ε-N-Methyl Lysine: an Additional Amino-acid in Human Plasma

We have found that plasma of fasting humans usually contains small amounts of a previously unrecognized amino-acid which we have tentatively identified as ε-N-methyl lysine. We first noted the presence of this compound when we began chromatographing plasma samples from adults with a new automatic amino-acid analyser technique[1], which uses an elution gradient of lithium citrate buffers instead of the usual sodium citrate buffers. Our procedure alters the order of emergence of certain amino-acids from the column, and we were surprised to observe the regular appearance on chromatograms of a small unidentified peak between lysine and histidine. 1-Methyl histidine, 3-methyl histidine and the dipeptides anserine and carnosine are all eluted after histidine with our technique, and no known ninhydrin-positive components of plasma should have been eluted between lysine and histidine.

We isolated the unknown compound from two specimens of plasma, selected because they contained relatively large amounts of it. One of the plasmas had been obtained from a normal male adult, and the other from a woman suffering from Huntington's chorea. We chromatographed a large volume of each of these plasmas on a 120 cm column of "Chromobeads *B*" resin on a Technicon amino-acid analyser, using the lithium buffer technique[1]. The zones of column effluent calculated to contain the unknown compound were collected from each of the preparative columns before the effluent was mixed with ninhydrin solution in the proportioning pump. These effluents were desalted on columns of 'Dowex 50 × 2', H^+, by a modification of the technique of Kakimoto and Armstrong[2]. After removal of the lithium citrate buffer, the unknown compound was eluted from the 'Dowex 50' columns with ammoniacal ethanol and taken to dryness on a rotary evaporator.

We could then chromatograph or electrophorese small amounts of the unknown compound on paper, as well as study its behaviour with different procedures on the amino-acid analyser. Table 1 shows that the unknown compound and authentic ε-N-methyl lysine (CalBiochem) behaved identically when chromatographed on paper in four different solvent systems, during high voltage paper electrophoresis, and when chromatographed on the amino-acid analyser with three different techniques. Both the compound isolated from plasma and authentic ε-N-methyl lysine gave a purple colour when paper chromatograms were sprayed with ninhydrin. Neither substance gave the red colour yielded by N-methyl α-amino-acids when sprayed with *p*-nitrobenzoyl chloride and pyridine[3]. The unknown compound was not altered by hydrolysis in 6 N hydrochloric acid for 16 h at 110° C. Thus it seems highly likely that the compound isolated from plasma of fasting humans is ε-N-methyl lysine.

Table 1. CRITERIA USED IN IDENTIFICATION OF ε-N-METHYL LYSINE ISOLATED FROM HUMAN PLASMA

Property		Compound isolated from plasma	Authentic ε-N-methyl lysine	Lysine
R_F* in	PAA	0·37	0·37	0·39
	IF	0·28	0·28	0·21
	BuAc	0·11	0·11	0·11
	IA	0·24	0·24	0·12
Electrophoretic mobility†		−6·7 cm	−6·7 cm	−5·5 cm
Emergence of peak on amino-acid analyser chromatogram:‡				
60 cm sodium column, 60° C		11 min after orn, 10 min before his	11 min after orn, 10 min before his	11 min after orn, 10 min before his
120 cm lithium column, 70° C		33 min after lys, 8 min before his	33 min after lys, 8 min before his	
120 cm lithium column, 65° C		34 min after lys, 16 min before his	34 min after lys, 16 min before his	

* Solvents used in paper chromatography were: PAA, pyridine–acetone–ammonium hydroxide–water (45 : 30 : 5 : 20); IF, isopropanol–formic acid–water (75 : 12·5 : 12·5); BuAc, *n*-butanol–acetic acid–water (12 : 3 : 5); IA, isopropanol–ammonium hydroxide–water (8 : 1 : 1).

† Electrophoresis was carried out on Whatman 3*MM* paper in 0·1 M borate buffer, *p*H 10·0, for 45 min at 3 kV (59 V/cm). −, Indicates migration towards the cathode.

‡ 65 cm column of Technicon amino-acid analyser was developed with sodium citrate buffers by a slight modification of standard method[4]. 120 cm column was developed with lithium citrate buffers, at 70° C for last 14 h of chromatogram[1], or at 65° C for last 14 h.

Orn = ornithine, lys = lysine, his = histidine.

Ambler and Rees[5] were the first to report natural occurrence of ε-N-methyl lysine; they found this amino-acid in acid hydrolysates of flagellin from *Salmonella typhimurium*. Later Murray[6] identified ε-N-methyl lysine residues in acid hydrolysates of histones prepared from calf and rabbit thymus, liver and kidney. To our knowledge, this amino-acid has not been reported previously in free form in either tissues or physiological fluids. Its presence in blood plasma could easily be missed because ε-N-methyl lysine is eluted simultaneously with lysine in the standard short column procedure on the Technicon amino-acid analyser (Table 1). When amino-acid analyses are performed on the two column system[7], ε-N-methyl lysine appears as a shoulder on the descending side of the lysine peak[6]. Thus small amounts of this amino-acid in plasma could also be hidden easily by the larger amounts of lysine present, when specimens of plasma are examined by the technique of Spackman *et al.*[7].

The concentration of ε-N-methyl lysine in the plasmas of most healthy adults ranged from barely detectable traces to 0·003 μmoles/ml., and was comparable in amount with aspartic acid or with 3-methyl histidine in human plasma. Three healthy adults out of twenty examined had fasting plasma concentrations of ε-N-methyl lysine of approximately 0·01 μmoles/ml. In adults suffering from Huntington's chorea or from chronic schizophrenia, we found fasting plasma levels of this amino-acid to be higher; approximately half of these patients exhibited concentrations of 0·01 to 0·02 μmoles/ml. The plasma of such patients contained almost as much ε-N-methyl lysine as it did methionine or α-amino-*n*-butyric acid. We have not detected ε-N-methyl lysine in specimens (2·0 ml.) of cerebrospinal fluid obtained from normal adults or from patients with Huntington's chorea.

Presumably free ε-N-methyl lysine in blood is derived from the degradation of histones. Whether the increased amounts of this amino-acid in the plasma of some patients with serious mental disease reflect abnormalities in endogenous metabolism of histones, or are related to the psychotropic drugs given to these patients, or to other environmental factors, merits further investigation.

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Present Knowledge concerning the Amino-acid Sequence of Cow κ-Casein

In a recent article, Hill and Wake[1] suggested that the basis for the micelle stabilizing property of cow κ-casein was its amphiphile nature, for the N-terminal two-thirds of the molecule were hydrophobic and the C-terminal