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Heat-induced changes in casein-derived phosphopeptides

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Summary: Phosphopeptides derived from casein may function as carriers for calcium and trace elements. In regard to such specific nutritive effects, the heat-induced changes in tryptic phosphopeptides liberated from bovine sodium caseinate as a model system were investigated. Both microwave and oven heating resulted in a marked loss of peptide-bound phosphorous (dephosphorylation) and a decrease of caseinophosphopeptides in the soluble part of the tryptic hydrolysate. It is concluded that hydrolysis of phosphoseryl to seryl residues was the prevailing degradation step to soluble proteolytic products, whereas lysinoalanyl-casein is claimed to be present almost exclusively in the pH 4.6-insoluble part of the tryptic digest.

Zusammenfassung: Phosphopeptide können als Carrier für Calcium und Spurenelemente dienen. Im Hinblick auf diese spezifischen nutritiven Effekte wurden die hitzeinduzierten Veränderungen tryptischer Phosphopeptide untersucht, die aus Natrium-Caseinat als Modellsystem freisetzbar waren. Sowohl die Mikrowellen als auch die Backofenerhitzung führte zu einer deutlichen Verringerung an peptidgebundenem Phosphor (Dephosphorylierung) und Abnahme der im tryptischen Hydrolysat gelösten Caseinophosphopeptide. Es wird die Schlußfolgerung gezogen, daß die Hydrolyse von Phosphoseryl- zu Serylresten der vorherrschende Abbauweg zu löslichen Proteolyseprodukten war, während Lysinoalanyl-Casein offensichtlich nur im pH-4.6-unlöslichen Anteil des tryptischen Hydrolysates vorlag.

Key words: phosphopeptides; casein; heating; dephosphorylation; nutritive value of proteins

Schlüsselwörter: Phosphopeptide; Casein; Erhitzung; Dephosphorylierung, nutritive Proteinqualität

Introduction

Food proteins are potential precursors of bioactive peptides that are inactive within the protein sequence, but can be released during intestinal digestion (6, 7). These findings offer new aspects for the evaluation of the nutritive value of dietary proteins (8). In particular, milk protein is a rich source of regulatory peptides such as casomorphins and caseinophosphopeptides. Phosphopeptides derived from casein can form organophosphate salts with calcium and trace elements and may function as carriers

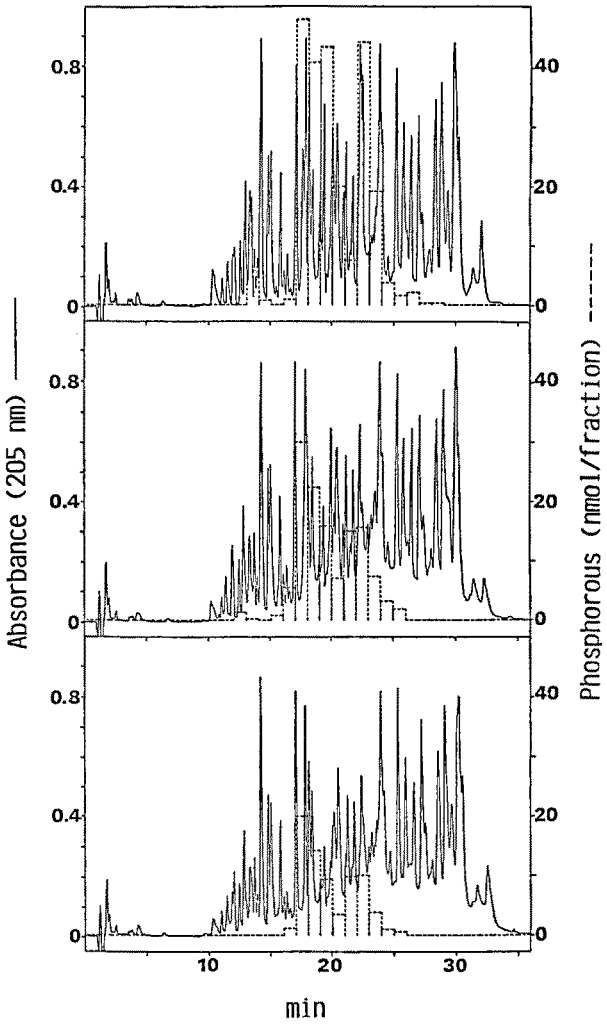

Fig. 1. HPLC elution pattern of peptides and peptide bound phosphorus in the tryptic digest of different heat-treated sodium caseinate: A) unheated; B) microwave heating, 180 W, 60 min; C) oven heating, 170 °C, 75 min.

for a variety of minerals (6, 9, 10). However, heat and or alkali treatment of milk products may lead to dephosphorylation of phosphoseryl residues in casein and to the formation of amino acids not normally found in the native protein (2). The present paper reports the effect of microwave and oven heating, respectively, on the dephosphorylation of bovine casein and liberation of caseinophosphopeptides during in vitro proteolysis of heated casein.

Methods

The heating experiments were performed in a model system with sodium caseinate (Alanate 180, Protein Division New Zealand; Dairy Board). 150 g caseinate (40 % water, w/w) was spread in a glass baking dish (10 × 23 cm). The portions were heated

Table 1. Content of peptide bound phosphorus and serine (μmol/ml) in the pH 4.6-soluble part (filtrate) of the tryptic digest of microwave or oven-heated sodium caseinate. The percentages of destruction (decline in relation to unheated control) are given in parentheses.

		μmol/ml (% destruction)					
		Microwave heating, 180 W			Oven heating, 170 °C		
	Unheated control	Minutes 30	45	60	45	60	75
Peptide bound phosphorus	11.8	10.1 (–14.4)	7.7 (–34.7)	6.3 (–46.6)	8.8 (–25.4)	7.1 (–39.8)	3.7 (–68.6)
Serine + phosphoserine*	28.4	28.4 (– 0)	27.6 (– 2.8)	26.9 (– 5.3)	27.0 (– 4.9)	25.9 (– 8.8)	24.1 (–15.1)

* About 40 % of seryl residues in casein are phosphorylated

either by use of microwaves at 180 W for 30, 45, or 60 min or by use of oven heating at 170 °C (top- and bottom heat) in 45, 60, or 75 min (Siemens, model Top-Line; 2450 MHz/600 W microwave oven, 1700 W baking oven). The determination of lysinoalanine in the heated, lyophilized samples is described elsewhere (3). Tryptic in vitro proteolysis of the same heated samples (5 % solution, w/v, of lyophilized powder) was performed by a titration method (pH-stat, Metrohm) according to reference (4). In the pH 4.6-soluble part (filtrate) of the tryptic digest, peptide separations (HPLC), amino acid analysis, determination of peptide bound phosphorus, free amino groups, and relative chain length of peptides were performed as described in reference (10).

Results

Caseinophosphopeptides could be identified by the relatively high content of peptide bound phosphorus in certain HPLC fractions of the pH 4.6-soluble part (Fig. 1). For both microwave and oven heating, there was a marked decrease of caseinophosphopeptides in all heated samples. This was accompanied by a decrease of peptide bound phosphorus (dephosphorylation) and decomposition of seryl residues (Table 1). Irrespective of the heating method, the content of peptide-bound phosphorus in the tryptic digest was negatively correlated with the content of lysinoalanine in sodium caseinate (Fig. 2). Accordingly, dephosphorylation and formation of lysinoalanine increases with increasing heating time. The lysine content (7.2 mol %) in the soluble tryptic digestion products (filtrate) did not change remarkably with increasing heating time using microwave or oven heating. There were no significant differences in digestibility measured as free amino groups and relative chain length (data not shown) between both heating techniques.

Discussion

Caseinophosphopeptides contain serine phosphate clusters that are essential as binding sites for minerals (6). Hence, partial dephosphoryla-

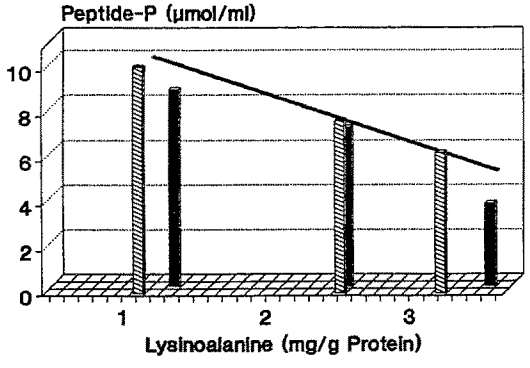

Fig. 2. Relationship between heat-induced changes in the content of peptide-bound phosphorus in the tryptic caseinate digest and lysinoalanine in sodium caseinate; (▨) microwave, (■) oven heating. The solid line represents the linear regression ($r = -0.93$) including all values irrespective of heating method.

tion might deteriorate the mineral binding ability of phosphopeptides. Indeed, it has been reported by Mendy (4) that in patients who had undergone surgical resection of the small intestine, the postprandial calcium absorption was markedly enhanced by a mixture of "native" caseinophosphopeptides. However, this was not the case after ingestion of a heated (in-bottle sterilized) phosphopeptide preparation.

Regarding the reaction mechanism of phosphoseryl destruction, heating of casein solutions at higher temperature (> 110 °C) promotes dephosphorylation of phosphoseryl residues by hydrolysis to serine or by β-elimination to dehydroalanine (2, 1). The formation of lysinoalanine results from the nucleophilic addition of a lysyl residue to an intermediate dehydroalanyl residue. Dehydroalanine may also react with other free amino groups leading to a β-N-alkyl-alanyl- protein or with the thiol group of cystein to form lanthionine (1).

According to the present results obtained with a caseinate model system, the data for dephosphorylation and (phospho)serine destruction in tryptic phosphopeptides are closely correlated to those for lysinoalanine formation in sodium caseinate. The measured decrease of serine can be largely attributed to the destruction of phosphoseryl residues. However, the amount of dephosphorylation was significantly higher as compared to the destruction of serine (see percentages of destruction in table 1). These findings suggest that hydrolysis of phosphoseryl esters to seryl residues – and not β-elimination to dehydroalanine – was the prevailing mechanism of heat-induced degradation of phosphorylated sequences that can be liberated during tryptic digestion. Structural studies of Manson and Carolan (5) have shown that phosphoseryl residues occurring in clusters, such as the sequence SerP-SerP-SerP common to caseinophosphopeptides, did not contribute preferentially to the formation of lysinoalanine.

Interestingly no remarkable losses of lysine were found in the pH 4.6-soluble fraction of the tryptic digested casein. It may be concluded that mainly those phosposeryl residues which occupy isolated positions in the sequence of casein were involved in lysinoalanyl formation, giving rise to pH 4.6-insoluble lysinoalanyl-casein.

The losses of lysine and the presence of hydrolysis-resistant lysinoalanyl of isopeptic cross-links in the pH 4.6-insoluble part of the tryptic digest are problems for additional experiments with the caseinate model system. Further studies are also needed to examine the mechanisms of heat-induced dephosphorylation in milk products and changes that take place in chemical, mineral binding, and nutritional properties of caseinophosphopeptides as digestion products of casein.

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