Reactivity of Taurine with Aldehydes and Its Physiological Role

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Chemical reactions of the amino group of taurine with aldehyde were investigated. Glucose, acetaldehyde, and malondialdehyde (MDA) were used as aldehydes. After taurine was reacted with the aldehydes, the amounts of remaining taurine and aldehydes were measured, and thereby the reactivity was evaluated. The amino acids such as glycine, α-alanine, and β-alanine were compared because of their structural resemblance to each other. Taurine showed a high reactivity with each one of the aldehydes tested.

It is known that protein is altered through reactions of the amino group with various aldehydes. Low density lipoprotein (LDL) was used as a model protein, and the inhibiting effect of taurine against the modification of LDL by MDA was examined. Our results indicate that taurine inhibited the production of LDL modified by MDA. It was shown that the inhibiting effects correlated with the reactivity of MDA with the amino acids.

Further, the taurine–glucose reaction product showed an antioxidative effect on the peroxidation of liposomes made of yolk phosphatidylcholine as a biomembrane model. The results suggest the possibility of an inhibiting effect of taurine against the modification of protein, as well as an antioxidative effect through the reactions of taurine with aldehydes *in vivo*.

Keywords taurine; aldehyde; chemical reaction; taurine–glucose reaction product; antioxidative effect; protein modification

Taurine is a sulfur-containing amino acid that has an amino group at the β position and a sulfonic group as an acid radical. It differs from natural amino acids, and is mostly present as free amino acid. Many studies on taurine have been reported. Various effects, such as lowering the blood sugar levels in an experimental diabetic model,[1] decreasing serum cholesterol,[2] the inhibition of peroxide lipid in serum,[3] and antioxidation,[4] have been reported. However, the details of these effects have not been clarified. It is possible that the effect of taurine is derived from its structural characteristics. We investigated the chemical reaction of the amino group of taurine *in vitro*, and discussed its physiological roles. Generally, the reaction between the amino group and the carbonyl group is called the amino-carbonyl reaction, or the Maillard reaction,[5] and much related research has been conducted in the field of food chemistry. In recent years it has been reported that the amino group of a protein reacts with reducing sugar non-enzymatically *in vivo*.[6,7] Further, it has been reported that not only reducing sugar, but also compounds containing aldehyde groups, reacted with the amino group of a protein, causing an alteration of the protein.[8,9] Moreover, various aldehydes or oxidants are generated in geriatric disorders, such as diabetes or senility, resulting in the alteration of proteins. Therefore, this reaction has received increased attention in both medical and physiological fields in recent years.

First of all, we examined the chemical reactions of taurine and aldehydes. Next, we investigated whether taurine has an inhibiting effect on protein modification as a result of the amino group competing with amino groups of the protein. Further, the antioxidative effect of the reaction product of taurine and glucose was examined.

Experimental

Materials Glycine, α-alanine, β-alanine, glucose, acetaldehyde, and 2-2′-azobis-(2-amidinopropane) dihydrochloride (AAPH) used were purchased from Wako Pure Chemical Industries, Ltd. Taurine was synthesized in our laboratory, and dicetyl phosphate (DCP) was purchased from Aldrich Chemical Co., Ltd. Malonaldehyde bis(dimethylacetal) was obtained from Tokyo Kasei Kogyo Co., Ltd. Low density lipoprotein (LDL) isolated from human blood plasma was obtained from PROTOGEN AG, and Coatsome NC-10 prepared by Nichiyu Liposome Co., Ltd. was used as yolk phosphatidylcholine (egg-PC). All other reagents were of the highest grade available.

Methods Reactivity of Taurine with Glucose: The amino acids were dissolved in 0.1 M phosphate buffer that had been adjusted to pH 7.4 with 1 N NaOH. Glucose was dissolved in the buffer. Each amino acid solution was mixed with glucose solution at a ratio of 1 : 1 (v/v). The final concentrations of amino acid and glucose were 250 and 250 mM, respectively. After reactions for 34 d at 50 °C, the amount of remaining glucose was determined by the HPLC method using a Hitachi, Ltd. L-6000 and a Differential Refractometer RI-8 Electronics Unit (Toyo Soda). An Ionpak KS-801 (Shodex) column was used, which was connected to an Ionpak KS-800P (Shodex), a precolumn. The column temperature was 80 °C. Deionized water was used as an eluent.

Reactivity of Taurine with Acetaldehyde: The solutions containing amino acids were prepared according to the method mentioned above. To prevent evaporation, acetaldehyde was dissolved in a phosphate buffer (pH 7.4) below 5 °C. Each amino acid solution was mixed with acetaldehyde solution at a ratio of 1 : 1 (v/v). The final concentrations of amino acid and acetaldehyde were 250 and 250 mM, respectively. A vial was filled with the solutions. After reacting for 30 min at 37 °C, the remaining acetaldehyde that reached a liquid/vapor equilibrium was determined by head space gas chromatography (GC), using a Shimadzu GC-9A. A G-column (G-950, GL Science Industry Ltd.) column was used. The column temperature was 110 °C. Hydrogen gas was used as the carrier gas.

Reactivity of Taurine with Malondialdehyde (MDA): The solutions containing amino acids were prepared according to the method mentioned above. MDA was prepared from malonaldehyde bis(dimethylacetal) according to the method described by Kikugawa, *et al.*,[10] and it was adjusted to pH 7.4 with 1 N NaOH. Each amino acid solution was mixed with MDA solution at a ratio of 1 : 1 (v/v). The final concentrations of amino acid and MDA were 250 and 250 mM, respectively. After reacting for 2 h at 37 °C, the amount of each remaining amino acid was determined by the HPLC method. The assay procedures for the amino acid determination were as follows: 0.2 M boric acid/potassium chloride/sodium hydroxide buffer (pH 9.0) was added to each solution. Then, an acetonitrile solution containing fluorescamine was added, and the mixture was stirred to derive a fluorecent substance. This was determined using HPLC (Hitachi, Ltd. 655A-12). Fluorescence was monitored with a fluorescence spectrometer (Hitachi, Ltd. F-1000) fitted with 390 and 485 nm filters for excitation and emission, respectively. A TSK gel LS410 (Tosoh, Ltd.) column was used. The column temperature was 50 °C. Acetonitrile : H_2O :

0.05 M tetra-*n*-butylammonium hydroxide solution (pH 7.4) = 36 : 54 : 10 (v/v/v) was used as an eluent.

Inhibiting Effect of Taurine on LDL Modification by MDA: Each amino acid was dissolved in 0.01 M phosphate buffered saline (PBS) adjusted to pH 7.4 with 1 N NaOH. MDA was prepared from malonaldehyde bis(dimethylacetal) and diluted with PBS. The solutions containing amino acid, MDA, and LDL were mixed. The final concentrations of amino acids, MDA, and LDL were 25, 25 mM, and 1 mg/ml, respectively. They were incubated for 3 h at 37 °C. Afterwards, LDL was dialyzed for 20 h at 5 °C against 0.01 M PBS containing 0.01% EDTA.[9] The amount of MDA per solution containing LDL was measured using the thiobarbituric acid method.[11] The results showed the amounts of MDA that modified LDL.

Preparation of Taurine-Glucose Reaction Product: Taurine (25 mmol) and glucose (50 mmol) were dissolved in 0.1 M PBS. After adjusting the solution to pH 7.4 with 1 N NaOH, a total volume of 60 ml was prepared. It was refluxed for 9 h at 100 °C. This solution was diluted by 10 times and was used for the experiment on antioxidative effects.

Peroxidative Reaction of Egg-PC: A thin membrane was prepared by using a chloroform solution containing 103.4 μmol egg-PC and 10 μmol DCP, and it was hydrated with 10 ml PBS, to prepare the liposome. A taurine–glucose reaction product was added to this liposome suspension at the ratio of 1 : 1 (v/v). PBS was added as a control, and 80 mM glucose and 40 mM taurine were added as a reference, instead of a taurine–glucose reaction product. AAPH (50 mM), with a water-soluble azo-compound as an oxygen radical source, was added to the liposome suspensions at a ratio of 1 : 1 (v/v). After reacting for 3 h at 37 °C, the amount of remaining egg-PC was determined by HPLC (Hitachi, Ltd. 655A-12). A Lichrosorb Si60 (Merck) column was used at 25 °C, and the eluent was methanol: 40 mM NaH_2PO_4 = 96 : 4 (v/v).

Results

Reactivities of Taurine with Aldehydes The reactivity of taurine with glucose was evaluated from the percentage of residual glucose (Fig. 1). The percentage of residual glucose decreased when each amino acid was reacted with glucose, confirming the reactivity of amino acids with glucose. Taurine reacted with glucose similarly to other amino acids. Similarly, the reactivity of taurine with acetaldehyde was evaluated from the percentage of residual acetaldehyde (Fig. 2). Taurine reacted with acetaldehyde similarly to other amino acids. And the reactivity of taurine with MDA was evaluated from the percentage of residual amino acids (Fig. 3). Taurine reacted with MDA similarly to other amino acids. In any case, the reactivity of taurine was almost the same as or even better than the other amino acids.

Inhibiting Effect of Taurine on LDL Modification by MDA When MDA is added to an LDL solution and incubated, LDL will be modified by MDA. We examined the effect of the addition of each amino acid to this solution to investigate whether the modification of LDL was inhibited. The amount of MDA modified with LDL was 32.2 nmol/ml LDL in the control, without the addition of any amino acids, as shown in Fig. 4.

Yet, when taurine was added to the solution containing LDL, the amount of MDA was 15.7 nmol/ml LDL, and the modification of LDL was significantly controlled ($p<0.001$). When the inhibiting effects were compared among the amino acids, taurine was potent, followed by: glycine, β-alanine, and α-alanine. A tendency for the inhibiting effect to be correlated with the reactivity of amino acids with MDA was shown.

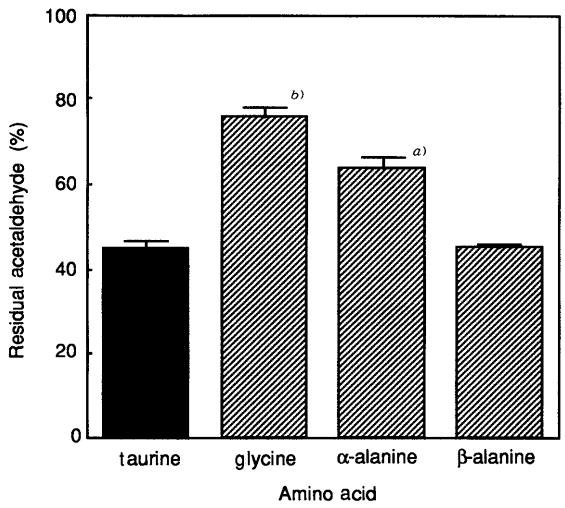

Fig. 2. Reactivity of Taurine and Acetaldehyde

The mixture solutions of amino acid and acetaldehyde (250 mM : 250 mM, pH 7.4) in full vials were cooled below the temperature of 5 °C. After reacting for 30 min at 37 °C, the remaining acetaldehyde that reached a liquid/vapor equilibrium was determined by the head space gas chromatography method. *a*) $p<0.01$, *b*) $p<0.001$ against taurine.

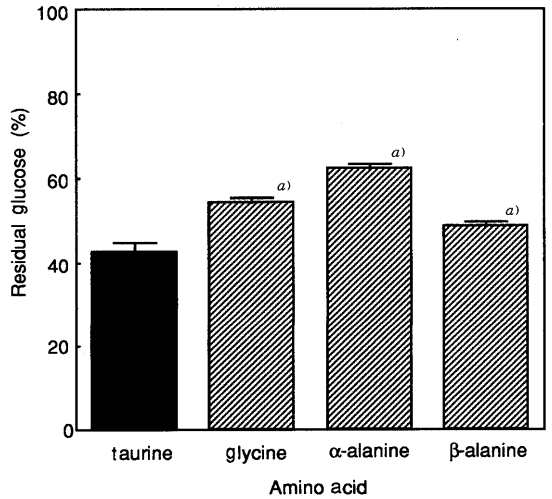

Fig. 1. Reactivity of Taurine and Glucose

The mixture solutions of amino acid and glucose (250 mM : 250 mM, pH 7.4) were reacted for 34 d at 50 °C. The amount of remaining glucose was determined by HPLC. *a*) $p<0.001$ against taurine.

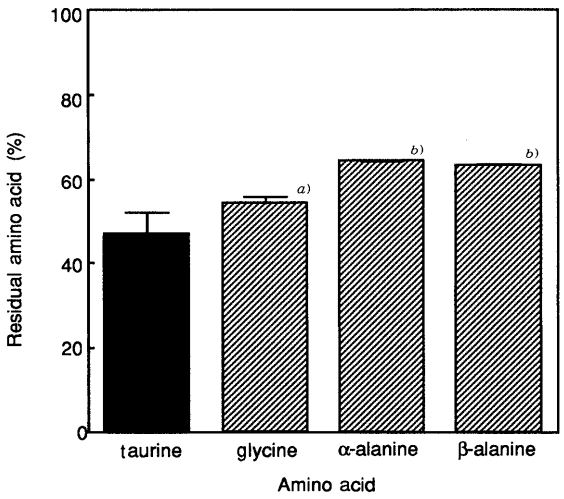

Fig. 3. Reactivity of Taurine and MDA

The mixture solutions of amino acid and MDA (250 mM : 250 mM, pH 7.4) were reacted for 2 h at 37 °C. The amount of amino acids was determined by HPLC. *a*) $p<0.05$, *b*) $p<0.01$ against taurine.

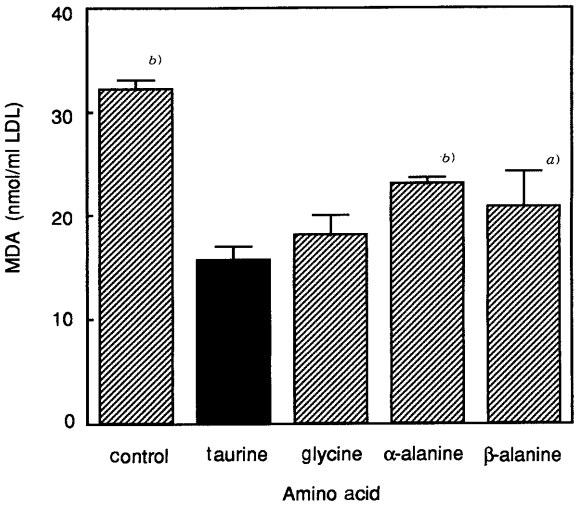

Fig. 4. Effect of Taurine Addition on LDL Modification

The mixture solutions of amino acid, MDA, and LDL (25, 25 mM and 1 mg/ml, respectively; pH 7.4) were incubated for 3 h at 37 °C. LDL was dialyzed for 20 h at 5 °C against 0.01 M PBS containing 0.01% EDTA. The amount of MDA per solution containing LDL was determined by the thiobarbituric acid method. *a*) $p<0.05$, *b*) $p<0.001$ against taurine.

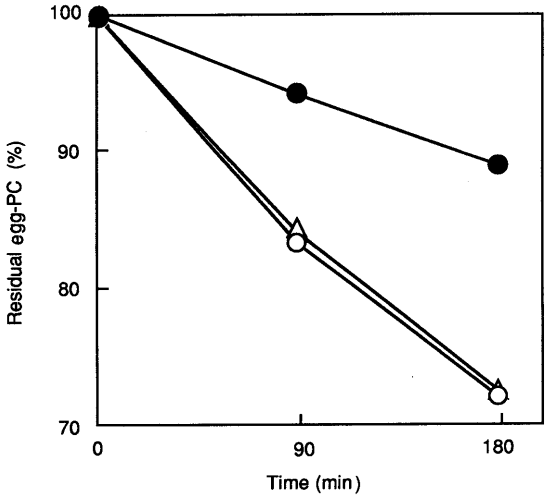

Fig. 5. Antioxidative Effect of Taurine–Glucose Reaction Product

AAPH as an oxygen radical source was added to the liposome suspension prepared with egg-PC. It was incubated for 3 h at 37 °C and the amount of residual egg-PC was determined. ○, no-addition; △, 40 mM taurine + 80 mM glucose; ●, taurine–glucose reaction product.

Antioxidative Effect of Taurine–Glucose Reaction Product

Egg-PC, which was a component of the liposome, was peroxidized by the oxygen radical generated by AAPH, and the percentage of residual egg-PC decreased to about 70% (Fig. 5). At this time, the effect of the addition of the taurine–glucose reaction product on the peroxidation of egg-PC was examined. When taurine and glucose were added, the percentage of residual egg-PC was almost equal to that of the control. On the other hand, when the taurine–glucose reaction product was added, the peroxidation of egg-PC was controlled and the percentage of residual egg-PC rose to about 90%.

Discussion

Changes in physiological activity due to the alteration of a protein, and its subsequent relationship with various diseases, have received considerable attention in recent years. Rahbar (1968) reported that HbAIc, a fraction of hemoglobin, was increased in diabetics.[12] The β chain N-terminal valine of hemoglobin reacts non-enzymatically with glucose to produce Amadri compounds.[7] In diabetes, the glucose concentration in the blood is maintained at a high level. It is also known that glucose reacts with various proteins (glycation), such as albumin, collagen, and myelin *in vivo*,[13] and that this is a cause of complications associated with diabetes. Moreover, acetaldehyde is an intermediate metabolite of ethanol, whose toxicity is problematic.[14] MDA is one of the metabolites of lipid peroxidation. It has been reported that the amount of MDA in serum increases during renal insufficiency,[15] suggesting a relationship to its pathogenesis.

In our study, we examined the chemical reaction of these aldehydes with taurine. We investigated whether taurine could control protein modification, because the amino group of taurine competed with the amino group of a protein in reactions with aldehydes. Further, the antioxidative effect of the taurine–glucose reaction product was examined.

Taurine showed a high reactivity for glucose, acetaldehyde, and MDA. As for the lowering effect on blood sugar levels by taurine, in an experimental diabetes model induced by alloxan or dehydroascorbic acid,[1] such an effect might have been partially due to the direct reaction of taurine with glucose. Hobara, *et al.* (1989) reported that the concentration of acetaldehyde in the blood and liver of a rat after the administration of ethanol to the stomach by gavage (1.5 g/kg weight) significantly decreased following the administration of taurine to the stomach by gavage (0.5 g/kg weight) within 30 min.[16] They concluded that this was due to the activation of aldehyde dehydrogenase by taurine.[17] We used a high concentration (250 mM) of taurine and acetaldehyde in our experiment, and incubated them for 30 min at 37 °C. A direct reaction between acetaldehyde and taurine was confirmed. The possibility is suggested that taurine scavenges acetaldehyde by a direct reaction *in vivo*.

The uptake of LDL to a macrophage is promoted by the alteration of apolipoprotein B. This changes foam cells, causing the deposition of cholesterol and eventually causing arteriosclerosis.[18] The inhibiting effect of taurine on LDL modification was evaluated *in vitro*. Our results demonstrated that the amount of MDA by which LDL was modified was significantly inhibited by the addition of taurine ($p<0.001$). Moreover, this inhibiting effect of LDL modification correlated with the reactivity of MDA and amino acids. In other words, taurine, with its high reactivity, showed a strong inhibiting effect. We speculated that taurine reacted with MDA, competing with the amino group of the protein of LDL, and that the amounts of MDA, which modified LDL, were relatively decreased. The serum cholesterol lowering effect of taurine has already been reported,[2] and the inhibiting effect of taurine is considered to be one of the mechanisms explaining the effect. Moreover, it is thought that the protein is modified by acetaldehyde.[8] The inhibiting effect of taurine on protein modification due to acetaldehyde may be expected, as well as that due to MDA.

As for the influence of taurine on lipid peroxidation, it has been reported that a high lipid peroxide value in serum decreased following the administration of taurine.[3]

Taurine is believed to control lipid peroxide formation by stabilizing the cell membrane. We examined the antioxidative effect of the taurine–glucose reaction product. It is generally known that there is an antioxidative effect in the Maillard reaction products of amino acids and a reducing sugar.[19] Moreover, Hayase *et al.* (1990) have reported that glycine–glucose reaction products display the strong scavenging actions of active oxygen species.[20] We examined the antioxidative effect of the taurine–glucose reaction product on the peroxidation of egg-PC by an active oxygen. An antioxidative effect of the taurine–glucose reaction product was demonstrated after the reaction for 9 h at 100 °C. It is thought that the reaction temperature influences the generation of Maillard reaction products,[21] and that the types of amino acid and the pH of the solution influence their antioxidative effect.[22] It is not clear whether the taurine–glucose reaction product exists *in vivo*. However, glycation *in vivo* indicates the possibility of the existence of a taurine–glucose reaction product *in vivo*. Further, Okamoto *et al.* (1992) reported that the protein by which glycation is caused scavenges active oxygen species.[23] This suggests the possibility that the taurine–glucose reaction product is produced *in vivo* and has an antioxidative effect.

From the facts described above, it is suggested that taurine plays various physiological roles by reacting with aldehydes. It is known that taurine is present in high concentrations in such animal tissues as retina and platelets.[4] In the future, we plan to investigate the relationship between concentrations of taurine and its reactivity, and to examine the physiological roles of taurine *in vivo*.

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