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SHORT COMMUNICATIONS

Bestatin transport in rabbit intestinal brush-border membrane vesicles

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Abstract—The effect of papain treatment on bestatin uptake by rabbit intestinal brush-border membrane vesicles (BBMVs) was studied. Papain treatment of BBMVs effectively diminished aminopeptidase activity but not bestatin uptake by a H^+/dipeptide cotransporter. Bestatin uptake by BBMVs was composed of two saturable components, and after papain treatment the high-affinity component disappeared while the low-affinity component was retained. These findings suggest that high- and low-affinity components represent bestatin binding to aminopeptidase and the true uptake by the H^+/dipeptide cotransporter, respectively.

Key words: bestatin; dipeptide; intestinal absorption; brush-border membrane; aminopeptidase; papain treatment

Bestatin is an immunostimulant given orally with anticancer chemotherapy [1, 2]. It is a dipeptide containing a β-amino acid, and is a potent inhibitor of several aminopeptidases [3–5]. In intestinal BBMVs,* dipeptides and oral cephalosporins are transported via a H^+/dipeptide cotransporter and bestatin is also a substrate for this transporter [6–11]. However, in contrast to other substrates, bestatin binds firmly to aminopeptidases [3, 5]. We also observed that the activity of aminopeptidase in rabbit BBMVs was inhibited by bestatin with an IC_{50} value of 5.0 μM (mean of two experiments). Therefore the apparent uptake of bestatin by BBMVs may include this binding [10].

Aminopeptidase in BBMVs can be removed by papain treatment [12, 13]. In this report we examined the effect of papain treatment to clarify the nature of the uptake of bestatin by BBMVs.

Materials and Methods

Bestatin and [^{3}H]bestatin (12.7 GBq/mmol) (Nippon Kayaku Co., Tokyo, Japan) were gifts. Papain (EC 3.4.22.2) 2× crystallized suspension was obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.).

BBMVs were isolated from the small intestine of male rabbits by a calcium precipitation method [8]. Papain treatment of BBMVs was carried out as described previously [14]. The uptake of bestatin by BBMVs was measured by a rapid filtration technique [10].

The radioactivity of [^{3}H]bestatin was determined by liquid scintillation counting, using an external standard to correct for quench. Protein was determined by the Bio-Rad Protein Assay Kit with bovine γ-globulin as the standard [15].

Results and Discussion

The effect of papain treatment of BBMVs on aminopeptidase activity was examined. At a papain concentration of 0.20 U/mg BBMV protein, aminopeptidase activity was diminished to less than 10% of control (untreated BBMVs, 1.88 ± 0.19; papain-treated BBMVs, 0.16 ± 0.08 μmol/mg protein/min, mean ± SE of eight experiments). We reported previously that bestatin uptake by rabbit intestinal BBMVs was actively driven by an inward H^+ gradient via the H^+/dipeptide cotransporter [10]. Figure 1A shows the effect of papain treatment on bestatin uptake by BBMVs. The uptake of bestatin was actively driven in the presence of an inward H^+ gradient in both papain-treated and untreated BBMVs. The bestatin uptake per milligram protein was markedly increased by papain treatment. Similar results were obtained for the uptake of cephradine, an aminocephalosporin which is also transported by the H^+/dipeptide cotransporter (data not

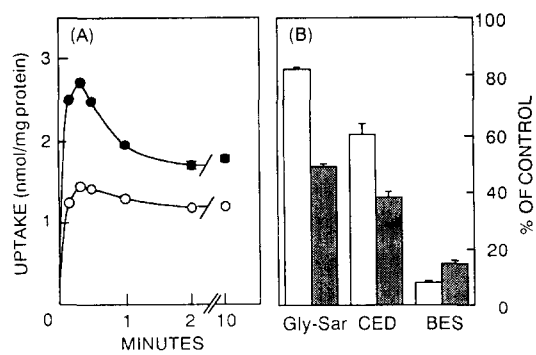

Fig. 1. Effect of papain treatment on bestatin uptake by BBMVs. BBMVs (20 μL, 175–192 μg protein for untreated BBMVs and 82–85 μg protein for papain-treated BBMVs), suspended in 100 mM mannitol, 100 mM KCl and 10 mM HEPES (pH 7.5), were incubated at 37° with the substrate mixture (200 μL) comprising 100 mM mannitol, 100 mM KCl, 10 mM Mes (pH 6.0) and 0.22 mM [^{3}H]bestatin. (A) Time course of bestatin uptake by untreated (○) or papain-treated (●) BBMVs. Each point represents the mean ± SE of three determinations. (B) The uptake of [^{3}H]bestatin for 10 sec was measured in the absence (control) or presence of 10 mM glycylsarcosine (Gly–Sar), cephradine (CED) or unlabeled bestatin (BES). Untreated BBMVs (open column); papain-treated BBMVs (dotted column). Each column represents the mean ± SE of three determinations.

* Abbreviations: BBMVs, brush-border membrane vesicles; Mes, 2-(*N*-morpholino)ethanesulfonic acid.

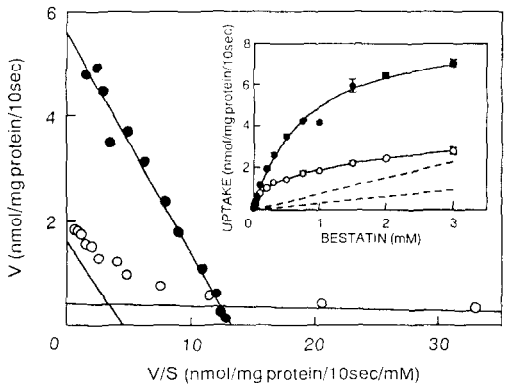

Fig. 2. Eadie–Hofstee plot of bestatin uptake by papain-treated or untreated BBMVs. BBMVs (20 μL, 245 μg protein for untreated BBMVs and 85 μg protein for papain-treated BBMVs), suspended in 100 mM mannitol, 100 mM KCl and 10 mM HEPES (pH 7.5), were incubated at 37° for 10 sec with the substrate mixture (200 μL) comprising 100 mM mannitol, 100 mM KCl, 10 mM Mes (pH 6.0) and varying concentrations of [^{3}H]bestatin. The data were plotted after correction for the non-saturable component. The inset shows concentration-dependent curves of bestatin uptake and the broken lines represent the non-saturable components. Untreated BBMVs (○); papain-treated BBMVs (●). Each point represents the mean ± SE of three determinations. The kinetic parameters were calculated as described previously [10], and the values of estimates ± SE were: $K_{m1} = 4.8 \pm 5.2$ μM, $V_{max1} = 2.48 \pm 0.77$ nmol/mg protein/min, $K_{m2} = 0.36 \pm 0.13$ mM, $V_{max2} = 9.67 \pm 0.96$ nmol/mg protein/min, $K_d = 1.89 \pm 0.35$ nmol/mg protein/min/mM in untreated BBMVs, and $K_m = 0.42 \pm 0.10$ mM, $V_{max} = 33.6 \pm 4.8$ nmol/mg protein/min, $K_d = 4.54 \pm 1.36$ nmol/mg protein/min/mM in papain-treated BBMVs.

shown). Inhibition of bestatin uptake by the substrate for the H^+/dipeptide cotransporter is shown in Fig. 1B. Glycylsarcosine, cephradine and unlabeled bestatin inhibited [^{3}H]bestatin uptake in both papain-treated and untreated BBMVs, indicating that bestatin is taken up by a H^+/dipeptide cotransport system in these BBMVs. The apparent uptake by untreated BBMVs should include a significant amount of bestatin binding to aminopeptidase, because the inhibitory effect of glycylsarcosine and cephradine, which do not bind to the enzyme as strongly as bestatin, was weaker in untreated than in papain-treated BBMVs. Thus, as described previously [12–14], papain treatment removed aminopeptidase and other membrane proteins without impairing bestatin transport by the H^+/dipeptide cotransporter.

Figure 2 shows the concentration dependence of bestatin uptake by BBMVs. The uptake was saturable in both papain-treated and untreated BBMVs. As described previously [10], bestatin uptake by untreated BBMVs was composed of two saturable components. After papain treatment, the high-affinity component disappeared, while the low-affinity component was retained. Taken together, these results indicate that the high- and low-affinity components of the uptake of bestatin by untreated BBMVs represent the binding to aminopeptidase and the true uptake by the H^+/dipeptide cotransporter, respectively.

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