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RAPID COMMUNICATIONS

Occurrence of Indigestible Pyroglutamyl Peptides in an Enzymatic Hydrolysate of Wheat Gluten Prepared on an Industrial Scale

Keywords: *Peptide; pyroglutamyl; indigestible; N-terminal-blocked; wheat; gluten; glutamine; pyroglutamate aminopeptidase; enteral nutrition*

INTRODUCTION

Glutamine is a preferred energy source for rapidly proliferating cells of the gastrointestinal tract and immune system. During episodes of malnutrition, surgical stress, immunosuppression, and critical illness, glutamine is considered as a conditionally indispensable amino acid, whereas it is classified as a nonessential (dispensable) amino acid under normal conditions (Lacey and Wilmore, 1990; Shabert and Ehrlich,1994). Therefore, there has been growing interest in supplementing critically ill patients with glutamine. Glutamine, however, easily forms a pyrrolidone ring, resulting in the occurrence of pyroglutamic acid in aqueous solution as shown in Figure 1. Pyroglutamic acid does not possess the beneficial activities of glutamine. To overcome the instability of glutamine in solution, glutamine-containing peptides such as alanylglutamine have been synthesized, and this peptide can be a good substitute for free glutamine (Scheppach et al., 1994). In addition, a peptide mixture derived from naturally occurring glutamine-rich proteins such as wheat gluten has been demonstrated to be a stable and inexpensive glutamine source for oral and enteral formula (Tanabe et al., 1993). To date, a few enzymatic hydrolysates of gluten have been prepared on an industrial scale. However, the fate of glutamine residue in such preparations has not been examined, because conventional amino acid analyses based on acid and alkaline hydrolysis completely degrade glutamine.

In this paper, we report the occurrence of a significant amount of indigestible pyroglutamyl peptides in an enzymatic gluten hydrolysate prepared on an industrial scale.

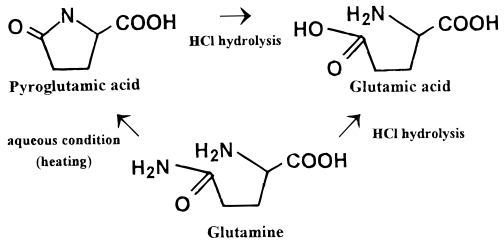

Figure 1. Chemical structures of glutamine, pyroglutamic acid, and glutamic acid.

MATERIALS AND METHODS

Materials. Enzymatic wheat gluten hydrolysate was prepared on an industrial scale according to the method of Motoi (1994) as summarized in Figure 2.

Exhaustive Proteolytic Digestion. To liberate glutamine from the enzymatic gluten hydrolysate, an exhaustive proteolytic digestion was performed according to the method described previously (Sato et al., 1992).

Isolation of N-Terminal-Blocked Peptides. An AG50WX8 strong cation exchanger (Bio-Rad, Hercules, CA) was packed in a minispin column (10 × 5 mm, i.d., AB-1150, Atto, Tokyo, Japan). The column, which was successively prewashed with 50% methanol and distilled water, was equilibrated with 10 mM formic acid. Peptide sample (50 μg/100 μL) was applied to the minispin column. N-Terminal-blocked peptides were eluted with 10 mM formic acid (100 mL × 3 times).

Pyroglutamate Aminopeptidase Digestion. The N-terminal-blocked peptide fraction was digested with 1 mU of porcine liver pyroglutamate aminopeptidase (Takara, Kyoto, Japan) in 100 μL of the attached reaction buffer at 37 °C for 3 h. The reaction was terminated by adding 10 μL of formic acid.

Determination of Pyroglutamic Acid. The pyroglutamic acid fraction was prepared by using a reversed phase HPLC

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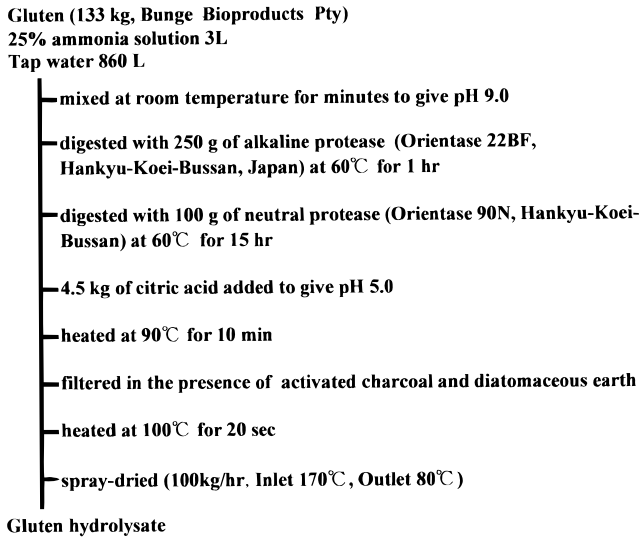

Figure 2. Preparation of gluten hydrolysate on an industrial scale [Bunge Bioproducts Pty is now known as Westin Bioproducts (Altona North, VIC, Australia)].

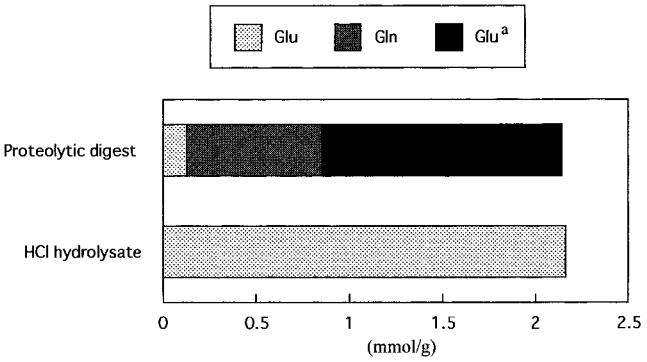

Figure 3. Recovery of glutamine and glutamic acid from the gluten hydrolysate prepared on an industrial scale by exhaustive proteolytic digestion and/or direct HCl hydrolysis. Glu[a] represents glutamic acid liberated from N-terminal-blocked fraction by HCl hydrolysis.

column (J'sphere ODS-H80, YMC, Kyoto, Japan) equilibrated with 10 mM HCl at 1 mL/min. The pyroglutamic acid fraction was applied to an ion-exclude column (HPICE-AS1, Dionex, Sunneyvale, CA) equilibrated with 1 mM octanesulfonic acid containing 2% (v/v) 2-propanol at 0.5 mL/min. Suppressed conductivity was monitored.

RESULTS AND DISCUSSION

As shown in Figure 3, an unexpectedly low amount of glutamine was liberated from the gluten hydrolysate prepared on an industrial scale by the exhaustive proteolytic digestion. Addition of some other proteinases and peptidases including mammalian digestive proteases such as pepsin and pancreatin to the reaction mixture did not improve the recovery of glutamine (data not shown). A significant amount of glutamic acid (Glu[a] in Figure 3), which can make up the low recovery of glutamine by exhaustive proteolytic digest, was liberated from the N-terminal-blocked peptide fraction by HCl hydrolysis, indicating that occurrence of indigestible N-terminal-blocked peptides rich in glutamine. These N-terminal-blocked peptides consisted predominantly of pyroglutamyl peptides, because pyroglutamyl aminopeptidase digestion of them liberated a significant amount of pyroglutamic acid (Table 1).

Tanabe et al. (1993) prepared wheat gluten hydrolysate by digestion with molsin and actinase E without a heating step. They reported >80% of glutamine could be liberated from their preparation by papain digestion. In the present study, direct pyroglutamate aminopeptidase digestion of the gluten hydrolysate without exhaustive proteolytic digestion resulted in liberation of pyroglutamic acid corresponding to the value from its exhaustive proteolytic digest (Table 1), indicating that only a negligible amount of the pyroglutamyl peptides was produced during the analytical exhaustive proteolytic digestion at 37 °C. On the basis of these facts, we hypothesize that peptides with N-terminal glutamine were produced by the industrial proteolysis of gluten and they were converted to pyroglutamyl peptides in the subsequent heating steps (see Figures 1 and 2) and consequently became resistant to the analytical proteolytic digestion including mammalian digestive proteases except for pyroglutamate aminopeptidase. Our preliminary experiment revealed that the N-terminal-blocked peptide could also be produced by heating the enzymatic gluten hydrolysate prepared on a laboratory scale by using analytical grade enzymes and gluten. On the basis of this finding, comprehensive experiments are now being carrried out to define the heating condition under which pyroglutamyl peptides are formed.

Table 1. Compositional Feature of the N-Terminal-Blocked Peptide Fraction from Exhaustive Proteolytic Digest of the Gluten Hydrolysate[a]

Glu[b]	1.14
Gly[b]	0.06
Pro[b]	0.18
pyroGlu[c]	0.41 (0.43)

[a] Values are means of three determinations (mmol/g). [b] Determined after HCl hydrolysis. [c] Determined after pyroglutamate aminopeptidase digestion without HCl hydrolysis. Value in parentheses was determined without analytical exhaustive proteolytic digestion.

The compositional feature of the indigestible N-terminal-blocked peptide fraction by HCl hydrolysis is shown in Table 1. It consisted predominantly of glutamic acid and small amounts of proline and glycine. As free and bound forms of pyroglutamic acid are converted to glutamic acid by acid hydrolysis (Lackey, 1992), the glutamic acid is possibly derived from pyroglutamyl, glutaminyl, and glutamyl residues (see Figure 1). On the assumption that all indigestible N-terminal-blocked peptides are pyroglutamyl peptides, the mean length of the indigestible pyroglutamic peptides in the wheat gluten hydrolysate can be estimated to be 3.4 residues.

Fürst and co-workers (Kuhn et al., 1996a,b) determined peptide-bound glutamine by derivatization of glutamine to acid stable L-2,4-diaminobutyric acid and reported the amount of peptide-bound glutamine in 14 protein/peptide-based enteral products. However, their method cannot distinguish glutamine in the digestible and indigestible peptides. In our preliminary experiment, the indigestible N-terminal-blocked peptides are distributed in some industrial peptide preparations derived from other proteins and commercial peptide-based products. Assessment of the distribution of the indigestible N-terminal-blocked (pyroglutamyl) peptides in other commercial products is now in progress.

Some short-chain pyroglutamyl peptides isolated from biological materials have been demonstrated to show significant biological activities, such as thyrotropin-releasing hormone, inhibitory activity against carcinoma and normal cells in vivo (Paulsen, 1993; Elgjo et al.,

1995), and enhancement of natural cytotoxicity of peripheral blood lymphocytes (Kojima et al., 1993). There are possibilities that some pyroglutamyl peptides in industrial preparations may have some beneficial and/or undesirable biological activities. There are, however, few data on the biological function of the peptides that have been produced unexpectedly during food processing, whereas extensive efforts have been focused to isolate some peptides with specific biological activities from analytical digests of food proteins.

Because of its low contents of lysine and threonine, it is unlikely that the wheat gluten hydrolysate can be used as a single protein source for human nutrition. Nevertheless, if a person were to consume >10 g of the present gluten hydrolysate a day, his or her consumption would exceed 1 g of the indigestible pyroglutamyl peptides. Some of the pyroglutamyl peptides in the present preparation and other industrial peptide preparations may be digested in the gastrointestinal tract by combination with pyroglutamyl aminopeptidase and other peptidases. However, pyroglutamyl aminopeptidase activity in the intestine is significantly lower than in the kidney and liver (Szewczuk and Kwiatkowska, 1970). Some pyroglutamyl peptides may be adsorbed into the blood and/or excreted with feces. The fate of the orally administrated pyroglutamyl peptides and their biological effects should be examined as soon as possible. To solve these problems, development of a large scale preparation procedure of the pyroglutamyl peptides for feeding trials and structural analysis of the pyroglutamyl peptides is necessary.

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