Transport of N-α(or N-ε)-L-methionyl-L-lysine and acetylated derivatives across the rabbit intestinal epithelium

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Intestinal absorption constitutes an important step in the biological utilization of L-Methionine and N-Acetyl-L-methionine covalently bound to ε-NH_2 groups of food protein lysyl residues. The transport of synthesized and purified N-α-(or N-ε-)L-Methionyl-L-lysine and N-α-(or N-ε-)Acetyl-L-methionyl-L-lysine was studied in vitro in rabbit ileum mounted in the Ussing chamber and compared to that of both free L-Methionine and L-Lysine or both free N-Acetyl-L-methionine and L-Lysine. Addition of all solutes to the mucosal reservoir, except N-ε-Acetyl-L-methionyl-L-lysine, led to an increase in the short-circuit current, the lowest response obtained by N-α-Acetyl-L-methionyl-L-lysine. In all cases, only the constitutive amino acids were recovered in the serosal chamber. When free L-Methionine and L-Lysine were added together to the mucosal reservoir, comparable fluxes were obtained; when any of the dipeptides were added, transport of lysine was the highest. Although the mucosal reservoir disappearance of N-α-L-Methionyl-L-lysine was faster than that of N-ε-L-Methionyl-L-lysine, the mucosal to serosal fluxes of their constitutive amino acids were not significantly different. However, the transepithelial flux of L-Methionine originating from the dipeptides was 40–50% less than that of the free amino acid, whereas L-Lysine flux was unchanged. Substantial tissue oxidation of L-Methionine unlike L-Lysine, which are slowly released from peptide hydrolysis by the brush border membrane aminopeptidases, likely altered the amino acid transport. Moreover, the observed higher transepithelial flux for L-Lysine relative to L-Methionine when both were derived from the dipeptides may have resulted from intracellular L-Methionine stimulation of L-Lysine absorption. As compared to the normal di- and isodipeptides, lower net fluxes of L-Methionine, and to a lesser extent of L-Lysine, were observed from the acetylated peptides, the former being zero even in the case of N-ε-Acetyl-L-methionyl-L-lysine. The results demonstrate the significant contribution of brush border aminopeptidases to the intestinal absorption of N-ε-L-Methionyl-L-lysine in the rabbit. Our findings also suggest that the availability of amino acids from acetylated peptides is limited by their transport rates and/or their hydrolysis from cytosolic N-acylpeptide hydrolase and N-acylase.

Keywords: isopeptide; acetylated dipeptide; intestinal absorption; methionine; lysine

Introduction

Covalent attachment of the essential amino acid methionine to dietary proteins has been shown to reduce deteriorative changes that occur during food processing and storage, and to improve the nutritional value of the proteins.[1-5] Moreover, replacing L-Methionine (Met) with N-Acetyl-L-methionine (Ac-Met) in covalently modified casein may prevent the Strecker reaction from forming methional and associated undesirable odors and flavors.[6] Unlike Met, grafted Ac-Met does not appear to improve the bioavailability of the amino acid in the rat.[3,7,8] Ac-Met attached to whole soybean proteins is biologically available, although to a lesser extent than free DL-Met added to unmodified soybean proteins.[9] The bioavailability of Met cova-

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lently attached to ε-amino groups of casein lysyl residues depends on the effectiveness of hydrolysis of the isopeptide bond in ε-methionyllysine by intestinal membrane bound aminopeptidase N.[4,7,10] In addition, mammalian tissues contain enzymes capable of hydrolyzing N-acylated peptides, and deacylating N-acylated amino acids, which together may contribute to the biological utilization of Ac-Met grafted to proteins.[11,12]

Free Ac-Met is reportedly as effective as free Met in improving the quality of vegetable proteins deficient in sulfur amino acids.[13] Relative to Met, however, oral loading with Ac-Met in the pig[14] and human[15] resulted in delayed plasma methionine peak levels. This lag might be due both to slow rates of hydrolysis of acyl groups from acylated amino acids by intestinal acylases[11,13] and of transport of N-substituted amino acids across the intestinal epithelium.[16,17] Previous studies have found that Met is more rapidly absorbed from the dipeptide N-α-L-Methionyl-L-lysine (α-Met-Lys) and the isodipeptide N-ε-L-Methionyl-L-lysine (ε-Met-Lys) than from their acetylated derivatives in rabbit intestinal brush border membrane vesicles.[18] The present study was undertaken in order to evaluate the contribution of cellular deacylating and hydrolyzing enzymic activities to the overall intestinal transepithelial passage of these methionine derivatives. For this purpose, we have compared their transepithelial passage across the rabbit ileum mounted in the Ussing chamber in vitro.

Materials and methods

Peptide synthesis (Table 1)

α-Met-Lys. A solution of N-ε-tert-butyl-oxycarbonyl-L-lysine-tert-butyl-ester hydrochloride (N-ε-Boc-Lys-OtBu.HCl; 2.0 g = 5.9 mmol) and tert-butyloxy-carbonyl-L-methionine N-hydroxysuccinimide ester (Boc-Met-OSu; 1.94 *g* = 5.6 mmol) in dimethylformamide (DMF, 10 ml) was cooled in an ice bath. N,N-diisopropylethylamine (DIPEA; 1.12 ml, 6.5 mmol) was added and the reaction mixture was allowed to warm to room temperature. The acylation was complete within 4 hr. The mixture was then evaporated in vacuo, diluted with ethyl acetate (EtOAc; 200 ml) and washed successively with a saturated solution of $NaHCO_3$ (2 × 100 ml), 1 M NaCl (50 ml), 10% citric acid (2 × 100 ml), and 1 M NaCl (50 ml), dried over Na_2SO_4 and concentrated in vacuo. The residue crystallized from EtOAc/hexane (1/1) at 4° C. Crystals were filtered, rinsed with hexane and dried in vacuo [Yield: 2.35 g (78%)]. Thin-layer chromatography (TLC) showed that the product was homogeneous. N-α-Boc-Met, N-ε-Boc-Lys-OtBu was dissolved in anhydrous trifluoroacetic acid (TFA) and the solution was kept at room temperature for 1 hr. Addition of petroleum ether precipitated α-Met-Lys which was then washed with ether and dried. This compound showed a single peak by reversed-phase HPLC analysis. A Waters-HPLC system (Milford, MA) consisting of two M 510 pumps, a M 720 solvent programmer, a WISP 710 B multisampler, a M 441 fixed-wavelength detector (214 nm), and a 730 two-channel chart recorder was employed for this purpose. The chromatography was performed under isocratic conditions with 0.1% H_3PO_4 and acetonitrile (98:2, vol/vol) using a Merck Lichrosorb C18 reversed-phase column (7 μm, 4 × 250 mm).

α-Ac-Met-Lys (N-α-Acetyl-L-methionyl-L-lysine). N-ε-Boc-Lys-OtBu (2.0 g = 5.9 mmol), N^{α}-9-Fluorenyl-methyloxycarbonyl-L-methionine (Fmoc-Met; 2.2 g = 5.9 mmol) and Benzotriazolyloxy-tris-dimethylamino-phosphoniumhexafluorophosphate (BOP; 2.5 g = 5.6 mmol) were dissolved in 10 ml DMF. The solution was cooled and triethylamine (TEA; 13 mmol) was added. The reaction was monitored by TLC and was complete within 4 hr. The solution was concentrated in vacuo, diluted with EtOAc, washed successively with a saturated solution of $NaHCO_3$, 1 M NaCl, 10% citric acid, and 1 M NaCl and dried over Na_2SO_4. The product N-α-Fmoc-Met, N-ε-Boc-Lys-OtBu was purified by chromatography on silicagel, crystallized from ether/hexane at 4° C and dried in vacuo [Yield: 3.5 g (91%)].

3.1 g (4.8 mmol) of the N-α-Fmoc-Met, N-ε-Boc-Lys-OtBu was dissolved in DMF containing 10% diethylamine to cleave off the Fmoc group (completed within 4 hr). The base and the solvent were removed in vacuo and the resulting product acetylated in 10 ml DMF in the presence of p-nitrophenylacetate (830 mg, 4.6 mmol) and 1-hydroxybenzotriazol (690 mg; 4.6 mmol). After an overnight incubation at an ambient temperature, the solvent was removed in vacuo, the residue dissolved in EtOAc (200 ml), the organic layer washed as described above, dried over Na_2SO_4, and concentrated in vacuo. After purification by silica gel chromatography, N-α-Acetyl-L-methionyl, N-ε-Boc-Lys-OtBu was crystallized from ether-hexane. Crys-

Table 1 Methionine dipeptides synthesized and used in transport experiments

Compound	Structure
N-α-L-Methionyl-L-lysine	CH_3-S-$(CH_2)_2$-CH(NH_2)-CO-NH-CH(COOH)-$(CH_2)_4$-NH_2
N-ε-L-Methionyl-L-lysine	CH_3-S-$(CH_2)_2$-CH(NH_2)-CO-NH-$(CH_2)_4$-CH(NH_2)-COOH
N-α-Acetyl-L-methionyl-L-lysine	CH_3-S-$(CH_2)_2$-CH(NH-$COCH_3$)-CO-NH-CH(COOH)-$(CH_2)_4$-NH_2
N-ε-Acetyl-L-methionyl-L-lysine	CH_3-S-$(CH_2)_2$-CH(NH-$COCH_3$)-CO-NH-$(CH_2)_4$-CH(NH_2)-COOH

tals were then filtered, rinsed with hexane, and dried in vacuo [Yield: 2.1 g (92%)]. Cleavage of the Boc-group and determination of product purity were executed as described for α-Met-Lys.

ε-Met-Lys and ε-Ac-Met-Lys (N-ε-Acetyl-L-methionyl-L-lysine). The experimental procedure was the same as for the synthesis of the corresponding dipeptides α-Met-Lys and α-Ac-Met-Lys, except that N-α-Boc-Lys-OtBu was used as the initial compound for the coupling reaction with either Boc-Met-OSu or Fmoc-Met. N-α-Boc-Lys-OtBu was synthesized as follows. Di-tert-butyl dicarbonate (2.8 g = 13.0 mmol) and TEA (3.8 ml = 27 mmol) were added to a solution of N-ε-carbobenzoxy-L-lysine-tert-butyl-ester hydrochloride (N-ε-CBZ-Lys-OtBu.HCl; 4.0 g = 10.8 mmol) in 80 ml water/acetone (1/1). The solution was stirred overnight at room temperature, then concentrated in vacuo, and the residue dissolved in EtOAc (200 ml). The organic layer was washed with saturated $NaHCO_3$ (2 × 100 ml), 1 M NaCl (50 ml), 10% citric acid (2 × 100 ml), and 1 M NaCl (50 ml), then dried over sodium sulfate and concentrated in vacuo. Filtration through a silicagel column gave a colorless oil which was shown homogeneous by TLC. The N-α-Boc,N-ε-CBZ-Lys-OtBu was dissolved in 200 ml ethanol containing 2 ml 6 N HCl. This mixture was hydrogenated over a 1-hr period in the presence of palladium as a catalyst. After filtration of the catalyst and removal of the solvent, N-α-Boc-Lys-OtBu was used for the synthesis of the isopeptides.

Ussing chamber experiments

Stripped tissues of rabbit ileum were prepared using a modified version of the Ussing apparatus.[19] Male New Zealand White rabbits weighing 2–3 kg were killed by intravenous sodium pentobarbital injection. Segments of distal ileum were removed, rinsed free of intestinal contents, and bathed in 95% O_2–5% CO_2 gassed cold Ringer solution. The external muscle layers were stripped off with fine forceps, and the tissue was opened along the mesenteric border. Pieces of stripped tissue were mounted as flat sheets between the two halves of a Lucite chamber (exposed area 3.14 cm^2) and bathed on both mucosal and serosal sides by 12 ml of isotonic Ringer solution at 37° C containing the following (in mM): 140 Na^+, 5.2 K^+, 1.2 Ca^{2+}, 1.2 Mg^{2+}, 120 Cl^-, 25 HCO_3^-, 2.4 HPO_4^{2-}, 0.4 $H_2PO_4^-$ (pH 7.4). The spontaneous transepithelial electrical potential difference (PD) was measured via Ringer solution in 4% agar bridges placed on both sides of the tissue and adapted to calomel half-cells connected to a high impedance voltmeter. PD was short-circuited throughout the experiment by a short-circuit current (Isc) via Ringer solution in 4% agar bridges placed in each reservoir, adapted to Ag/AgCl electrodes and connected to an automatic voltage clamp (WPI, New Haven, CT). Delivered Isc was corrected for fluid resistance and continuously monitored on a chart recorder. Isc is a measure of ionic movements across the epithelium. A modification of Isc indicates a change in ion transport. Every 50 s the tissue was automatically clamped at ± 1 mV to calculate the electrical conductance (G) of the tissue according to Ohm's law. After the stability of the electrical parameters of the tissue was monitored for 30 min, methionine derivatives were added to the mucosal reservoir for a final concentration of 1.25 mM; Ringer only was used for the control chambers. Aliquots were collected at different times from the mucosal and serosal reservoirs, adjusted to 1% TFA (vol/vol) to terminate the reaction and centrifuged to remove the insoluble material. The sample was then dried in a speed vac concentrator and finally resuspended in an appropriate buffer prior to HPLC or amino acid analysis.

Sample analysis

The concentration of Met, Lys, α-Met-Lys, and ε-Met-Lys in both mucosal and serosal reservoirs was determined by means of a Beckman system 6300 autoanalyzer equipped with a sodium high performance cation exchange column. These compounds were eluted at 27.5, 44.3, 42.6, and 46.4 min, respectively, under the standard gradient conditions provided by the manufacturer. Ac-Met, α-Ac-Met-Lys, and ε-Ac-Met-Lys were assayed at a wavelength of 214 nm by HPLC. The compounds were eluted at an ambient temperature using a linear gradient of 0–30% acetonitrile in 0.1% H_3PO_4 for 20 min and a flow rate of 1 ml/min. Under these conditions, the retention time of the three methionine derivatives was 15.2, 12.2, and 13.4 min, respectively.

Metabolic transformation of Met by rabbit small intestine

Enzymic oxidation of Met or Lys in homogenates from rabbit intestinal mucosa was determined as in ref 20, using L-[1-^{14}C]Met or L-[U-^{14}C]Lys (0.5 μCi/ml, 28 mM) as a substrate (L-[U-^{14}C]Lys was used instead of L-[1-^{14}C]Lys which was not commercially available).

Statistical evaluation

All results are expressed as means ± SD. Where applicable, the experimental data were statistically evaluated by the Tukey's Studentized range test with a P level lower than 0.05 considered significant.

Chemicals and reagents

L-[1-^{14}C]Met (51.5 mCi/mmol) and L-[U-^{14}C]Lys (282 mCi/mmol) had a radiochemical purity of 98% and were provided by the Commissariat à l'énergie atomique (Gif-sur-Yvette, France) and Amersham (Les Ulis, France), respectively. Most of the reagents used for peptide syntheses and commercial α-Met-Lys utilized for controls were from Bachem (Bubendorf, Switzerland). All other chemicals for the experiments were of the highest purity available.

Table 2 Electrical parameters recorded after introduction of 1.25 mM dipeptide, isodipeptide, their acetylated derivatives, or free amino acid to the mucosal side of rabbit ileum mounted in the Ussing chamber

	Ringer	α-Met-Lys	ε-Met-Lys	α-Ac-Met-Lys	ε-Ac-Met-Lys	Lys/Met	Lys/Ac-Met
Isc	26.4 ± 3.5	49.3 ± 1.4	50.9 ± 4.3	34.5 ± 2.9	28.5 ± 3.9	55.7 ± 2.8	49.2 ± 1.4
(μA/cm²)	a	*b	*bd	*c	*ac	*d	*b
PD	−1.4 ± 0.1	−2.8 ± 0.4	−2.6 ± 0.2	−2.2 ± 0.3	−1.3 ± 0.4	−3.2 ± 0.5	−2.3 ± 0.3
(mV)	a	*bcd	*bcd	*bc	a	*d	*bc
G	18.6 ± 2.0	17.6 ± 2.1	19.5 ± 0.2	15.8 ± 2.1	21.6 ± 2.8	17.2 ± 3.6	21.9 ± 2.9
(mS/cm²)	ab	ab	a	b	a	ab	a

Values represent means ± SD for tissues from 8 animals. N-α-L-Methionyl-L-lysine (α-Met-Lys), N-ε-L-Methionyl-L-lysine (ε-Met-Lys), N-α-Acetyl-L-methionyl-L-lysine (α-Ac-Met-Lys), N-ε-Acetyl-L-methionyl-L-lysine (ε-Ac-Met-Lys), mixtures of L-Lysine and L-Methionine (Lys/Met) or N-Acetyl-L-methionine (Lys/Ac-Met), or Ringer only as a control, were added to the mucosal reservoir at time 0. Isc, PD and G were measured between t = 10 and t = 120 min. Values with the same letter within a row (a–d) are not significantly different ($P > 0.05$).
* Statistically different from control ($P < 0.01$).

Results

Electrical parameters

The dipeptide, the isodipeptide, their acetyl derivatives, or a mixture of free Lys and free Met or free Lys and free Ac-Met (1.25 mM) were added to the mucosal side of the rabbit ileum mounted in the Ussing chamber. As shown in *Table 2*, the Isc value was increased following the addition of all the substrates, except ε-Ac-Met-Lys, as compared to the control. The highest value of Isc was observed in the presence of the mixture of free Lys and Met, but it was statistically indistinguishable from the value observed with ε-Met-Lys. In fact, α-Met-Lys, ε-Met-Lys, and the mixture of free Lys and Ac-Met produced a similar increase in Isc, which was hardly smaller than that observed with the Lys/Met mixture. The lowest and statistically significant increase in Isc was observed in the presence of α-Ac-Met-Lys. Furthermore, in no case was a marked alteration of tissue conductance (G) observed.

Mucosal degradation of the substrates

After addition of the peptides to the mucosal side of the tissue, their concentration and that of free Lys, Met, or Ac-Met in the mucosal reservoir was measured as a function of time (*Figure 1*). Results showed that α-Met-Lys readily vanished from the solution bathing the mucosal side of the tissue (100% disappearance after 60 min) with a concomitant appearance of the constitutive amino acids (*Figure 1A*), whereas ε-Met-Lys disappearance (30% after 60 min) and subsequent free amino acid release proceeded less rapidly (*Figure 1B*). There was no marked difference in the profiles of free Met and Lys disappearance from the solution bathing the intestinal mucosa (disappearance of 34 and 29%, respectively, after 180 min; *Figure 1C*). A 40-to-60-min lag period preceded the disappearance of α-Ac-Met-Lys from the solution (disappearance of 64% after 180 min) which was accompanied by the release of free Lys and Ac-Met, and to a minor extent of free Met (*Figure 1D*). In contrast, the mucosal concentration of ε-Ac-Met-Lys was minimally altered even after long-term incubations, with an average disappearance of 13% after 180 min (*Figure 1E*). Disappearance of free Ac-Met from the mucosal reservoir was somewhat slower than that of free Lys (disappearance of 12 and 27%, respectively, after 180 min; *Figure 1F*).

In order to examine the possibility of a transaminase-catalyzed catabolic transformation of Met in the ileum of the rabbit, as previously reported in many tissues of the chick[20] and the rat,[21,22] formation of [1-^{14}C]2-keto-4-methylthiobutanoate ([1-^{14}C]KMB) from L-[1-^{14}C]Met was measured in homogenates from the intestinal mucosa and comapred to the oxidation of L-[U-^{14}C]Lys. As shown in *Figure 2*, the time-course of Met oxidation to KMB was linear for a 120-min period and plateaued thereafter. We calculated from the initial rate measured during the period over which the oxidation time-course was linear that the rabbit ileum contains enough enzyme activity to transform 2 nmol of Met/mg of protein per hour. In contrast, L-Lys was poorly oxidized by the mucosal homogenates.

Transepithelial passage of Lys and Met

After addition of the peptides or mixtures of amino acids to the mucosal compartment of the Ussing chambers, no significant amount of intact peptide nor Ac-Met was detected in the serosal reservoir throughout the incubation period. By contrast, a mucosal to serosal net flux of both free Lys and Met was observed following such additions (*Table 3*). Despite the observed variations, the transport of Lys covalently attached to Met through a peptidic or an isopeptidic bond was not statistically different from that of the equivalent free amino acid when incubated with either free Met or Ac-Met. On the other hand, large variations in the net flux of Met were found depending on the molecular form initially added to the mucosal reservoir. The highest value, which was elicited from the mixture of free amino acids, represented 2.0- and 2.7-fold ($P < 0.05$) that obtained from α-Met-Lys and ε-Met-Lys, respectively. Met net flux from both peptides differed by 38%, but the difference was not significant due to the large individual variations.

Mucosal to serosal transport of both amino acids

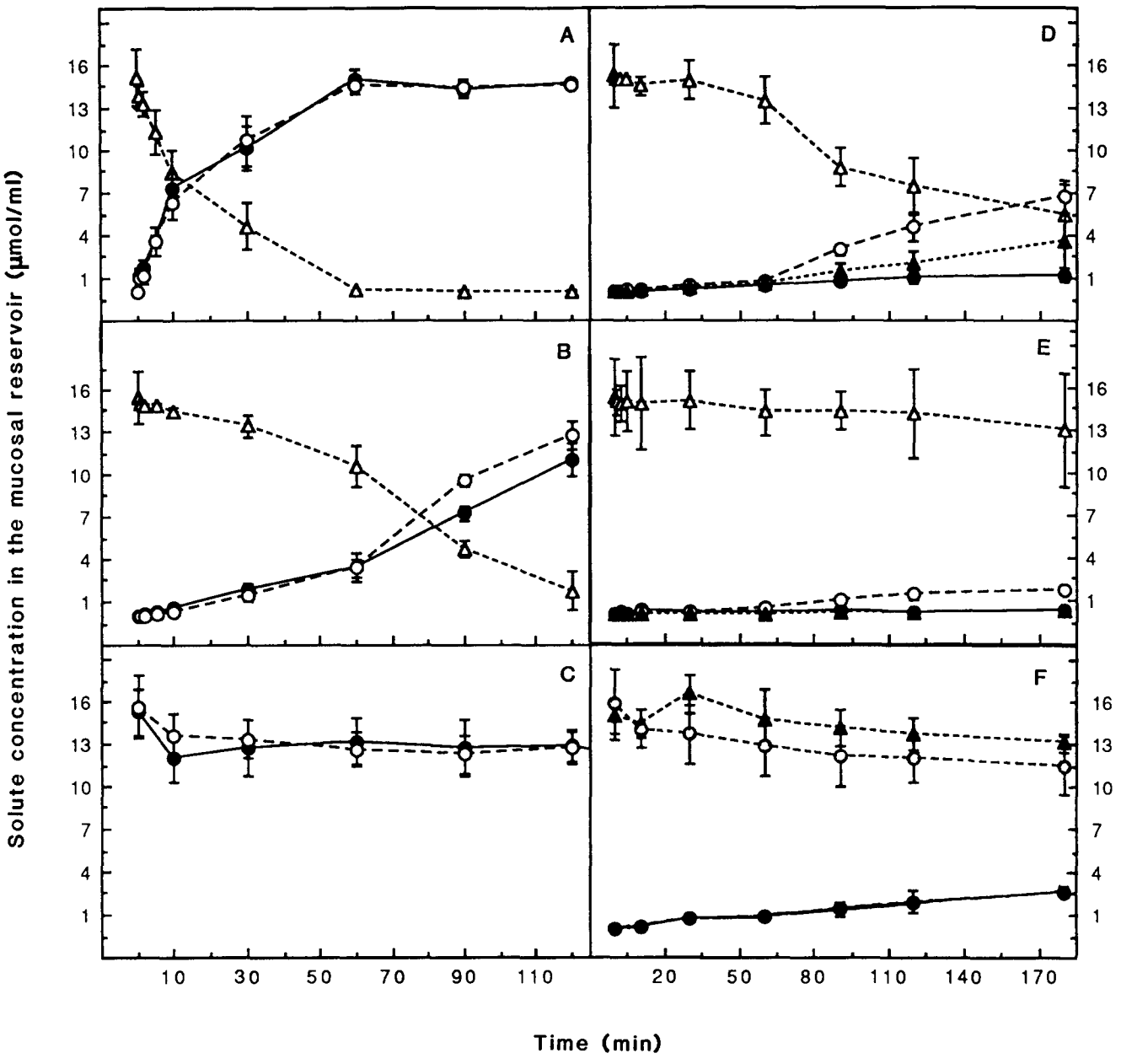

Figure 1 Variation of solute concentration as a function of time on the mucosal side of stripped rabbit ileum mounted in Ussing chambers after addition of 1.25 mM of either N-α-L-Methionyl-L-Lysine (A), N-ε-L-Methionyl-L-Lysine (B), free L-Methionine and L-Lysine (C), N-α-Acetyl-L-methionyl-L-Lysine (D), N-ε-Acetyl-L-methionyl-L-Lysine (E), or free N-Acetyl-L-methionine and L-Lysine (F). Results are expressed as µmol of peptide (△), N-Acetyl-L-methionine (▲), L-Methionine (●), or L-Lysine (○) in the total volume of the reservoir (12 ml). Values are expressed as means ± SD of duplicates from 4–8 animals.

was markedly reduced when the acetylated peptides were substituted for the normal peptides in the mucosal reservoir. The decrease amounted to 64 and 55% for the fluxes of Met and Lys, respectively, in the case of α-Ac-Met-Lys. With the acetylated isodipeptide, Met flux did not differ significantly from zero, while the mucosal to serosal net transfer of Lys fell by 92%. Finally, the transepithelial passage of Met from its acetylated derivative was only 1.2-times greater than that from α-Ac-Met-Lys.

Except when the two amino acids were added in their free form to the mucosal reservoir, the transepithelial transport of Lys was significantly greater than that of Met. Lys/Met flux ratios averaging 2.5 were found in the case of the dipeptide, the isodipeptide and the acetylated dipeptide. Larger differences in Lys and Met tansport were obtained from the other substrates.

Discussion

The present study demonstrates that comparable amounts of Met cross the intact intestinal epithelium regardless of whether the amino acid is initially cova-

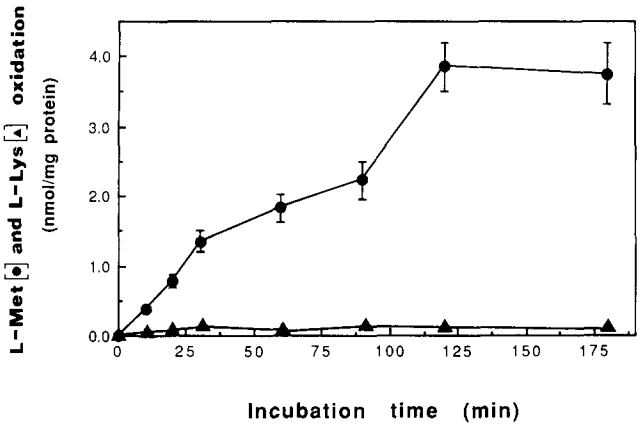

Figure 2 Time-course of L-Methionine (L-Met ●) and L-Lysine (L-Lys ▲) oxidation in homogenates from rabbit ileal mucosa. Incubations were carried out at 37° C in 50 mM sodium pyrophosphate (pH 8.5) containing 28 mM L-[1-^{14}C]Methionine or L-[U-^{14}C]Lysine. The [1-^{14}C]2-keto-4-methylthiobutanoate, which was formed from L-[1-^{14}C]Methionine, and the oxidation products of L-[U-^{14}C]Lysine were assayed as the quantity of $^{14}CO_2$ that had evolved on addition of 30% H_2O_2 (as reported previously in ref. 20). Values represent means ± SD of duplicates from 3 animals. When not indicated, the SD was lower than the corresponding mean value.

lently attached to the ε- or to the α-amino group of Lys. Similar phenomena occur with the transepithelial flux of Lys. In contrast, the absorption of the constitutive amino acids of the acetylated isodipeptide, Ac-ε-Met-Lys, is slower than that from the equivalent acetylated dipeptide, Ac-α-Met-Lys. Even if transport of intact peptides does occur, only the constitutive amino acids are recovered in the serosal reservoir of the chamber.

Uptake of dipeptides theoretically occurs by means of one or both of the following mechanisms: [1] hydrolysis by brush border membrane aminooligopeptidases, followed by uptake of the free amino acids; and [2] uptake of the intact peptide by the appropriate peptide transport system followed by cytosolic hydrolysis.[23,24] The substantial release of free amino acids on the mucosal side of the tissue after addition of the dipeptide or the isodipeptide and the associated electrogenic effect indicates that the first mechanism was predominant in both peptide transport. Previous findings showing that α-Met-Lys and ε-Met-Lys are readily hydrolyzed by brush border membrane vesicles prepared from rabbit small intestine[18] and that both peptides make almost equally as good a substrate for pure aminopeptidases[7,10] also support this hypothesis. Furthermore, it has been demonstrated that the intravesicular Na^+-dependent accumulation of Met is less important from peptides than from an equivalent mixture of free Met and Lys due to the time necessary for peptide hydrolysis.[18] This time factor may have also contributed to our finding of a smaller mucosal to serosal flux of the Met that originated from the peptides as compared to that from the free amino acid mixtures. However, since our findings showed that Lys was transported through the intestinal epithelium regardless of the form it was presented, the hypothesized peptide hydrolysis step must not have been sufficient in itself to alter the flux of Met. The latter may indeed undergo catabolic transformations following its absorption by enterocytes in rabbit ileum mounted in Ussing chambers, as suggested by the substantial rates of Met oxidation to KMB in homogenates from rabbit small intestinal mucosa. Such catabolic transformations have been observed in both the chick[20] and rat,[21,22] while small intestinal metabolization of Lys appears to be absent in the three species (our results and reference 25).

The comparable transepithelial fluxes for free Met and Lys could have resulted in part from stimulation of Lys entry into the enterocytes, in addition to catabolic transformation of the sulphur amino acid. Previous studies have shown that, at millimolar concentrations, Lys is mainly transported by a low capacity, high affinity Na^+-independent carrier, unlike Met whose absorption primarily involves a high capacity,

Table 3 Mucosal to serosal transport of L-Lysine (Lys) and L-Methionine (Met) after introduction of 1.25 mM dipeptide, isodipeptide, their acetylated derivatives or free amino acid to the mucosal side of rabbit ileum mounted in the Ussing chamber

Transported amino acid	α-Met-Lys	ε-Met-Lys	α-Ac-Met-Lys	ε-Ac-Met-Lys	Lys/Met	Lys/Ac-Met
Lys	226 ± 128	181 ± 72	101 ± 21	19 ± 9	210 ± 35	163 ± 61
	(8)	(7)	(7)	(5)	(3)	(4)
	abc, 1	bdgh, 1	fhik, 1	jk, 1	adef, 1	cegij, 1
Met	98 ± 55	71 ± 20	36 ± 14	1.2 ± 1.5	193 ± 52	44 ± 21
	(9)	(7)	(7)	(6)	(3)	(4)
	ab, 2	acd, 2	ceg, 2	fg, 2*	1	bdef, 2

Values (nmol/h.cm^2) represent means ± SD for tissues from the number of animals stated within brackets. N-α-L-Methionyl-L-lysine (α-Met-Lys), N-ε-L-Methionyl-L-lysine (ε-Met-Lys), N-α-Acetyl-L-methionyl-L-lysine (α-Ac-Met-Lys), N-ε-Acetyl-L-methionyl-L-lysine (ε-Ac-Met-Lys), mixtures of L-Lysine and L-Methionine (Lys/Met) or N-Acetyl-L-methionine (Lys/Ac-Met), or Ringer as the control, were added to the mucosal reservoir at time 0. The rate of Lys and Met appearance in the serosal reservoir was measured between t = 30 and t = 180 min and adjusted by the control value. Values with the same letter within a row (a–k) or number within a column (1–2) are not significantly different ($P > 0.05$).

* Not statistically different from zero.

low affinity Na^+-dependent transporter.[26] However, it appears that intracellular neutral amino acids are able to increase both Lys efflux across the basolateral membrane[25] and entry across the brush border membrane of enterocytes.[27] Met is particularly effective in stimulating unidirectional mucosal to serosal flux of Lys across rat small intestine mounted in Ussing chambers.[28] However, to what extent such allosteric interactions may account for the observations made in the present study cannot be precisely determined.

The time course of appearance in the mucosal reservoir of the Lys, Ac-Met and Met that originated from the acetylated peptides, and the values for the mucosal to serosal flux of the constitutive amino acids clearly shows that peptide hydrolysis preceded the deacylation step. Furthermore, α-Ac-Met-Lys and, to a lesser extent, ε-Ac-Met-Lys slowly disappeared from the solution bathing the mucosal side of the tissue. These observations thereby confirm previous suggestions that the step for hydrolysis of these peptides does not take place at the surface of the brush border membrane, and that in vesicles they are absorbed via a Na^+-independent and nonelectrogenic pathway at rates largely inferior to those measured with the corresponding nonacetylated peptides.[18] Although previously demonstrated for many other nonhydrolyzable dipeptides,[29,30] activation of acetylated dipeptide transport by a H^+-gradient has never been demonstrated. Because substantial amounts of Lys were recovered in the serosal reservoir prior to Met, it appears from the present study that α-Ac-Met-Lys was hydrolyzed through a N-acylpeptide hydrolase-catalyzed reaction in the cytosol of the intestinal cells following its absorption. N-acylpeptide hydrolase has been identified in several tissues of the rabbit,[31,32] but to our knowledge never in the intestinal epithelium. Furthermore, our results suggest a strong preference of the rabbit intestinal N-acylpeptide hydrolase for the peptidic bond as compared to the isopeptidic bond. As a consequence of the very slow hydrolysis of ε-Ac-Met-Lys, the mucosal to serosal flux of Met from this peptide was not significantly different from zero.

The appearance of Met from the two acetylated peptides in the serosal reservoir of the Ussing chambers was severely delayed by the deacylation step of the released Ac-Met. Since similar transepithelial net fluxes of Met were observed from α-Ac-Met-Lys and Ac-Met, one can assume that the absorption plus hydrolysis of the former proceeded as rapidly as uptake from the latter. Results from the present study show that rabbit enterocytes contain N-acylase active towards Ac-Met. Such enzyme activity has already been described in the rat, chick, human, and monkey small intestine[13] and generally is thought to be present in the cytosol of cells.[33] Furthermore, in the rabbit, the capacity to deacylate various acylamino acids has been demonstrated in tissues other than the small intestine.[31]

In conclusion, the biological availability of Lys and Met linked via a peptide or an isopeptide bond does not greatly differ in the rabbit in view of the transepithelial net flux of both amino acids from these different sources. It is likely that covalent attachment of Met to food vegetable proteins or casein may improve their nutritional quality and benefit the growing rabbit, as for the growing rat.[7] In contrast, it does not appear that α-Ac-Met-Lys and ε-Ac-Met-Lys are utilized by this species as well as the corresponding nonacetylated peptides. However, studies focusing solely on intestinal transport are not sufficient to definitively draw such a conclusion. Furthermore, we feel there is a strong possibility that the catabolic process is amplified in isolated small intestine mounted in Ussing chambers, resulting in an underestimation of peptide transport activities. One therefore cannot discard the eventuality that in vivo significant amounts of acetylated peptides could be recovered in the portal blood of animals fed modified proteins. The contribution of hepatic and peripheral N-acylpeptide hydrolases and N-acylases to the biological utilization of these peptides would then be of importance.

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