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In vitro transport of β-lactoglobulin across the jejunum of lactose-fed rats

JF Huneau *, D Tomé

INRA, Laboratoire de Nutrition Humaine et Physiologie Intestinale, et INSERM U290, Hôpital St-Lazare, 75010 Paris, France

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Summary — Changes in protein absorption through the intestinal mucosa occur with aging and might reflect modifications in enterocyte membrane characteristics. We have observed that bovine β-lactoglobulin (β-LG) was efficiently transported across the small intestine of adult rats *in vitro* and that 12–16% of the absorbed protein was recovered intact or as large hydrophobic peptides. Ten percent lactose-feeding resulted in decreased tissue conductance and significantly reduced (–58%, $P < 0.05$) β-LG transport across rat small intestinal mucosa. The amounts of β-LG absorbed, either as amino acids and peptides or as intact protein, were reduced to the same extent. Therefore, the effect of lactose feeding might be related to a decrease in protein endocytosis at the brush-border level, rather than to reduced protein transport across tight junctions.

intestine / β-lactoglobulin / lactose / transport / rat

Résumé — Transport *in vitro* de β-lactoglobuline à travers le jéjunum de rats nourris au lactose. *L'absorption intestinale des protéines varie en fonction de l'âge des animaux, peut être en raison de modifications affectant les caractéristiques membranaires des entérocytes. Nous avons observé que la β-lactoglobuline bovine (β-LG) est absorbée efficacement par le jéjunum de rat adulte. Douze à quinze pour cent de la protéine absorbée* in vitro *sont retrouvés intacts ou sous forme de gros peptides hydrophobes. L'introduction de 10% de lactose dans l'alimentation provoque une diminution de la conductance tissulaire et réduit de façon significative (–58%, $P < 0,05$) le transport de la β-LG. Cette réduction affecte de façon similaire les composantes intacte et dégradée du transport de cette protéine. L'effet du lactose pourrait être lié à une réduction de la captation des protéines par endocytose au niveau de la bordure en brosse des entérocytes plutôt qu'à une diminution d'un éventuel transport paracellulaire des protéines.*

intestin / β-lactoglobuline / lactose / transport / rat

* Correspondence and reprints

INTRODUCTION

Bovine milk represents an important source of dietary proteins for humans. In bovine milk, β-lactoglobulin (β-LG) represents the major whey protein, with a concentration of up to 3.7 g/l. β-LG is considered to be responsible for allergic reactions to milk (Fällström *et al,* 1978; Huang *et al,* 1985), and is also suspected to play a role in the transport of ligands through the intestinal epithelium (Papiz *et al,* 1986; Said *et al,* 1989).

β-LG has been demonstrated to be especially resistant to digestion *in vitro* and *in vivo* (Miranda and Pelissier, 1983; Koritz *et al,* 1987; Reddy *et al,* 1988). *In vitro* experiments have led to the conclusion that an uptake of intact β-LG occurs in adult small intestine (Stern and Walker, 1984; Marcon-Genty *et al,* 1989). Up to 0.43 $\mu g \cdot h^{-1} \cdot cm^{-2}$ antigenic β-LG crossed rabbit ileum mounted in the Ussing chamber (Marcon-Genty *et al,* 1989), and using isolated ileal loop *in situ,* up to 2.03 $ng \cdot ml^{-1}$ of antigenic β-LG was recovered in the portal blood per cm^2 of ileal mucosa (Gotteland *et al,* 1989).

Both histologic (Cornell *et al,* 1971; Walker *et al,* 1972) and metabolic (Heyman *et al,* 1982; Marcon-Genty *et al,* 1989) evidence has shown that protein transport across the epithelium is mainly transcellular and proceeds by endocytosis, intracellular transport and exocytosis through the basolateral membrane. Therefore, it has been suggested that modifications in enterocyte membrane composition may affect protein absorption by the gut (Udall and Walker, 1982).

Intestinal epithelium renewal and maturation is affected by many factors such as hormones (Johnson, 1976), microflora (Abrams *et al,* 1963; Meslin *et al,* 1974) and food (Ecknauer *et al,* 1981). Lactose, a disacharide which is known to bypass hydrolysis in adult rat small intestine, may induce changes in intestinal epithelium maturation since 10% lactose feeding has been shown to provoke a significant increase in the epithelium renewal time of the conventional adult rat ileum (Meslin *et al,* 1981).

The aim of the present study was to investigate the effect of lactose-feeding on intestinal protein absorption by the adult rat intestine. For that purpose, the jejunums of lactose-fed and control rats were mounted in Ussing chambers in order to investigate transepithelial transmission of β-lactoglobulin.

MATERIALS AND METHODS

Chemicals

[^{14}C]-formaldehyde (0.74 GBq/mmol) was purchased from Amersham (Les Ulis, France) and bovine β-LG was obtained from Sigma (La Verpillière, France). Radiolabeling of β-LG with ^{14}C was achieved by reductive alkylation of lysine amino groups using [^{14}C]-formaldehyde, as previously described (Jentoft and Dearborn, 1979). Labeled β-LG had a specific activity of 3.9 10^6 cpm/mg.

Animals and diets

Ten male conventional Fisher F344 rats were housed with a constant temperature and lighting cycle, and fed lactose-free commercial pellets (UAR ref 0340, Epinay/Orge, France) from weaning until the age of 9 wks. They were then randomly divided into 2 groups and received for a 4 wk feeding period, either a semi-synthetic (control) diet (57.5% corn starch, 20.5 % casein, 9% corn oil, 5% cellulose, 5% minerals, 8% vitamin mixture), or the same semi-synthetic diet, in which 10% lactose was substituted for an equiv-

alent amount of starch. The animals had free access to water and food. Daily weight gains were similar with both diets (4.1 and 4.0 g/d, for control and lactose diets, respectively).

Ussing chamber experiment

At the end of the feeding period, rats were fasted overnight and anesthetized with an ip injection of sodium pentobarbital (5 mg/100 g body weight). The jejunum (beginning 10 cm distal to the Treitz ligament) was removed, rinsed with cold saline, and mounted in the Ussing chamber, as previously described (Li *et al,* 1989). Both faces of the tissue (exposed area, 0.5 cm^2) were bathed with 10 ml of bicarbonate Ringer containing (in mM): Na^+140, Cl^-120, K^+5.2, Ca^{2+}1.2, Mg^{2+}1.2, HCO_3^-25, HPO_4^{2-} 2.4 and $H_2PO_4^-$ 0.4, pH 7.4, and gassed with O_2/CO_2 (95.5). Short-circuit current (Isc), which reflects ion transport (mainly Na^+ and Cl^-) across the mucosa (Field *et al,* 1971), was continuously monitored. Transepithelial potential difference (PD) was measured at regular intervals and tissue conductance was calculated according to Ohms law: G = Isc/PD, where G is an indicator of tight junction leakiness (Madara, 1989). After checking the stability of electrical parameters for 30 min, 10 mg of β-LG trace labeled with ^{14}C β-LG (final activity 10^6 cpm/mg) were added to the mucosal compartment. Aliquots from both serosal and mucosal reservoirs were collected every 20 min for radioactivity measurements. After 2 h, the entire contents of both reservoirs were collected and subjected to HPLC analysis. Ninety-seven and 98% of the radioactivity added on the mucosal side of the tissue, was recovered in the same compartment at the end of the experiment for control and lactose-fed rats, respectively.

High performance liquid chromatography

Five-ml samples were diluted with the same volume of buffer (water + 0.07% trifluoroacetic acid), and injected into a Waters c18 μbondapack column (3.9 x 30 cm) with a 0.5 ml/min flow rate. Elution of retained material was achieved with a 2-step (0-35 and 35–67%) linear gradient of acetonitrile, at a flow rate of 2 ml/min. Two-ml fractions were collected for radioactivity measurements. Standards of radiolabeled β-LG were chromatographied in the same way.

Statistical analysis

Results are expressed as mean ± SD. Linear regression analysis was used to calculate the rate of protein transport. Analysis of variance and Tukey Studentized *t*-test were used to analyze the results.

Table I. Electrical parameters of control and lactose-fed rats' jejunum in the Ussing chamber. Effect of 1 mg/ml β-lactoglobulin (β–LG). Values are means ± standard errors for $n = 5$ tissues and represent the average value recorded during the 2 h experiments. * significantly different from paired control, $P < 0.05$.

	Control rats		*Lactose-fed rats*	
	Ringer	*β-LG*	*Ringer*	*β-LG*
Isc (μA/cm^2)	42.7 ± 8.1	46.5 ± 7.3	25.8 ± 6.3*	24.4 ± 12.3*
PD (mV)	2.1 ± 0.5	2.0 ± 1.0	1.8 ± 0.3	1.4 ± 0.7
G (mS/cm^2)	20.1 ± 1.3	23.0 ± 2.3	14.7 ± 3.0*	15.5 ± 2.6*

RESULTS

^{14}C β-LG was added, at a final concentration of 1 mg/ml, on the mucosal side of both the control and lactose-fed rat jejunum mounted in the Ussing chamber (table I). Lactose-fed rat tissue exhibited a significantly reduced Isc and G, compared to control tissue. The mucosal addition of β-LG did not induce any variation in electrical parameters in either control, or lactose-fed, rat jejunum.

After the addition of radiolabeled β-LG on the mucosal side of the tissue, the radioactivity that appeared on the serosal side of the tissue was measured at regular intervals (fig 1). The amount of radioactivity recovered from the serosal compartment increased as a function of time. After 40 min, this amount was significantly smaller for the jejunum of lactose-fed rats than for controls, and this difference increased as the experiment progressed. At steady state, the rate of absorption of radiolabeled material was significantly reduced (–58%, $P < 0.05$) in lactose-fed rats compared to controls (8.0 ± 1.7 and 19.0 ± 2.1 μg β-LG•h^{-1}•cm^{-2}, respectively).

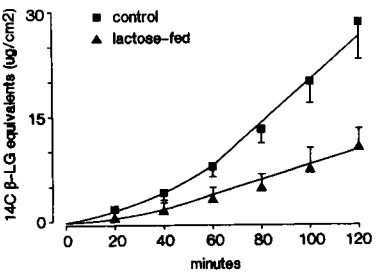

Fig 1. Time dependant accumulation of radiolabeled material in the serosal reservoir of the Ussing chamber for both control and lactose-fed rats. ^{14}C labeled β-LG was added in the mucosal compartment at $t = 0$ min. Absorption is linearly related with time after 60 min.

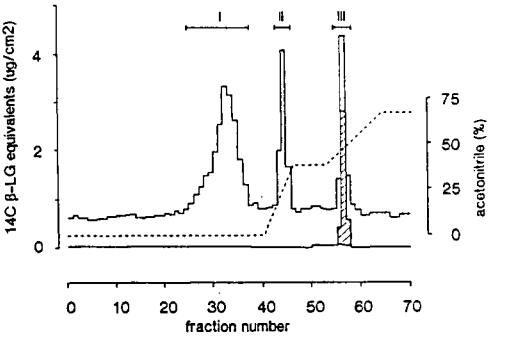

Fig 2. Reverse-phase HPLC analysis of the serosal compartment contents of an Ussing chamber 120 min after the addition of ^{14}C β-LG to the mucosal compartment. 2 ml fractions were collected. Hatched figure represents elution pattern of the labelled β-LG standard. Dotted line represents the % of acetonitrile present in the elution solvent.

Reverse phase HPLC analysis of the serosal contents, collected after 120 min of incubation, revealed the existence of 3 main fractions (fig 2). Fraction I represent unretained hydrophilic material and consisted primarily of amino acids and small peptides. Fraction II and fraction III were eluted with 30% and 46.5% acetonitrile, respectively. The former, most likely consisted of small peptidic fragments, whereas the latter contained mostly intact β-LG and large hydrophobic peptides, as can be assumed by chromatography of β-LG standards. The relative importance of each of these 3 fractions was similar for both lactose-fed and control rats (table II). Fraction III represented 16 ± 8 and 12 ± 7% of the radioactive material for control and lactose-fed rats, respectively. For lactose-fed rats, the amount of protein recovered in fraction III, and fractions I + II, represent 33 and 41% of protein recovered from the

Table II. Amount of radioactive material (in μg equivalents of β-lactoglobulin) recovered in each fraction after reverse phase HPLC analysis of serosal compartment contents. Results represent means ± standard errors for $n = 5$ experiments. Results are expressed in terms of the total serosal compartment contents (10 ml). 90% of the radioactivity injected in the HPLC was recovered.

	Control rats	*Lactose-fed rats*
Total absorbed	27.5 ± 6.6	11.2 ± 2.4
Fraction I	13.8 ± 3.4 (50%)	5.6 ± 2.8 (50%)
Fraction II	9.4 ± 3.0 (34%)	4.2 ± 2.0 (38%)
Fraction III	4.3 ± 2.2 (16%)	1.4 ± 1.1 (12%)

controls. Tissue conductance did not correlate with either the quantity of radioactive material present on the serosal side of the jejunum at the end of the experiment, or the amount of radioactivity recovered in fraction III ($r = 0.21$, NS and $r = 0.18$, NS, respectively).

DISCUSSION

This study shows that β-lactoglobulin is readily absorbed by the small intestine of adult rats, and confirms that part of the absorbed β-LG is recovered on the serosal side of the tissue as intact protein or large hydrophobic fragments. It also demonstrates that β-LG absorption may be reduced by lactose-feeding.

It is well known that protein absorption occurs in the small intestine of adult animals (Cornell *et al,* 1971; Warshaw *et al,* 1974; Heyman *et al,* 1982; Marcon-Genty *et al,* 1989). In the present study, the rate of β-LG absorption through rat jejunum *in vitro*, as determined by transfer of radioactive material, was slightly higher than that previously reported for rabbit ileum (Marcon-Genty *et al,* 1989). The nature of the absorbed material provides information on the intracellular processing which occurs during protein transport (Walker *et al,* 1972; Stern and Walker, 1984). Our results indicate that most of the absorbed β-LG is recovered as amino acids and small peptides, and that about 12–15% is transported as intact protein or large hydrophobic peptides. It has been previously reported that 6–9% of the β-LG that is absorbed by rabbit ileum *in vitro*, is transported as antigenic material (Marcon-Genty *et al,* 1989).

Lactose-feeding of lactase-deficient animals has been shown to affect different aspects of intestinal function, such as mineral absorption (Armbrecht and Wasserman, 1976; Andrieux *et al,* 1982; Debiec *et al,* 1985), steroid absorption (Iritani and Wells, 1966) and epithelial cell turnover (Meslin *et al,* 1981). In the present study, we observed reduced tissue conductance and short-circuit current, and decreased β-LG absorption *in vitro* by lactose-fed rat jeju-

num, compared to the control. Tissue conductance is known to reflect ion permeation through tight junctions (Madara, 1989), and it has been previously observed that G decreases with cell turnover. Therefore, one could assume that lactose-feeding of adult rats results in reduced leakiness of the intestinal epithelium, and that this is a possible consequence of the previously described slowdown of epithelial cell turn-over (Meslin *et al,* 1981). The reduction of β-LG absorption may not result from a change in paracellular transport of proteins since the intact and degraded components of protein transport were decreased to the same extent and no correlation was observed between protein transport and tissue conductance. An alteration of proteolytic activity of epithelial cells also seems unlikely, since the proportion of protein that crossed the mucosa as small peptides or amino acids was the same in control and lactose-fed rats. Rather the difference in β-LG transport across jejunal mucosa could be a result of decreased protein uptake at the brush-border level. This hypothesis is in agreement with the assumption that protein transport across intestinal mucosa is mainly intracellular and that endocytosis is the main limiting step.

The origin of the modification of β-LG uptake by the brush-border membrane of lactose-fed rat intestine remains unclear. Lactase activity of the intestinal mucosa of adult rats is low and lactose is known to be poorly absorbed in the small intestine (Kim *et al,* 1978). Unhydrolyzed lactose has been shown to enhance phosphate diffusion through direct modification of the intestinal brush-border membrane characteristics of the rat (Debiec and Lorenc, 1985). Such a modification might be responsible for the lactose-induced decrease in β-LG absorption. Decreased endocytosis may also result from a slowdown in epithelial cell turnover, previously described (Meslin *et al,* 1981). In this latter case, the effect of lactose should represent a consequence of its action on gut microflora, since the increase in epithelial cell renewal time has not been observed in germ-free rats (Meslin *et al,* 1981). In adult rats, lactose-feeding is known to shift the microflora to a fermentative type in the large bowel and to a lesser extent in the small intestine (Adrian and Frangne, 1978; Wise *et al,* 1984; Morishita and Shiromizu, 1987). However, there is no simple hypothesis to explain how such a shift could result in decreased epithelial turnover.

These results illustrate the impact of particular nutrients on intestinal function. Whatever the mechanism, lactose seems to strengthen the intestinal barrier against luminal macromolecular antigens and might, therefore, contribute to intestinal maturation in the young mammal.

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