In Vitro Enzymatic Digestion of Benzyl- and Phenylisothiocyanate-Derivatized Food Proteins

Harshadrai M. Rawel, Jürgen Kroll,* and Insa Schröder

Institute of Nutritional Science, University of Potsdam, A.-Scheunert Allee 114-116, D-14558 Bergholz-Rehbruecke, Germany

The interactions of different isothiocyanates (ITCs, benzyl- and phenyl-ITC) with selected food proteins such as egg white proteins, myoglobin, and legumin have been investigated. The first aspect summarizes the changes in physicochemical properties of the derivatized proteins such as a decrease in solubility and in the amount of free ϵ-amino groups coupled with an increase in the hydrophobicity. In addition, the isoelectric points were shifted to lower pH values, demonstrated for legumin by isoelectric focusing of legumin. The main feature of this paper represents the proteolytic degradation (tryptic, chymotryptic, and peptic) of ITC-derivatized proteins (egg white proteins, myoglobin, and legumin), which leads to different degrees of digestion of the derivatives. The enzymatic progress has been monitored by RP-HPLC and SDS−PAGE. Proteolytic digestion was inhibited by ITC derivatization (trypsin more inhibited than chymotrypsin = pepsin), due to the presence of derivatized lysine side chains that proved difficult to split.

Keywords: *Egg white proteins; myoglobin; legumin; isothiocyanates; protein derivatization; tryptic, chymotryptic, and peptic digestion; RP-HPLC; SDS−PAGE; IEF*

INTRODUCTION

Glucosinolates are native components present in *Brassica oleracea* (Cruciferae) vegetables (cabbage, Brussels sprouts, broccoli, cauliflower, kohlrabi, turnip, swede, etc.) that upon hydrolysis by the plant's own enzyme myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) yield different products depending on the reaction conditions (Fenwick et al., 1983; Stoewsand, 1995). Important factors influencing this enzymatic degradation include the pH (Benn, 1977; McGregor et al., 1983), the structure of the glucosinolate, and the presence of compounds that modify the action of the enzyme (Tookey and Wolff, 1970; Tookey, 1973; Tookey et al., 1980). From a physiological point of view glucosinolates are relatively nontoxic (Bell, 1984), but their hydrolysis products adversely affect animal growth and reproduction and cause goiter and abnormalities in internal organs of animals (Fenwick et al., 1989; Mawson et al., 1994a,b; Stoewsand, 1995; Jongen, 1996). Daily consumption of glucosinolates in human beings has been estimated to be >300 mg (Sones et al., 1984). One of the hydrolysis products released by the action of myrosinase represents the group of isothiocyanates (ITCs; Tookey et al., 1980), which may react with proteins during food preparation or after intake with digestive proteins. Thus, ITCs from crushed seeds from Cruciferae family may react with proteins under mild conditions as present during oil production from rapeseed and mustard seeds (Björkmann, 1973; Kishore Kumar Murthy and Narasinga Rao, 1986). The most ITC binding occurs at high pH, as documented for model proteins by Kishore Kumar Murthy and Narasinga Rao, (1986). In case intact glucosinolates are consumed as part of the diet, hydrolysis products may also be formed, because myrosinase also occurs in several microbial species inhabiting the gastrointestinal tract (Larsen, 1981).

Björkmann (1973) and Kishore Kumar Murthy and Narasinga Rao (1986) as well as Kawakishi and Kaneko (1987) have reported on the reactions of ITCs with different proteins in which the most reactive and accessible sulfhydryl, ϵ-amino, and terminal α-amino groups were involved. ITCs react even under mild conditions with amino groups or sulfhydryl groups of proteins to form thiourea or dithiocarbamate derivatives, respectively. From the ITCs (allyl-, butyl-, phenyl-, and benzyl-ITC) tested, benzyl- and phenyl-ITC showed the strongest interaction with egg white proteins (Kroll et al., 1994a,b). Reactions of ITCs with bovine sarcoplasmic proteins and myoglobin isolated from horse heart have also been investigated and documented (Rawel and Kroll, 1995; Kroll and Rawel, 1996). These reactions were evaluated by determining the solubility, by the change in free amino and sulfhydryl groups, and by their chromatographic and electrophoretic behavior. The results show that the electrophoretical mobility increased, the isoelectric point shifted to lower pH values, and the molecular weight changed.

Looking at these reactions of ITC with proteins from a physiological point of view, it is of importance to estimate their impact on the rate of proteolytic cleavage of the peptide bond. Kishore Kumar Murthy and Narasinga Rao (1986) reported that this cleavage is sensitive to changes in the conformation of a protein molecule because it depends on the state of localized areas in the molecule. The main objective of this paper is to demonstrate the influence of benzyl- and phenyl-ITC derivatization on digestibility of food proteins (egg white proteins, myoglobin, and legumin from faba beans) with the main enzymes of the gastrointestinal

* Author to whom correspondence should be addressed (e-mail kroll@www.dife.de; fax 033200/88573; telephone 033200/88262).

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tract (trypsin, α-chymotrypsin, and pepsin) on the basis of in vitro experiments. Benzyl- and phenyl-ITCs were chosen since they showed the highest reactivity in previous derivatization experiments (Kroll et al., 1994a).

MATERIALS AND METHODS

Investigated Proteins, Enzymes, and ITCs. Whole egg white proteins and purified main components (ovalbumin and lysozyme from Fluka Chemie AG, Buchs, Switzerland), myoglobin from horse heart (Fluka Chemie AG), and legumin from broad beans (*Vicia faba* L.), extracted and lyophilized according to the method of Bikbov et al. (1986), were used. The pH value of the protein solutions was adjusted to 9 using 0.5 M NaOH. Under continuous stirring at room temperature, benzyl- and phenyl-ITC (62.5 mg/g of protein, Fluka Chemie AG) dissolved in ethanol was added. The final ethanol concentration in the solution was 16.6% (v/v). After 3 h of reaction time, the sample was dialyzed for 18−20 h and finally lyophilized (Kroll et al., 1994a,b; Kroll and Rawel, 1996). The nonderivatized proteins (controls) were prepared under the same conditions but without addition of ITC.

Trypsin from porcine pancreas (14 900 units/mg), pepsin from porcine stomach mucosa (3100 units/mg; Sigma Chemical Co., St. Louis, MO), and α-chymotrypsin from bovine pancreas (53.1 units/mg; Fluka Chemie AG) were the proteolytic enzymes applied.

Physicochemical Characterization. The protein content in the solutions was determined according to a modified Lowry method (Lowry et al., 1951). The control protein preparation was used in each case to calibrate the regression curves (correlation = 0.97−0.99) after its protein content had been determined by semimicro-Kjeldahl analysis (Kjeldatherm System KT 40, Gerhardt Laboratory Instruments, Bonn, Germany). The solubility profile of the lyophilized samples under varying pH conditions was determined in a 0.05 M sodium phosphate/citric acid buffer system by removing the insoluble material through centrifugation at 10 700 rpm (9088*g*, 10 min, Megafuge 2.0R, Heraeus, Hanau, Germany). Changes in the content of free amino groups according to Adler-Nissen (1972) were analyzed using trinitrobenzenesulfonic acid (TNBS) in a 1% sodium dodecyl sulfate (SDS) solution of the samples. The determination of possible reaction products of ITC and sulfhydryl groups of proteins was conducted by identifying dithiocarbamates as done methodically for fungicides (Engst and Schnaak, 1974). The principle of this method involves the decomposition of dithiocarbamates followed by absorption of the released carbon disulfide in ethanolic Cu−acetate−diethanolamine solution and finally the photometric determination of the complex Cu−dithiocarbamate. In the case of legumin, the reaction at the sulfhydryl groups was monitored by using the DTNB method (Hoffmann, 1977). The change in hydrophobicity was followed by RP-HPLC. The details of the RP-HPLC conditions for the egg white proteins and myoglobin are presented in our preceding papers (Kroll et al., 1994a,b; Kroll and Rawel, 1996). RP-HPLC of legumin−ITC derivatives was conducted with a Jasco chromatographic system using a 20−54% acetonitrile gradient (both eluents acidified with HCl, 1 mL of concentrated HCl/1000 mL of eluent; gradient: 5 min, 100% A; 20 min, 0−100% B; 15 min, 100% B; 5 min, 0−100% A; 10 min, 100%A) on a Grom-Sil 300 RP-C18 column (250 × 4 mm; 300 Å; 5 μm, UV detection, 220/254 nm) at 25 °C.

SDS−PAGE according to the method of Laemmli (1970) was applied for molecular weight determination. Isoelectric focusing (IEF) of the control and ITC-treated protein was carried out in a pH range from 3 to 10 (PAGE, T = 8%) as described in Kroll and Rawel (1996). The sample application for legumin and myoglobin was on the anionic side, whereas that for lysozyme was on the cationic side. Gel permeation chromatography (GPC) was performed using 0.025 M Tris-HCl, pH 8.8, as eluent (HPLC column, Tosohaas TSK gel G 3000 pW_{XL}). These methods were also applied to unreacted legumin as well as to benzyl- or phenyl-ITC-treated protein, and some of the relevant results are presented and discussed in this paper. MALDI-TOF-MS experiments of the proteins and their complete tryptic digests were performed as described in detail in Rawel et al. (1998).

Tryptic and Chymotryptic Digestion. Four milligrams of control whole egg white proteins, ovalbumin, lysozyme, and their benzyl-ITC-treated products was dissolved in 0.25 mL of 8 M urea and incubated for 3 min at 100 °C, diluted with 0.65 mL of 0.025 M Tris-HCl, pH 7.5, and mixed for 15 min. To every 90 μL of this protein solution was mixed 10 μL of trypsin (14 900 units/min) or chymotrypsin (53.1 units/min) in 0.05 M HCl to give different enzyme/substrate (E/S) ratios. Stock solutions of the two enzymes containing 1 mg of solids/mL were diluted to obtain the necessary E/S ratios. The E/S ratios (documented in the figures) were different for each protein and optimized in each case. The hydrolysis was performed at 37 °C for 5 min. The reaction was stopped by addition of 0.1 mL 0.5 M Tris-HCl (pH 6.8, 4% SDS, 5% β-mercaptoethanol) and incubation at 100 °C for 10 min. The enzymatic degradation was monitored by SDS−PAGE using a 14% T gel according to the method of Laemmli (1970). The change in the concentration of the protein bands was estimated using densitometer scanning software (Bio-Rad, Fluor-S MultiImager, Hercules, CA).

Tryptic hydrolysis (40 μL of trypsin, 1 mg/mL, 14 900 units/min) of legumin and myoglobin as well as their ITC-derivatized products (1.6 mg/360 μL; 2 M urea, 0.1 M Tris-HCl, pH 8, 0.02 $CaCl_2$) was investigated by incubation at 35 °C. After different lengths of digestion (E/S = 1:40), the reaction was stopped by addition of 400 μL of trichloroacetic acid (20% TCA). The reaction mixtures were allowed to stand for 10 min and then centrifuged (9088*g*, 10 min, Megafuge 2.0R, Heraeus, Hanau, Germany). RP-HPLC of the TCA soluble peptides was performed on a Micra-NPS C18 column (33 × 4.6 mm, 1.5 μm, flow rate = 0.5 mL/min, UV detection at 220 nm) with a column temperature of 25 °C using a Jasco chromatographic system. A distilled water/acetonitrile (both eluents acidified with 0.1% trifluoroacetic acid v/v) gradient was applied under the following conditions: 100% water, 2 min; 0−70% acetonitrile, 10 min; 70% acetonitrile, 4 min; 0−100% water, 4 min; 100% water, 10 min (equilibration). The injection volume of the samples was 5 μL. The total peak area of the peptides was used to quantify the extent of tryptic digestion.

Peptic Digestion. Analogous to tryptic and chymotryptic analysis, the protein solutions (4 mg/ 25 μL) were prepared in 8 M urea and incubated at 100 °C for 3 min. To 25 μL of each of these solutions was added 55 μL of 0.1 M citric acid or 0.1 M HCl and mixed for 15 min. To 80 μL of the resulting mixtures was added 20 μL of pepsin in phosphate buffer (pH 5.5, stock solution: 1 mg of solid/mL, diluted as necessary, 3100 units/min) to give the required enzyme/substrate ratio. The hydrolysis was performed at 37 °C and monitored by means of SDS−PAGE analogous to the procedure described above.

Statistical Analysis. The digestions and analysis were repeated three times and analyzed by means of standard deviation (SD). A maximum of ±5% SD from the averaged values was generally tolerated. The averaged values are evaluated in the respective figures.

RESULTS AND DISCUSSION

Characterization of Physicochemical Properties of the ITC-Treated Proteins. The contents of free amino groups in control whole egg white, ovalbumin, and lysozyme were 0.299 mmol/g of protein (±2.2% SD), 0.118 mmol/g of protein (±0.9% SD), and 0.291 mmol/g of protein (±3.4% SD), respectively. The amounts decreased after benzyl-ITC derivatization for each protein sample to 0.161 mmol/g of protein (±2.7% SD), 0.094 mmol/g of protein (±2.2% SD), and 0.196 mmol/g of protein (±3.5% SD), showing that 46, 20, and 33% of the amino groups were derivatized, respectively. A

decrease of 34% was observed for myoglobin with 1.245 mmol/g of protein (±0.7% SD) in the control and 0.820 mmol/g of protein (±2.2% SD) in the benzyl-ITC-derivatized sample (Kroll and Rawel, 1996). In agreement with the above-mentioned results, it can also be shown that ITC reacts preferentially with the free amino groups of legumin to form thiourea derivatives (reaction 1).

$$\text{R–N=C=S + Protein–NH}_2 \rightarrow \text{R–NH–C(=S)–NH–Protein} \quad (1)$$

thiourea derivatives

In this case, a decrease in their content from 0.290 mmol/mg of protein (±0.9% SD) in the control legumin to 0.176 mmol/g (±1% SD) for benzyl-ITC-treated product and to 0.143 mmol/g (±4.8% SD) for phenyl-ITC-treated sample was observed.

The reaction of benzyl-ITC at sulfhydryl groups leading to the formation of dithiocarbamate derivatives was documented by the release of carbon disulfide from derivatized samples (reaction 2).

$$\text{R–N=C=S + Protein–SH} \rightarrow \text{R–NH–C(=S)–S–Protein} \quad (2)$$

dithiocarbamic acid ester

In this respect 463.3 ± 17.4, 308.9 ± 15.2, and 20.1 ± 1 μg/g of protein were released from the derivatives of whole egg white proteins, ovalbumin, and lysozyme, respectively. The release of carbon disulfide was not observed for their controls. Myoglobin contains no cysteine (CySSyC) and, therefore, no dithiocarbamate formation or consequent release of carbon disulfide was witnessed from the control or from its benzyl-ITC derivative.

The contents of sulfhydryl groups as determined according to the DTNB method were 0.055 mmol/g of protein (±4.2% SD) for whole egg white proteins and 0.051 mmol/g of protein (±2.6% SD) for ovalbumin. Their amounts decreased after benzyl-ITC derivatization to 0.01 mmol/g of protein (±2.4% SD) and 0.007 mmol/g of proteins (±3.7% SD), respectively. This shows that at least 82−86% of the sulfhydryl groups were blocked. In the case of lysozyme and myoglobin no free SH groups were detected with this method. A 10-fold increase in the amount of sulfhydryl groups of legumin from 0.0003 mmol/g of protein to 0.003 mmol/g (±2.5% SD) was noted after benzyl- and phenyl-ITC derivatization (DTNB method). This means that ITC causes a splitting of the disulfide bond, followed by a reaction with the free sulfhydryl, and finally displacement by DTNB.

The influence of ITC derivatization on solubility in a broad pH range (pH 2−8.5) is documented for whole egg white proteins in Kroll et al. (1994a) and for myoglobin in Kroll and Rawel (1996). In both cases the solubility decreased after derivatization. The solubility of the ITC-derivatized legumin at pH values >6 (e.g., to 40% by benzyl-ITC and to 90% by phenyl-ITC; at pH 7) also decreased, in agreement with the results obtained for the other ITC-derivatized proteins.

Both ITC-derivatized legumins are different with regard to their molecular weights as estimated by GPC. Soluble high molecular weight components were detected in the phenyl-ITC-treated legumin contrary to the benzyl-ITC derivatives, which remained partly insoluble. On the other hand, SDS−PAGE [in the absence and presence of a reducing agent (β-mercaptoethanol)] of both of these samples showed no relevant change in the electrophoretic band pattern compared to the unreacted legumin.

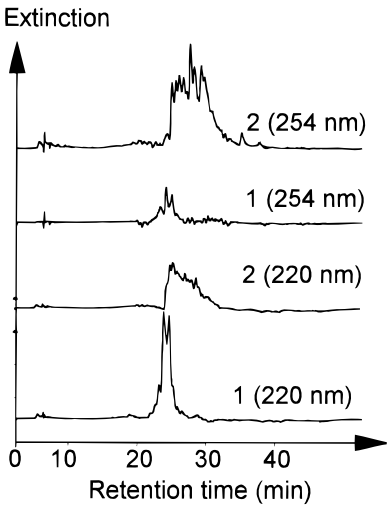

Figure 1. RP-HPLC of ITC-derivatized legumin: 1, legumin control; 2, 62.5 mg of phenyl-ITC/g of legumin.

Molecular weight determinations with MALDI-MS for myoglobin are documented in Rawel et al. (1998). Myoglobin contains an N-terminal glycine and 19 lysine residues. Thus, the maximum possible mass after benzyl-ITC derivatization is calculated to be *m*/*z* 16 951 + (20 × 149) = 19 931. The highest mass found in the case of derivatization with 62.5 mg of benzyl-ITC was *m*/*z* 18 289, which accounts for the addition of a maximum of nine benzyl-ITC groups per molecule of myoglobin (Supporting Information). Lysozyme from egg white contains an N-terminal free amino group and six lysine and eight cysteine (four CySSyC) residues as possible reaction sites. Mass determination with MALDI-MS showed after derivatization with 62.5 mg of benzyl-ITC an addition of a maximum of three benzyl-ITC groups per molecule (*m*/*z* 14 755, Supporting Information).

A further molecular property that can be affected by the reaction with ITC is its hydrophilic−hydrophobic character, which can be illustrated by RP-HPLC. The relative hydrophobicity increased after ITC derivatization as documented by higher retention times for derivatized egg white proteins (Kroll et al., 1994a), ovalbumin and lysozyme (Kroll et al., 1994b; Supporting Information), and myoglobin (Kroll and Rawel, 1996). RP-HPLC of phenyl-ITC derivative of legumin at 220 nm results in an expansion and in an increase of the retention time of the main peak (Figure 1). Interesting aspects can be observed during the RP-HPLC analysis at 254 nm after reaction of legumin with phenyl-ITC (Figure 1). Unreacted control legumin showed only a weak absorption as compared to the phenyl-ITC-treated protein, which absorbs strongly at 254 nm. It can be further observed that those fractions with higher retention times also show higher peak heights, indicating an increase in hydrophobic character with increasing ITC derivatization.

The decrease of free amino groups caused a decrease in the net charge of the proteins, affecting the isoelectric

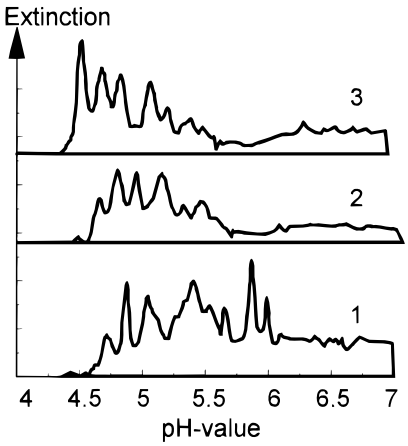

Figure 2. IEF of ITC-derivatized legumin: 1, legumin control; 2, 62.5 mg of benzyl-ITC/g of legumin; 3, 62.5 mg of phenyl-ITC/g of legumin.

points of ITC-derivatized proteins, which are shifted consequently to lower pH values. The unmodified control myoglobin had isoelectric points between 7 and 7.5. A subsequent benzyl-ITC derivatization shifted the isoelectric range to 5.13−5.6 (Kroll and Rawel, 1996). The control lysozyme showed an isoelectric point around 10.6. After benzyl-ITC derivatization, four fractions with a shifted isoelectric range between 8 and 10 were detected (Supporting Information). A similar trend was also obtained for legumin, for which phenyl-ITC, in agreement with a more intensive reaction with the free amino groups, induced a stronger shift of the isoelectric points to lower pH values during IEF (Figure 2).

Effect of ITC Derivatization on Proteolytic Hydrolysis. An important aspect of the ITC reactions with proteins is their influence on the digestibility by proteolytic enzymes. This was tested using the typical proteases of the intestinal tract such as trypsin, α-chymotrypsin, and pepsin. The results of tryptic degradation of egg white proteins at different E/S ratios are presented in Figure 3 (SDS−PAGE evaluation). Tryptic hydrolysis of whole egg white proteins was performed, but ovalbumin as the main protein fraction was chosen as an indicator to simulate the hydrolysis of whole egg white proteins (Figure 3A). Principally the ITC derivatization leads to a lower enzymatic digestibility of the proteins, although with increasing enzyme concentration the protein degradation was accelerated. The ITC-derivatized ovalbumin in whole egg white proteins is less resistant to tryptic hydrolysis as compared to the commercially available and partly purified (70%) ovalbumin fraction due to partial denaturation (compare panels A and B of Figure 3). In both cases, the breakdown of the ITC-derivatized ovalbumin is especially increased when using a high E/S ratio (1:20). This observation demonstrates that an inhibitory action of the ITC derivative or of the protein-type proteinase inhibitors (trypsin inhibitors) is likely to be present. Lysozyme, a compact globular protein, is difficult to digest even under partly denaturing conditions (incubation at 100 °C in the presence of urea before proteolysis). Only ∼10% is digested at an E/S ratio of 1:5 (Figure 3C). Under the conditions applied, the derivatized lysozyme is not tryptically digested (Figure 3 C). The tryptic degradation of legumin also declined after ITC derivatization as shown in Figure 4 (RP-HPLC evaluation). There is only a slight difference between digestion of benzyl- and phenyl-ITC-derivatized products.

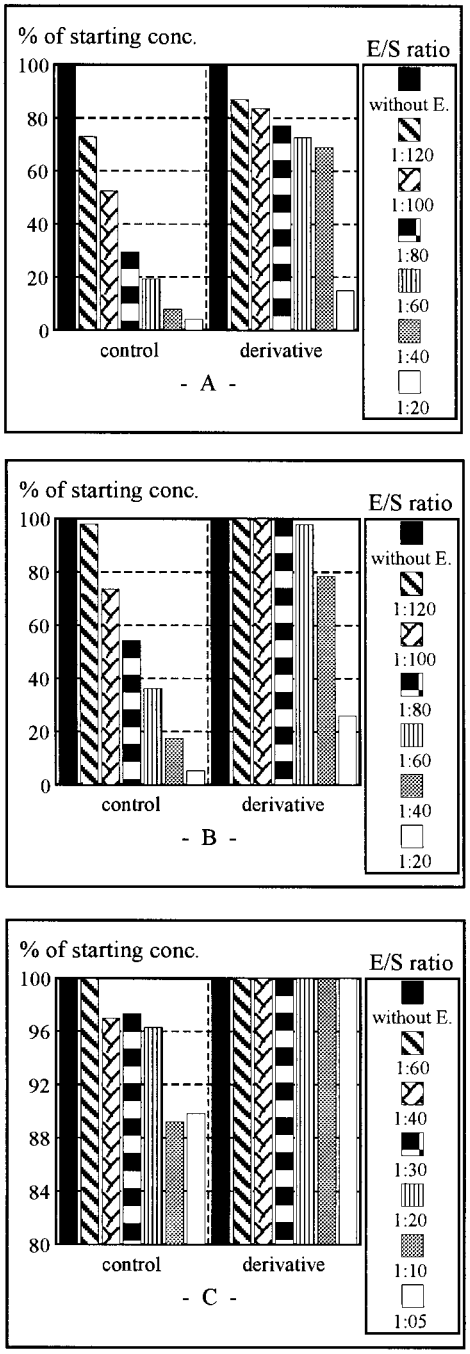

Figure 3. Tryptic hydrolysis of ITC-derivatized egg white proteins: A, ovalbumin in egg white proteins; B, commercially available ovalbumin; C, lysozyme.

Trypsin splits preferentially those peptide linkages that contain either lysine or arginine as amino side chains (Bond, 1989). Because it has already been shown that ITC reacts with ϵ-amino groups of lysine side chains (Kroll et al., 1994a,b), we can summarize that ITC derivatization prevents, or at least makes difficult, tryptic degradation. Tryptic hydrolysis of myoglobin is also affected in a similar way (decrease of proteolysis due to derivatization) and can be illustrated by RP-HPLC of the TCA soluble degradation products (Figure 5). Additional information was obtained by preliminary experiments using tryptic hydrolysis of myoglobin benzyl-ITC derivatives, followed by characterization of the resulting peptides with MALDI-MS. The comparison

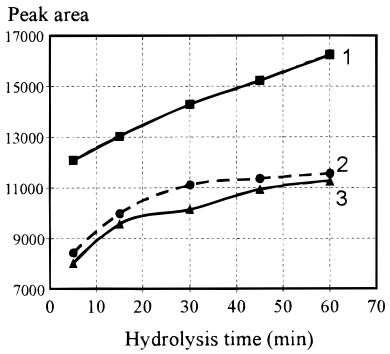

Figure 4. Tryptic hydrolysis of ITC-derivatized legumin: 1, legumin control; 2, 62.5 mg of benzyl-ITC/g of legumin; 3, 62.5 mg of phenyl-ITC/g of legumin (E/S ratio = 1:40; RP-HPLC of TCA soluble peptides).

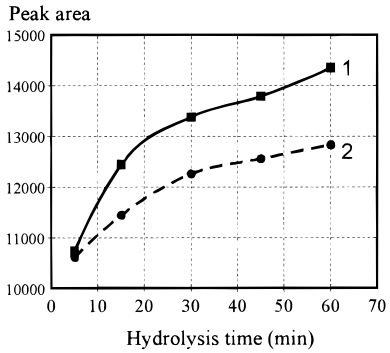

Figure 5. Tryptic hydrolysis of ITC-derivatized myoglobin: 1, myoglobin control; 2, 62.5 mg of benzyl-ITC/g of myoglobin (E/S ratio = 1:40; RP-HPLC of TCA soluble peptides).

of these experimental results with those of the theoretical breakdown products generally obtained by tryptic hydrolysis of myoglobin (sequence, data bank) showed that when the ϵ-amino group of lysine is blocked, no splitting of this particular peptide bond is possible (Supporting Information). The results further showed that at least two lysine residues in the myoglobin amino acid sequence were not derivatized (Supporting Information).

The effect on the chymotryptic hydrolysis of benzyl-ITC-treated whole egg white proteins, ovalbumin, and lysozyme is shown in Figure 6. Generally, the chymotryptic digestion declined after ITC derivatization. Again, the results of whole egg white proteins are documented by the change in the concentration of ovalbumin (Figure 6A). Ovalbumin in whole egg white is again more susceptible to chymotryptic hydrolysis compared to commercially available ovalbumin (effect of denaturation, compare panels A and B of Figure 6). A similar behavior was also obtained for lysozyme, although higher E/S ratios were needed here (due to compact globular structure). Chymotrypsin is known to have primary specificity for those peptide bonds that contain aromatic amino acid residues such as tryptophan, tyrosine, and phenylalanine (Bond, 1989). The reaction of ITC with these amino acids is limited, although tryptophan and tyrosine have been reported to react (Rawel and Kroll, 1995; Kishore Kumar Murthy and Narasinga Rao, 1986). The amino acid analysis of the ITC-derivatized egg white protein fractions (Kroll et al., 1994b) and myoglobin (unpublished data) shows that, in the first place, a reaction at the lysine side chains can be confirmed (tryptophan not accessed in both cases, due to the applied acidic hydrolysis methods).

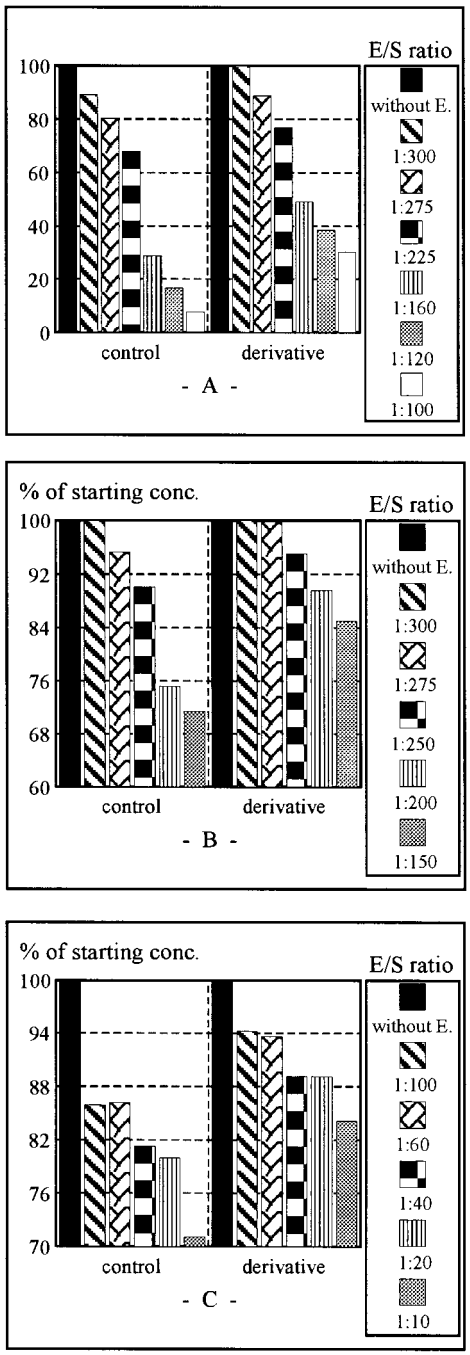

Figure 6. Chymotryptic hydrolysis of ITC-derivatized egg white proteins: A, ovalbumin in egg white proteins; B, commercially available ovalbumin; C, lysozyme.

Pepsin is a nonspecific protease but prefers aromatic and other hydrophobic residues, especially phenylalanine and leucine (Bond, 1989). Even in this case, there is a measurable influence of the ITC derivatization on the peptic hydrolysis, for example, of the egg white proteins documented in Figure 7. Similar behavior was observed as for tryptic and chymotryptic digestion. In this case, the reaction of ITC with amino groups and sulfhydryl side chains may cause structural changes such that some of the potential splitting sites are difficult to access.

Kishore Kumar Murthy and Narasinga Rao (1986) also tested the effect of proteolytic enzymes (α-chymotrypsin, papain, and trypsin) on untreated and allyl-ITC-derivatized mustard 12S protein and found that

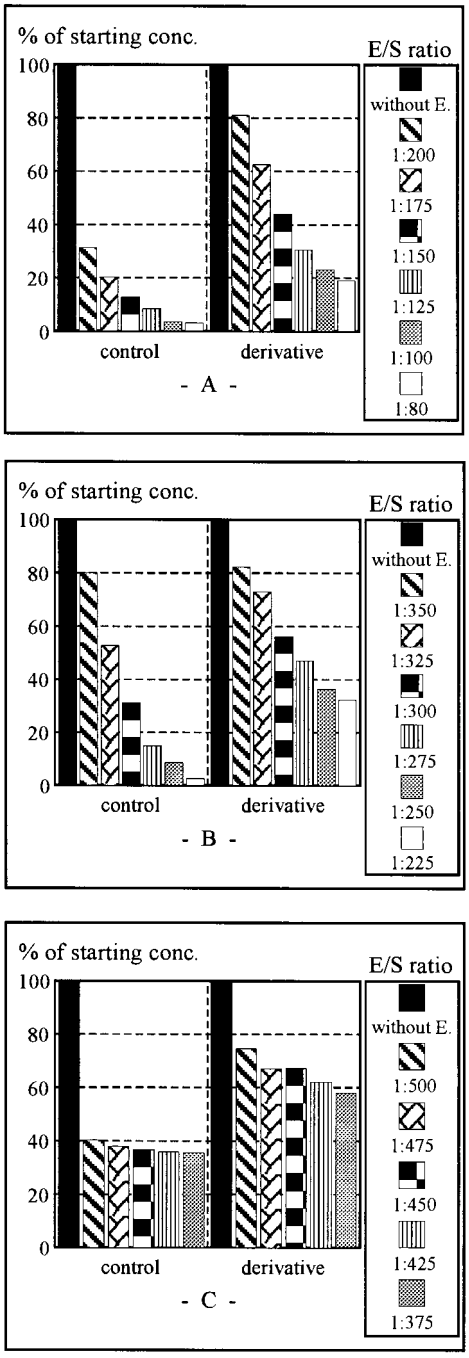

Figure 7. Peptic hydrolysis of ITC-derivatized egg white proteins: A, ovalbumin in egg white proteins; B, commercially available ovalbumin; C, lysozyme.

only tryptic digestion was effected. They attributed the decreased hydrolysis by trypsin to a combination of the effect of the blocking of the ϵ-amino groups of the lysine residues in the protein and of inhibitory effects of the ITC-derivatized products. There is good agreement on tryptic digestion among their results, data obtained by Kawakishi and Kaneko (1987), and our data. Contrary to Kishore Kumar Murthy and Narasinga Rao (1986), Kawakishi and Kaneko (1987) demonstrated that the α-chymotrypsin digestibility of BSA allyl-ITC derivatives is negatively influenced, which is in agreement with our results. However, only a slight decrease was observed for the peptic hydrolysis in their experiments. Finally, preliminary feeding experiments with rats also showed similar effects on the digestibility of ITC-derivatized proteins, underscoring the physiological importance of these observations (Hernández-Triana et al., 1996). The results of this study further showed that besides a decrease in lysine availability, the bioutilization of nitrogen and deposition of energy were also affected significantly.

In conclusion, the reactions of ITCs with proteins, whereby free amino and sulfhydryl groups are derivatized in the first place, lead to changes in the physicochemical properties (solubility, hydrophobicity, and electrophoretical behavior). This influenced the proteolytic digestion negatively, as shown for the main enzymes of the gastrointestinal tract (trypsin, chymotrypsin, and pepsin). Further research includes animal feeding experiments to investigate toxicological and nutritional as well as physiological influence of the ITC-treated proteins.

ABBREVIATIONS USED

BSA, bovine serum albumin; DTNB, dithiobis(2,4-nitrobenzoic acid); E/S, enzyme/substrate; GPC, gel permeation chromatography; IEF, isoelectric focusing; ITC, isothiocyanate; MALDI-TOF-MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; RP-HPLC, reverse phase high-performance liquid chromatography; SD, standard deviation from the average value; TCA, trichloroacetic acid.

Supporting Information Available: Results of MALDI-MS of benzyl-ITC derivatives of myoglobin and lysozyme, RP-HPLC chromatograms of benzyl-ITC derivative of lysozyme, isoelectric focusing of lysozyme after benzyl-ITC derivatization, and information on peptides identified after tryptic digestion of benzyl-ITC-derivatized myoglobin using MALDI-MS with consideration of theoretical breakdown products possible from myoglobin's amino acid sequence (5 pages). Ordering information is given on any current masthead page.

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