Guanidination of Lysine in Selected Dietary Proteins

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Lysine in proteins can be converted to homoarginine by the guanidination reaction with O-methylisourea (MIU). Food proteins in which all the lysine has been converted to homoarginine can be used in nutrition studies to directly determine endogenous lysine excretion from the gut. The pH of the reaction solution is known to affect the completeness of conversion of lysine to homoarginine. Optimum pHs of 11.0, 10.7, and 10.8 were found for sodium caseinate, gelatin, and soy isolate protein, respectively, when 2% (w/v) of each protein in 0.6 M MIU was incubated at 20 °C for 144 h. As the protein concentration in 0.6 M MIU was increased above 2% (w/v), the extent of conversion declined markedly for sodium caseinate and the soy isolate but there was only a very small decline for gelatin. With gelatin, a maximum conversion of lysine to homoarginine of 95% was achieved, indicating that guanidinated gelatin is a suitable protein source for directly determining the endogenous excretion of lysine in the mammalian gut.

Recently a novel method was described (Hagemeister and Erbersdobler, 1985; Siriwan and Bryden, 1987) allowing determination of endogenous lysine flow in the small intestine of mammals and birds. The latter measure is required for calculation of true estimates of lysine absorption. The method involves transforming lysine units in dietary protein to homoarginine units. Body proteins do not contain homoarginine; therefore, any unabsorbed homoarginine must have come from the diet rather than the endogenous secretions. The endogenous excretion of lysine has been estimated indirectly by difference between the true and apparent coefficients of homoarginine and lysine absorption, respectively. Being a difference method, however, small errors in the determination of either lysine or homoarginine can lead to a large error in the estimation of endogenous lysine flows. If the conversion of lysine to homoarginine was complete, the endogenous excretion of lysine could be determined directly.

Although the conversion reaction employing O-methylisourea (MIU) has been described (Kapfhammer and Muller, 1934; Greenstein, 1935; Hughes et al., 1949; Mauron and Bujard, 1964), the optimal reaction conditions for dietary proteins have not been fully characterized. The effects of incubation time, temperature, and the MIU concentration with respect to protein have been studied (Klee and Richards, 1957; Kenchington, 1958; Shields et al., 1959; Kassell and Chou, 1966; Maga, 1981). Reaction at room temperature (ca. 20 °C) for 4 days is recommended, and a 5% (w/v) solution of protein in 0.5 M MIU apparently ensures an adequate reagent to substrate ratio. The effect of incubation time on the guanidination of casein has also recently been investigated in our laboratory (Moughan and Rutherfurd, unpublished results). A 2% (w/v) sodium caseinate solution containing 0.6 M MIU was incubated at 20 °C, pH 11.0, for 4, 24, 48, 72, 96, and 144 h. The extent of conversion of lysine to homoarginine increased markedly between 4 (60% conversion) and 24 h (97% conversion) and then plateaued at a 98% conversion after 72 h. In spite of pH having a significant effect on the guanidination reaction and the optimum pH varying with protein source (Hughes et al., 1949; Klee and Richards, 1957; Means and Feeney, 1971; Maga, 1981), this factor has not been widely studied.

The aim of the present study, was to determine the optimum pH for guanidination of lysine in casein, gelatin, and isolated soybean protein. It was hoped that, for at least one of the proteins studied, complete conversion of lysine to homoarginine would be achieved. A further aim was to determine the effect of decreasing the MIU to protein ratio on the conversion of lysine to homoarginine, to ascertain whether use of lower amounts of the relatively expensive reagent impaired conversion.

MATERIALS AND METHODS

Materials. O-Methylisourea sulfate salt was purchased from Sigma Chemical Co. (St Louis, MO) and $Ba(OH)_2 \cdot 8H_2O$ from British Drug House Chemicals Ltd. (Poole, England). Sodium caseinate was obtained from the Dairy Research Institute (Palmerston North, New Zealand), gelatin from Veterinary Distributing Corp Ltd. (Palmerston North, New Zealand), and the isolated soybean protein (isolated soy protein PP 620, Protein Technologies International) from Columbit Marketing Ltd. (Auckland, New Zealand).

Preparation of 0.6 M MIU. The 0.6 M MIU (free base) was prepared by a modified procedure based on the methods reported by Chervenka and Wilcox (1956), Mauron and Bujard (1964), Kassell and Chow (1966), and Hurrell and Carpenter (1974). An approximately 38% (w/v) solution of barium hydroxide was prepared with boiling distilled deionized water that had been degassed (boiled for at least 10 min in a conical flask fitted with a soda lime trap) to prevent the formation of barium carbonate. The barium hydroxide solution was reheated until maximum dissolution had occurred and then quickly added to sufficient MIU for a 0.6 M solution. The solution was left to stand for 30 min before the resulting barium sulfate precipitate was removed by centrifugation at 6400*g* for 5 min at room temperature in a Sorval RC2-B centrifuge equipped with a GSA rotor. The yellow supernatant was removed and the precipitate washed with a minimum volume of distilled water and recentrifuged. The washings and supernatant were pooled. At this stage the MIU solution was between pH 12.5 and 13.5 and was adjusted to pH 11.0 by the gradual addition of 2 M HCl. The solution was made up to 98% of the final volume with distilled deionized water to give 0.61 M MIU, which was diluted to 0.6 M upon addition of the protein.

Reaction of 0.6 M MIU with Intact Proteins at Different pH Levels. Duplicate 2% (w/v) solutions of sodium caseinate, gelatin, and isolated soy protein containing 0.6 M MIU were prepared and adjusted to different pHs by addition of either 2 M HCl or 2 M NaOH. The ranges examined were pH 10.2–

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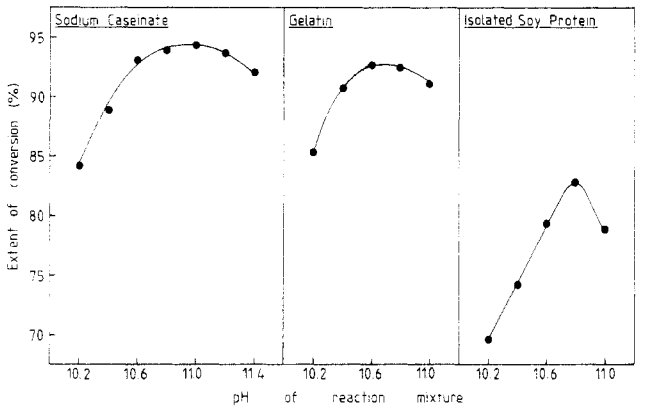

Figure 1. Effect of pH on the extent of conversion of lysine to homoarginine in intact casein, gelatin, and isolated soy protein (incubation at 20 °C for 144 h, 2% (w/v) protein in 0.6 M MIU). Values are the means of duplicate analysis. On average, the difference between duplicates was 3.10% of the mean.

11.4 and 10.2–11.0 for the sodium caseinate and gelatin and for the isolated soy protein, respectively. The solutions were incubated at 20 ± 1 °C for 6 days during which time the pHs were checked daily and adjusted as necessary. After 6 days, unreacted MIU was removed by dialysis against distilled water over 2 days at 20 °C, with several changes of water. The proteins were then freeze-dried, ground with a mortar and pestle, and stored at −20 °C while awaiting amino acid analysis.

Reaction of 0.6 M MIU with Intact Proteins at Different Protein Concentrations. Duplicate batches of 0.6 M MIU each containing 2, 5, 10, 15, and 20% (w/v) sodium caseinate or 2, 5, and 10% (w/v) gelatin or isolated soy protein were prepared as described above. Satisfactory dissolution of the gelatin and isolated soy protein was not achieved at concentrations above 10% (w/v). The material was incubated for 6 days at 20 ± 1 °C at the respective optimum pH. The pH was checked daily and adjusted as necessary. After 6 days, the protein was recovered and dried as described above.

Determination of the Extent of the Reaction. Samples (2–5 mg) of the guanidinated material were subjected to amino acid analysis (ion-exchange chromatography) on a Beckman 119 BL amino acid analyzer. The proteins were hydrolyzed in 0.5 mL of 6 M HCl containing 0.1% phenol in vacuum-sealed glass tubes for 24 h at 110 ± 2 °C. The extent of conversion of lysine to homoarginine was calculated from the levels of lysine and homoarginine present in the sample by the following equation:

$$\text{extent of conversion (\%)} = \frac{\text{nmol homoarginine}}{\text{nmol homoarginine} + \text{nmol Lys}} \times 100$$

RESULTS AND DISCUSSION

The optimum pH for the conversion of lysine to homoarginine in sodium caseinate, gelatin, or isolated soy protein varied (Figure 1). For sodium caseinate, an optimum pH of 11.0 was found, while for gelatin and soy isolate protein, pHs 10.7 and 10.8, respectively, maximized the conversion. It is apparent that to achieve maximum conversion of lysine to homoarginine, not only does the optimum pH vary with protein source but so does the importance of maintaining the pH at that optimum. The sharp pH peak observed with isolated soy protein suggests a strict control of pH at 10.8 is necessary for maximum guanidination, while, for casein and gelatin, maintaining the pH at their respective optima appears not to be so crucial. It is notable that the maximum conversion of lysine to homoarginine at pH 11.0 for the sodium caseinate was 94.5%, which is somewhat lower than the value (98%) obtained in preliminary work adopting the same experimental conditions (Moughan and Rutherfurd, unpublished results). A high conversion rate was found for sodium caseinate and gelatin, but the maximum conversion was considerably lower for the soy isolate protein. This may be because of the different tertiary structures of the proteins (Maga, 1981) among other factors.

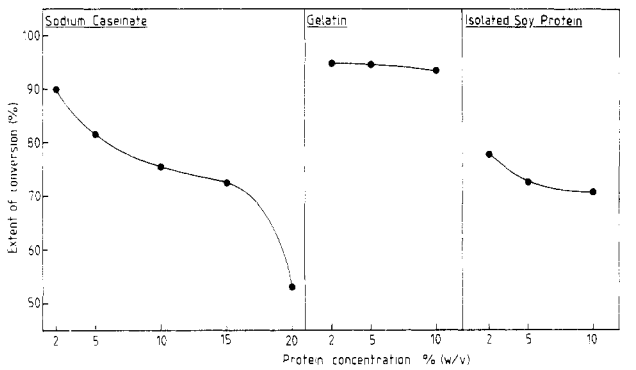

Figure 2. Effect of the protein concentration in the reaction mixture on the extent of conversion of lysine to homoarginine in intact casein, gelatin, and isolated soy protein (incubation at 20 °C for 144 h in 0.6 M *O*-methylisourea at pH 11.0 for casein, 10.7 for gelatin, and 10.8 for isolated soy protein). Values are means of duplicate analysis. On average, the difference between duplicates was 3.9% of the mean.

The effect of protein concentration on the extent of conversion of lysine to homoarginine in sodium caseinate, gelatin, and isolated soy protein is shown in Figure 2. For sodium caseinate there was a rapid decline in percentage conversion for protein concentrations greater than 2% (w/v). A marked decline was also observed for the soy isolate protein, but for gelatin a significant decline in conversion rate was not observed. It was expected, based on the literature, that the protein concentration could have been increased without a decline in the conversion of lysine to homoarginine. The present results, however, at least for sodium caseinate and soy isolate, do not support this, and it would appear worthwhile to examine the effect of decreasing the protein concentration below 2% (w/v) or rereacting the converted material with fresh MIU as described by Hettinger and Harbury (1965). The maximum rates of conversion of lysine to homoarginine at the 2% (w/v) protein concentration were different from those found under seemingly identical conditions in the pH study. There was no apparent reason for this discrepancy.

Complete conversion of lysine to homoarginine was not achieved for any of the proteins studied, which is in accord with the conclusions of Maga (1981). Nevertheless, gelatin, which has a low natural lysine content, underwent near-complete conversion (95%). Further, it can be shown that a semisynthetic diet containing 10% guanidinated gelatin as the sole protein source would contain only 0.014% lysine, thus allowing accurate direct determination of endogenous lysine. When such a diet is fed to animals, their lysine requirement is at least partly met by the partial metabolism of homoarginine to lysine. For gelatin, a 5% (w/v) solution of the protein in 0.6 M MIU may be used to prepare material for feeding to animals. We conclude that guanidinated gelatin is a suitable protein source for directly determining endogenous lysine excretion from the gut.

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Registry No. MIU, 2440-60-0; MIU sulfate, 294227-58-5; L-lysine, 56-87-1; L-homoarginine, 156-86-5; barium hydroxide, 12009-08-4.

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Retinol, Total Carotenoids, β-Carotene, and Tocopherols in Total Diets of Male Adolescents in The Netherlands

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Over a period of 2.5 years, every 3 months 221 different food items forming a "market basket" were purchased, prepared, and divided into 23 food commodity groups. The market basket was based on a study of the dietary intake of 18-year-old males. In the (homogenized) food groups vitamin A, β-carotene, and total carotenoids as well as α-, β-, γ- and δ-tocopherol were determined by chemical analysis. The analyzed total daily amount of vitamin A (1481 retinol equivalents or 4937 IU) can be considered as more than sufficient when compared to Dutch recommendations for male adolescents. Also the vitamin E intake (22.4 IU) meets the requirements. The group meat and meat products shows the highest contribution to the daily amount of retinol (37%); for the carotenoids, the groups leafy vegetables and root vegetables are the most important sources. The group butter, margarine, oils is the most important source for the average daily supply of all analyzed tocopherols (32–49% contribution). Regarding seasonality, highest daily amounts of most vitamins were observed in the months November/December, while lowest daily intakes seem applicable in May.

During the period 1976–1978 the first total diet study in The Netherlands with the market basket approach was carried out (van Dokkum et al., 1982; de Vos et al., 1984).

The aim of total diet studies is to monitor the exposure to additives and contaminants through habitual diets and to estimate the health risk for the consumer, by comparing the actual, analyzed contents with the acceptable daily intake (ADI) as established by FAO/WHO.

Total diet studies as defined and recommended by the FAO/WHO (WHO, 1976, 1985) are also well suited for evaluating the nutritional quality of well-defined diets (EuroNut, 1988).

In the 1976–1978 study, of all vitamins only α-tocopherol was determined; the present study was designed to include other fat-soluble vitamins as well.

In this paper we report results of the second total diet study, carried out in 1984–1986, regarding vitamins A and E. In this study the approach of a "market basket" survey was chosen, based on a dietary intake by a defined population group. The composition of the diet analyzed comprised the average total diet of 18-year-old males. This group probably has the highest food consumption as compared to other age categories and consequently is likely to have the highest intake of additives and con-

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