spherical cation-exchange resin type PA-35 at 55° with pH 5.25 citrate buffer (137.3 gm trisodium citrate, 26.2 ml conc. HCl and 0.4 ml caprylic acid per 4.0 l) at a flow rate of 1.0 ml/min. The effluent was monitored continuously for ninhydrin positive material using the standard Beckman Model 116 amino acid analyzer system. Fractions were collected prior to mixing with ninhydrin by use of an 8:1 stream splitter. Radioactivities in 0.5 ml samples were determined after mixing with 10 ml of Aquasol (New England Nuclear) using a Beckman LS-133 liquid scintillation counter. Fractions containing quaternary ammonium compounds were detected by appearance of a yellow precipitate upon mixing equal volumes of sample and 2% phosphomolybdic acid (17)

Results and Discussion

Reductive methylation of casein has been shown to decrease its susceptibility in vitro to hydrolysis by bovine trypsin (15), nevertheless in the present case radioactivity from ϵ-N-[^{3}H]-methyl- and dimethyllysine residues of the described sample were rapidly detected in serum and reached a maximum corresponding to a concentration of about 0.12 μmoles/ml only 30 min after ingestion. Samples of major tissues taken $5\frac{1}{2}$ hrs later showed a wide and relatively uniform distribution of the label (Table I) with only about 6% of the total in the feces and gut contents. An estimate based on the relative amount of the various tissues suggested that better than 70% of the ingested label was retained after $5\frac{1}{2}$ hrs.

Radioactivity in the liver was present in TCA soluble, TCA insoluble and ether soluble fractions. The ether soluble fraction, with approximately 6% of the label in the liver, after hydrolysis appeared to contain only two radioactive components. Thus, as shown in Figure 1, two peaks were observed upon cation exchange chromatography at approximately 4 and 29 to 30 ml, respectively, identical to the elution volumes of carnitine and choline. A similar sample upon chromatography on Bio-Rad AG 50W-X12, as recently described (17), gave similar results with the two radioactive components again eluting with authentic carnitine and choline

Table I. Tissue Distribution of [^{3}H] -label from Ingested [^{3}H]-methyl-and dimethyllysine in the Chicken

Tissue	CPM/g (x 10^{-3})[a]
Adipose (Mesenteric + retroperitoneal)	111
Brain	51
Heart	58
Kidney	112
Liver	72
Muscle (breast)	85
Spleen	48

[a] As determined by liquid scintillation counting of homogenized tissues in 0.5 ml of NCS plus 10 ml of omnifluor in toluene as described in the text.

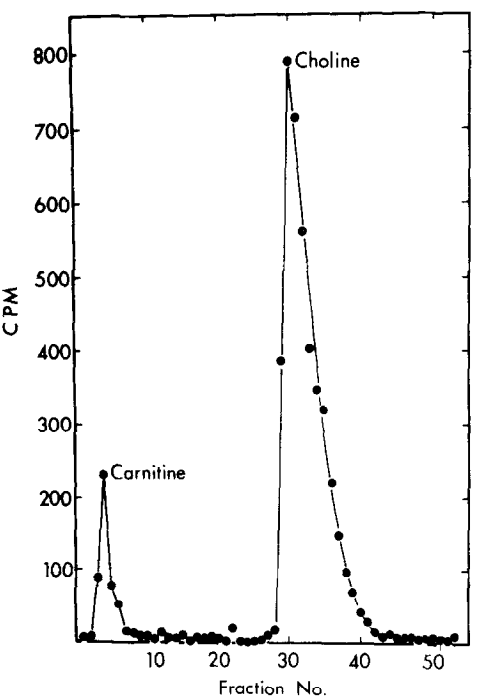

Figure 1. Cation Exchange Chromatography of the Hydrolyzed Ether-Soluble Fraction. The radioactivity in counts per min. of 1 ml fractions obtained from the acid hydrolyzed, ether-soluble fraction from 640 mg of wet liver upon chromatography on type PA-35 resin as described in the text.

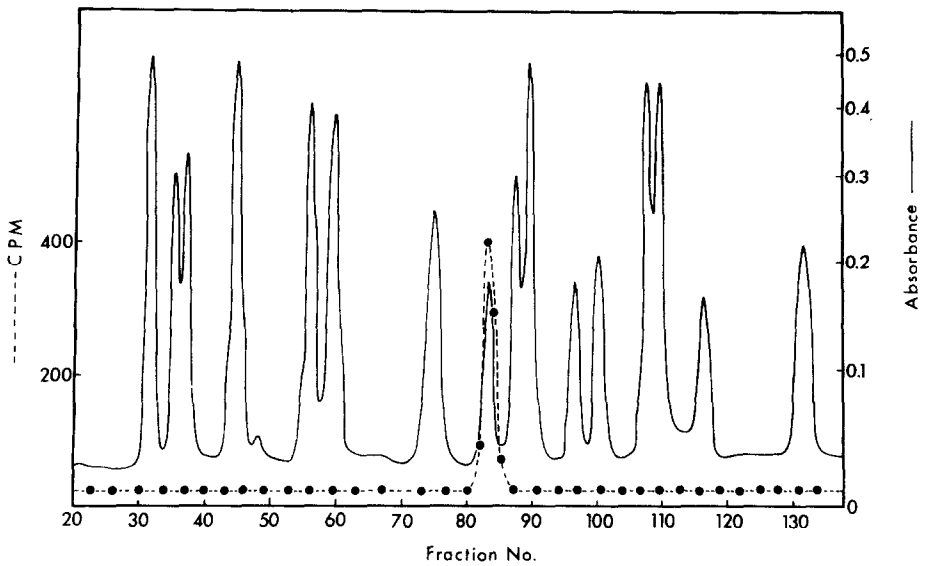

Figure 2. Cation Exchange Chromatography of the Hydrolzyed TCA-Insoluble Fraction. An elution profile for the acid hydrolyzed, TCA-insoluble fraction from 64 mg, wet weight, of liver obtained with a Beckman Model 116 Amino Acid Analyzer is shown in terms of the 570 nm absorbance after reaction with ninhydrin (solid line) and from counts per min. in 1 ml fractions (dash line) as described in the text.

The TCA insoluble fraction contained approximately 35% of the label in the liver. Hydrolysis and amino acid analysis revealed the presence of only one major radioactive component which eluted in the same volume as methionine[1] (Figure 2).

Most of the radioactivity in liver (i. e. ~60%) was present in the TCA soluble fraction which after hydrolysis and cation exchange chromatography appeared to contain a number of radioactive components (Figure 3). The three largest peaks eluted at the same volumes as carnitine, betaine and choline, respectively and were in ratios of approximately 5:3:6. Other compounds in smaller peaks have not been identified but those at 31 and

1. The degradation of a [^{14}C] - methyllysine containing derivative of porcine elastase by rat liver has recently been reported to give rise to small amounts of labeled methionine (19).

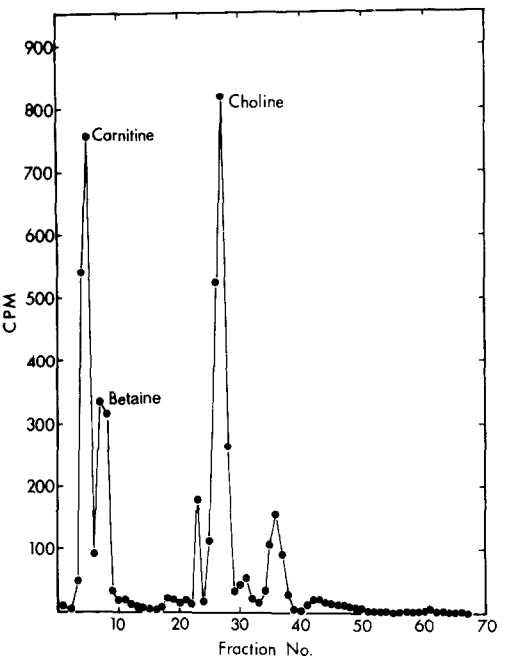

Figure 3. Cation Exchange Chromatography of the Hydrolyzed TCA-Soluble Fraction. Radioactivity in 1 ml fractions obtained from the acid hydrolyzed, TCA-soluble fraction from 64 mg, wet weight of liver upon chromatography on type PA-35 resin as described in the text.

36 ml eluted at the same volumes observed for authentic dimethyl- and monomethyllysine, respectively.

The results described above appear to indicate the existence of pathways in the domestic chicken whereby methyl groups of dietary ϵ-N-methyl- and ϵ-N-dimethyllysine residues are utilized for the synthesis of several one-carbon pool compounds. The apparent formation of carnitine in the present case and the apparent lack of its formation from these same methylated amino acid residues in rat liver (10) may reflect a variety of factors including the species difference. Thus, for example, unlike most mammalian species, young chickens have a specific dietary requirement for choline which is thought to reflect very low levels of S-adenosylmethionine: phosphatidylethanolamine methyltransferase activity (20,21).

The rapid absorption and high incorporation of label into tissue suggests the existence of an efficient salvage mechanism for these minor dietary amino acid residues. Although present only in small amount in the few dietary proteins which have been examined (22), this rapid and efficient incorporation into the one-carbon pool suggests that their presence in the diet may be nutritionally significant for the chicken, presumably as an alternate source of one-carbon units. Additional studies to characterize the metabolic pathways and possible nutritional consequences support the above results and will be reported in detail later.

1. Murray, K. (1964) Biochemistry 3, 10-15.
2. Paik, W. K. and Kim, S. (1967) Biochem. Biophys. Res. Commun. 27, 479-483.
3. DeLange, R. J., Glazer, A. N. and Smith, E. L., (1969) J. Biol. Chem. 244, 1385-1388.
4. Huszer, G., (1972), J. Biol. Chem. 247, 4057-4062.
5. Kakimoto, Y. and Akazawa, S. (1970) J. Biol. Chem. 245, 5751-5758.
6. Paik, W.K., and Kim, S. (1970) J. Biol. Chem. 245, 6010-6015.
7. Horne, D. W., and Broquist, H. P. (1973) J. Biol. Chem. 248, 2170-2175.
8. Tanphaichitr, V., and Broquist, H. P. (1973) J. Biol. Chem. 248, 2176-2181.
9. Paik, W. K., Nochumson, S. and Kim. S. (1977) Trends Biochem. Sci. 2, 159-161.
10. LaBadie, J. H., Dunn, W.A. and Aronson, N. N. (1976) Biochem. J. 160, 85-95.
11. Kim, S., Benoiton, J., and Paik, W. K. (1964) J. Biol. Chem. 239, 3790-3796.
12. Paik, W. K. and Kim. S. (1974) Arch. Biochem. Biophys. 165, 369-378.
13. Neuberger, A. and Sanger, F. (1944) Biochem. J. 38, 125-129.
14. Lee, H. S., Sen, L. C., Clifford, A. J., Whitaker, J. R. and Feeney, R. E. (1978) J. Nutr. 108, 687-697.
15. Means G. E. and Feeney, R. E., (1968) Biochemistry 7, 2192-2201.
16. Lin, Y., Means, G. E., and Feeney, R. E. (1969) J. Biol. Chem. 244, 789-793.
17. ElAmin, B., (1977) M. S. Thesis, The Ohio State University, Columbus, Ohio. p. 13.
18. Hulse, J. D., Ellis, S. R., and Henderson, L. M. (1978) J. Biol. Chem. 253, 1654-1659.
19. Gan, J. C. and Fretz, J. C. (1978) Fed. Proc. 37, 1811 (abstract no 2964.
20. Jukes, T. H., Oleson, J. J., and Dornbush, A. C. (1945) J. Nutr. 30, 219-223.
21. Molitoris, B. A. and Baker, D. H. (1976), J. Nutr. 106, 412-418.
22. Matsuoka, M. (1972) Seikagaku 44, 364 as quoted by Paik, W. K. and Kim, S. (1975) Advances in Enzymology 42, 227-286.