3:15 Nutritional digestibility of insoluble collagen - influence of hydrochloric acid

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Introduction

When evaluated in rat experiments the digestibility of meat protein is 96% and gelatin 89% (Mauron, 1973). The content of intramuscular collagen varies from about 2 to 20% of the protein in different muscles and in Swedish sausages 35% of the protein can be of collagenous origin (Lawrie, 1979; Fuchs & Kuivinen, 1980). Native collagen is considered to be more or less indigestible (Loewit, 1970; Cheftel, 1979; Kies, 1981; Ashgar & Henrickson, 1982). The denaturation of collagen to gelatin, as e.g. by cooking, makes it susceptible to proteolytic enzymes and thus digestible (Paul, 1972; Rogowski, 1980; Bender, 1980; Prandl, 1980; Ashgar & Henrickson, 1982). In vitro studies have shown that pepsin can, to a large extent, solubilize collagen fibers by cleaving in the non-helical telopeptide region, leaving the collagen helix intact (Weiss, 1976; Etherington, 1977). In vitro studies have shown that the swelling effect of hydrochloric acid on hide collagen was maximal at pH 2 (Gustafsson, 1956) and above pH 4.5 insoluble collagen was totally resistant to degradation by, for example, cathepsins (Bailey & Etherington, 1980). The pH in the human stomach during digestion varies depending on individuals and their diet composition (Gitler, 1964; Davenport, 1977).

The present study was performed in order to establish whether the degradation of insoluble collagen is pH-dependant a) when solubilized in vitro by pepsin b) when digested by rats under conditions where gastric acid production has been inhibited.

Materials and Methods

Gelatin (swine skin, 175 Bloom) and insoluble collagen (bovine Achilles tendon) prepared according to Einbinder & Schubert (1951) were purchased from Sigma. Beef, M. psoas major, was freed of connective tissue and visible fat, minced and freeze-dried. Gelatin, insoluble collagen and meat were studied in vitro and in vivo. In the in vivo experiments, ANRC Reference Protein High Nitrogen Casein (Sheffield Chem. USA) was used as reference protein and fat extracted, lyophilized whole egg was used for the determination of metabolic nitrogen.

In vitro assay

Samples of 0.08 g nitrogen were mixed with 50 ml of hydrochloric acid (HCl) and 50 mg pepsin (porcine pepsin NF, Merck). HCl concentration varied between 0.03 M - 0.0001 M. pH was measured before and after incubation and an average was calculated. The samples were incubated for 2 h at 37°C and the pH was adjusted to 7.0 with NaOH. The samples were filtered through a glass filter with a porosity of 15 - 40 µm and the nitrogen solubilized by pepsin was measured. After incubation with pepsin some samples were adjusted to pH 6.8, 25 ml of 0.1 M sodium phosphate buffer pH 6.8 and 50 mg pancreatin (porcine pancreas 4NF, Sigma) was added and incubation was performed for a further 2 h at 37°C. Trichloroacetic acid (final concentration 25% w/v) was used for protein precipitation and the samples were filtered. The nitrogen solubilized by pepsin/pancreatin/TCA was measured. Samples were analyzed in triplicate. Corrections were performed for enzyme-blanks.

In vivo assay

True digestibility was evaluated by nitrogen balance studies on growing rats. Omeprazole (Hässle, Sweden) was administered by intragastric intubation every 12th h, as described by Laser Reuterswärd & Fabiansson (1984). Diets were given at a level of 1.5% nitrogen (dry basis) of the diet. When using omeprazole samples of meat, gelatin and insoluble collagen were mixed with egg protein as shown in Table 1. Casein was supplemented with methionine (0.22% of the diet) and insoluble collagen with methionine, tryptophan, isoleucine and histidine (totally 0.45% of the diet).

Chemical analyses

Original samples, solutions after enzymatic digestion and faeces were analyzed for nitrogen according to Kjeldahl. Hydroxyproline was analyzed according to Stegeman (1958) as modified by Weber (1973) and the collagen content of original samples was calculated as described by Laser Reuterswärd et al. (1982).

Results and Discussion

The meat sample contained 2.4% collagen of the total protein. The results of in vitro solubilization are shown in Fig. 1. Insoluble collagen was about 90% soluble in pepsin at lower pH-values but at pH 2.5 the solubility started to decrease and was 37% at pH 3.2. The TCA-solubility after pepsin and pancreatin treatment was likewise about 90% at lower pH-values and also started to decrease at pH 2.5. Treatment with pancreatin further solubilized collagen but only to a small extent. The results showed that all collagen solubilized by pepsin was degraded to TCA-soluble peptides by pancreatin. For the gelatin and meat samples a much weaker pH effect was obtained, the solubilities still being high at higher pH-values.

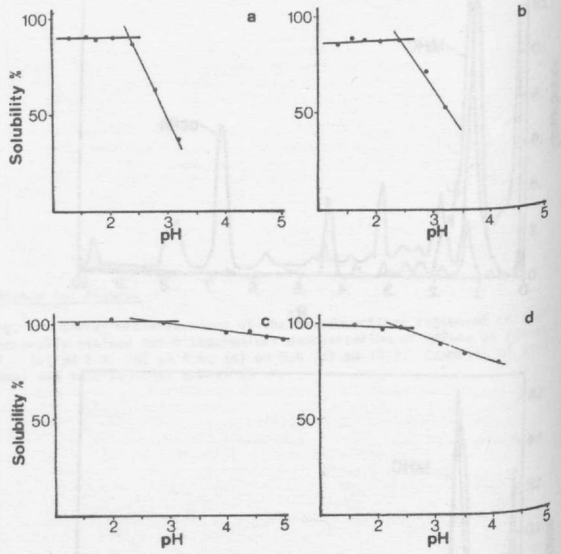

Fig. 1. Nitrogen solubility, as a % of total nitrogen in sample. Sample (a) insoluble collagen, (b) insoluble collagen, (c) gelatin and (d) meat. Incubation for 2 h at 37°C with pepsin in hydrochloric acid at different pH levels. In samples (b), (c) and (d) this was followed by incubation in pancreatin for 2 h at 37°C and precipitated by trichloroacetic acid (25% w/v final concentration). n = 3, standard deviations less than 6.0.

In vivo results are shown in Table 1. Insoluble collagen, without inhibition by omeprazole, was highly digestible, 95%. The calculated digestibility values (egg protein excluded) were, after inhibition of gastric acid production, for meat, gelatin and insoluble collagen, 97%, 99% and 71%, respectively. The value for insoluble collagen was thus significantly ($P < 0.001$) decreased after inhibition by omeprazole. A value of 71%, obtained in vivo, corresponds to a pH of 2.8 in vitro (Fig. 1b). The inhibition by omeprazole results in anacidity for 6 - 8 h after each dose and at least 80% inhibition of gastric acid output over 24 h (Carlsson, personal communication). Thus most of the time pH in the stomachs of rats was quite high.

Protein in diet	Nitrogen content in diet %	n	True digestibility %
Without omeprazole			
Reference casein	1.50	5	100.0 (0.3)
Insoluble collagen	1.50	5	95.2 (2.2)
With omeprazole			
Whole egg	0.72	7	99.9 (2.4)
Meat/Whole egg	0.78/0.72	6	98.4 (2.0)
Gelatin/Whole egg	0.78/0.72	5	99.4 (1.9)
Insoluble collagen/ Whole egg	0.78/0.72	6	85.0 (3.2)

Table 1. True digestibility in rats fed different proteins with and without the inhibition of gastric acid production by omeprazole. Standard deviations within brackets.

Four effects of HCl should be important for digesting insoluble collagen in the gastrointestinal tract of rats. The first effect is to break one of the intermolecular crosslinks of insoluble collagen, the aldimine, which is labile against acid. The aldimine is occurring in tendon in equal proportions as the other major crosslink, the 'keto', but this form is stable against acid (Weiss, 1976; Sims & Bailey, 1981).

The second effect is that of swelling, which has been shown to be maximal at pH 2 for hide collagen (Gustafsson, 1956). Swelling has been indicated to be necessary for the action of enzymes in the interfibrillar space of collagen (Bailey & Etherington, 1980). The action of pancreatin and elastase is known only to occur in the non helical telopeptide region of insoluble collagen (Weiss, 1976; Bailey & Etherington, 1980) and a limited action would therefore occur at the high pH level in the small intestine. Collagenases are the only enzymes cleaving collagen in the intact helix acting at neutral pH (Bailey & Etherington, 1980). However, Fullmer, et al. (1966) stated that they could find no collagenolytic activity in the alimentary tract of rats.

The third effect of HCl is to provide an optimum pH for pepsin activity. Pepsin, like pancreatin, also only cleaves the undenatured collagen in the telopeptide region (Weiss, 1976). It has been shown that fibrous bovine tendon collagen could be degraded by human pepsins at a pH of up to 3.8 (Etherington et al., 1980). This may indicate that in the gastrointestinal tract of the rat pepsins occur which are also active at a higher pH than 2 which is generally considered as the optimum pH for pepsin activity.

The fourth effect of HCl is that of denaturation/gelatinization of the collagen monomers solubilized by pepsin. Denaturation of collagen fibers only occurs after solubilization (Etherington, 1977). The denaturation temperature of soluble monomers is about 39°C for mammalian collagen (Bailey, 1983) but the exact temperature of bovine tendon is not available from the literature. The denaturation temperature is known to be pH-dependent, decreasing at lower pH (Dick & Nordwig, 1966). The body temperature of the rat is 37 - 38°C (Falkmer & Waller, 1972). Inhibition of gastric acid production could thus have resulted in a less extended denaturation after solubilization than at normal acidity. The *in vitro* study (Fig. 1) showed that the collagen solubilized by pepsin was also TCA-soluble after pancreatin treatment independent of the pH during pepsin digestion. It is thus probable that denaturation of solubilized collagen is not a limiting step in the further digestion of collagen fibers. Most critical for the digestion of insoluble collagen should be the initial steps of swelling and solubilization by pepsins.

In conclusion, results *in vitro* and *in vivo* both indicate that when the pH is low a considerable amount of insoluble collagen can be digested. The production of hydrochloric acid is of importance *in vivo* for the digestion of insoluble collagen but not for gelatin and meat proteins. The application of these data to other collagens needs further study as does the importance of pH for the digestion of insoluble collagen in humans.

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