Zeitschrift für
Ernährungswissenschaft Z Ernährungswiss 33:310–312 (1994)

Kurzmitteilung

Prevention of amino acid racemization during guanidination – a prerequisite for measurement of protein digestibility by homoarginine labeling

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Vermeidung von Aminosäurerazemisierung während der Guanidinierung – Vorbedingung für die Messung der Proteinverdaulichkeit mittels Homoargininmarkierung

Summary: Homoarginine labeling (guanidination) is used to calculate true prececal protein digestibility. A particular worry is that guanidination of proteins at alkaline pH might cause formation of D-amino acids. If D-amino acids show decreased protein digestibility in vivo, as seen in vitro, then the homoarginine method would underestimate protein digestibility. Therefore, the degree of protein racemization was measured during guanidination of casein at pH values between pH 9 and 11 and temperatures between 4 ° and 65 °C. Optimal conditions for the guanidination reaction were 4 °C and pH 10.5–11 or 22 °C and pH 10. A higher pH value at 22 °C or temperatures above 22 °C at each pH leads to the formation of appreciable amounts of D-amino acids.

Zusammenfassung: Die Homoargininmarkierung (Guanidinierung) dient zur Bestimmung der wahren präcaecalen Proteinverdaulichkeit. Die Proteinguanidinierung erfolgt bei alkalischem pH, was möglicherweise zur Bildung von D-Aminosäuren führt. Da D-Aminosäuren enthaltende Proteine *in vitro* eine verminderte Verdaulichkeit zeigen, könnte die Homoargininmethode eine falsch niedrige Proteinverdaulichkeit ergeben. Daher wurde das Ausmaß der Proteinrazemisierung während der Guanidinierung von Casein bei pH-Werten zwischen 9 und 11 und bei Temperaturen zwischen 4 und 65 °C bestimmt. Optimale Guanidinierungsbedingungen lagen bei 4 °C und einem pH-Wert von 10,5–11 oder bei 22 °C und pH 10. Höhere pH-Werte bei 22 °C führten ebenso wie Temperaturen über 22 °C zur Bildung nicht mehr vernachlässigbarer Mengen von D-Aminosäuren.

Key words: Homoarginine labeling – guanidination – racemization – D-amino acids – protein digestibility

Schlüsselwörter: Homoargininmarkierung – Guanidinierung – Razemisierung – D-Aminosäuren – Proteinverdaulichkeit

Abbreviations:
HA: Homoarginine
DM: Dry matter
Asx Aspartic acid + asparagine
Glx Glutamic acid + glutamine

Introduction

Homoarginine-labeling is used to estimate true prececal digestibility of proteins (5). This method is based on transformation of protein-bound lysine into homoarginine by means of guanidination. Homoarginine is absorbed and eliminated like other amino acids, but it is not incorporated in endogenous proteins and does not re-enter the intestine. Only traces of this amino acid are naturally found in mammals. Thus, use of homoarginine-labeled food proteins makes it possible to distinguish precisely between proteins of exogenous and endogenous origin in the chyme. This avoids an underestimation of protein digestibility (9). Guanidination is performed by using O-methylisourea at alkaline pH (4). These reaction conditions may cause racemization of the amino acid residues, especially if the reaction temperature is increased to shorten the reaction time (6). If D-amino acids show decreased protein digestibility in vivo, as seen in vitro, then the homoarginine method would yield falsely low results. Therefore, a model protein was guanidinated under a variety of temperatures and pH values to determine the pH- and temperature-dependency of protein racemization and homoarginine formation and to find optimal reaction conditions.

Materials and methods

Formation of D-amino acids during guanidination was determined using casein as a model protein, because this protein is particularly sensitive to racemization due to its phosphoserine content (1, 6). It was isolated from skim milk in our research center to completely avoid protein damage due to technical processing (8). Casein was guanidinated with 0-methylisourea for 96 h at 4°, 2°, 30° or 65 °C and pH 9, 9.5, 10, 10.5 or 11 and afterwards hydrolyzed with 6 N HCl at 105 °C for 24 h (9). Homoarginine and lysine were measured in the hydrolysate before and after guanidination with an amino acid analyzer (LC 5001, Biotronik, Maintal, Germany) to determine the degree of labeling (9). The ratio of D- to L-amino acids was estimated by chiral phase capillary gas chromatography (Dani 8521, DANI, Monza, Italy) on chirasil-L- and D-val stationary phases using N(O)-trifluoroacetyl- and pentafluoropropionyl-amino acid propyl esters (2, 7). To determine the degree of racemization due to protein hydrolysis, a portion of the casein was not guanidinated, but otherwise treated according to the usual procedure.

Results and discussion

Measurable amounts of D-amino acids already appear during protein hydrolysis without guanidination (Table 1). The effects on amino acid racemization and homoarginine formation of various guanidination conditions are summarized in Table 1. The amino acids listed in this table are those most sensitive to racemization. This is true in particular for Asx and serine. Except at 65 °C, no appreciable amounts of D-amino acids were found when labeling was carried out at pH 9 or 9.5, but the yield of homoarginine was unsatisfactory. Optimal conditions were 96 h, 4 °C and pH 10.5–11 or 22 °C, pH 10, both of which yielded a sufficient degree of labeling without the formation of relevant amounts of D-amino acids. When the guanidination reaction is performed at room temperature (22 °C), the pH must be kept below pH 10.5 to avoid racemization of Asx and serine. At 30 °C increasing amounts of D-Asx and D-serine were observed, and a higher temperature should be avoided in any

Table 1. Amount of D-amino acid formation after guanidination (96 h) at various temperatures and pH values. Mean of six estimations (HA: n = 2); standard deviations in brackets

°C	pH	HA %[1)]	D-As	xD-Serine %	D-Gl	xD-Alanine
Controls[2]		–	2.8 (0.2)	2.4 (0.1)	1.2 (0.1)	1.0 (0.3)
	9	25.6	2.2 (0.3)	2.9 (0.6)	1.0 (0.5)	0.1 (0.6)
	9.5	44.3	1.9 (0.1)	1.8 (0.6)	1.6 (0.7)	1.4 (0.8)
4	10	88.4	2.2 (0.3)	2.3 (0.4)	1.8 (0.7)	1.1 (0.5)
	10.5	93.6	2.7 (0.0)	2.2 (0.8)	1.6 (0.6)	0.7 (0.2)
	11	96.9	2.7 (0.1)	1.7 (0.0)	1.2 (0.5)	1.4 (0.9)
	9	40.2	2.7 (0.4)	2.0 (0.3)	1.1 (0.3)	1.8 (1.3)
	9.5	75.2	2.6 (0.3)	2.8 (0.2)	0.9 (0.2)	1.0 (0.6)
22	10	100.0	2.8 (0.4)	2.4 (0.3)	1.2 (0.3)	1.0 (0.2)
	10.5	100.0	4.3 (0.9)	3.7 (0.3)	1.2 (0.3)	0.9 (0.4)
	11	100.0	8.3 (0.8)	12.9 (0.4)	1.3 (0.4)	1.8 (0.1)
	9	56.3	2.7 (0.8)	3.1 (0.6)	1.4 (0.4)	0.9 (0.3)
30	10	98.1	3.9 (1.2)	3.9 (0.4)	1.3 (0.1)	1.4 (0.6)
	11	100.0	8.4 (0.4)	10.5 (0.5)	11.7 (0.1)	1.6 (0.3)
	9	67.2	8.1 (0.2)	9.9 (0.4)	2.0 (0.3)	0.8 (0.1)
65	10	85.7	13.5 (0.7)	25.8 (0.8)	3.2 (0.3)	1.6 (0.4)
	11	98.0	22.5 (0.4)	41.4 (1.7)	10.8 (0.6)	4.8 (0.7)

[1)] $\% \text{ HA} = \dfrac{\text{nmole HA / g DM} * 100}{\text{nmole HA / g DM + nmole lysine / g DM}}$

[2)] D-Amino acids created by protein hydrolysis without guanidination

case in order to prevent temperature artifacts. When the most frequently used guanidination conditions are applied, i.e., 4 °C and pH 10.5 for 96 h (9), there is no need to worry about a possible impact on protein digestibility due to amino acid racemization.

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Received July 12, 1994
accepted September 30, 1994

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