A Fundamental Study of Quantitative Desulfurization of Sulfur Containing Amino Acids by Raney Nickel and its Character
Shinji Ohmori*, Kazuko Takahashi, and Mikiko Ikeda
Faculty of Pharmaceutical Sciences, Okayama University, Taushima-Naka-1, Okayama 700, Japan
Toshihiko Ubuka
Okayama University Medical School, Japan
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The desulfurization of several naturally occurring sulfur-containing amino acids by Raney nickel was studied under various conditions. Raney nickel, which was prepared by treating Al-Ni alloy with 5 N NaOH at 60 °C for 30 min, and was not washed with water, was most active and desulfurized, in quantitative yield, methionine, homocysteine, homocystine, homocysteine sulfinic acid, S-(2-carboxy-n-propyl)-L-cysteine, cysteine, cysteine sulfonic acid and S-methylcysteine sulfoxide. Raney nickel prepared from 100 mg of Al-Ni alloy desulfurized quantitatively up to 40 μmol methionine at 60 °C for 30 min. The desulfurization occurred effectively in the pH range of 7 and 13, but not below 7. Methionine sulfone, cysteic acid, and homocysteic acid were not subject to the reaction. The Raney nickel was deactivated by H₂S, and H₂O₂, or combustion. Desulfurization activity was not enhanced by hydrogen gas.

Introduction
Raney nickel catalyst alone removes sulfur in reasonable yield from organic sulfur compounds, such as thiois, thioethers, disulfides, hemithioacetals, hemithioketals, diketals, thioamides, thiolenes, thioazoles, sulfoxides, sulfones, isothiocyanates, thionoates, and also organoselenium and tellurium compounds. This desulfurization reaction, therefore, has been used for the solution of structure, and synthetic work. Elaborate, and excellent reviews were published by Hauptmann and Waltner [1], and Pettit and Tamelen [2] for desulfurization reactions, and Bonner and Grimm [3] for its mechanisms.
In 1947, Vogler [4] reported that 5 g methionine was desulfurized in a 60% yield by 150 g Raney nickel at 100 °C for 90 min. In a series of studies by Wieland, Amanitin and Phalloidin, poisonous cyclopeptides from mushrooms, were subjected to the desulfurization reaction by Raney nickel [5–8]. In these reports, large excess of Raney nickel (1 g) did not quantitatively desulfurize 20 mg of these peptides even under drastic condition. In the course of our studies on the structure of new sulfur-containing amino acids [9, 10] it was noticed that neither Raney nickel W-6 [11] nor W-2 [12] could efficiently cleave thioether bond of the new sulfur-containing amino acids and methionine. For example, 20 mg of these amino acids were desulfurized in an only 15% yield by 100 mg of these catalysts under the described condition [2], and the increased quantities of Raney nickel did not sufficiently cleave them. However, it was found that Al-Ni alloy incompletely treated with 5 N NaOH or Al-Ni alloy itself could effectively desulfurize them [10]. After several years, Perlstein et al. [13] reported that a modified Raney nickel also did not desulfurize methionine essentially, while cysteine and cystine were completely converted to alanine. Recently, Otieno [14] reported that W-6 Raney nickel catalyst degraded cysteine rapidly, but methionine up to 53% at 25 °C in 6 hours.

We desired to cleave thioether bonds of some glutathione-S-conjugated peptides quantitatively for their determination. For this reason, it was sought such condition that as little amount of Raney nickel as possible is able to desulfurize the sulfide bond thoroughly under mild conditions.

Experimental
Al-Ni alloy was purchased from Katayama Kagaku Co. Ltd. (Osaka). Methionine, methionine sulfoxide, alanine, 2-aminoobutanoic acid (ABA), and other reagents were obtained from Wako Pure Chemical Ind. Ltd. (Osaka). Isobutylchlorormofamate was purchased from Tokyo Kasei Co. (Tokyo). S-(2-Carboxy-n-propyl)-L-cysteine (CPC), homocysteic acid, homocysteine sulfonic acid, cysteic acid, cysteine sulfonic acid, and methionine sulfone were prepared in our laboratory [10, 15, 16]. S-Methylcysteine sulfoxide (methiin) was kindly supplied from Dr. S. Mizuhara.
A Shimadzu gas chromatograph, model GC-4CMPFE, equipped with a flame ionization detector, was used for the determination of amino acids. Amino acid analysis was also carried out with a Hitachi KLA-5 amino acid analyzer (column, 9×550 mm) at 55 °C using sodium citrate buffer (pH 3.25 and 4.25).

Desulfurization procedure of sulfur-containing amino acids and determination of the reaction product

Al–Ni alloy (100 mg) was added to 5 ml of 5 N NaOH, which was continuously stirred at 60 °C for 90 min. After centrifugation at 1000 × g for 5 min, the supernatant was decanted. Sulfur-containing amino acid up to 50 μmol dissolved in 2 ml of water was added to the residue, and the mixture was continuously stirred at 60 °C for 30 min. After centrifugation at 1000 × g for 5 min, an aliquot (0.2 ml) of the supernatant was subjected to GLC analysis of alanine or ABA according to the method of Makita et al. [17], except that aspartic acid was used as an internal standard.

When the alloy itself was used for the desulfurization, the alloy (100 mg) was directly added to amino acid solution (2.0 ml) in 5 N NaOH. After stirring at 60 °C for 120 min, the mixture was centrifuged and analyzed for alanine or ABA as described above.

Gas chromatography

The analytical conditions were as follows. The glass column (3 mm × 1 m) was packed with 1.5% Silicone OV-17 on Shimalite W (AWDMCS), 80 to 100 mesh. The FID, and the injector block were maintained at 230 °C, and the column at 180 °C. The flow rate of carrier gas (nitrogen) was 60 ml/min. The FID was operated under the air pressure of 0.8 kg/cm², and the pressure of hydrogen gas of 0.6 kg/cm². The electrometer setting was continuously kept at range 10⁻², attenuation 128 or 256.

GC-mass spectrometry

Identification of desulfurization products was performed by a Shimadzu LKB 9000 GC-mass spectrometer, after derivatization with isobutylchloroformate and diazomethane as described by Makita et al. [17].

The GC conditions were as same as described above, except that helium gas was used as carrier gas.

Results

Identification of desulfurized compound from sulfur containing amino acids

Desulfurized products from sulfur-containing amino acids tested were identified by GC-mass spectrometry, and it was found that ABA was formed from methionine, homocysteine, homocysteine sulfinic acid, and alanine from CPC, cysteine, cystine, cysteine sulfinic acid and methionine.

Study on the desulfurization conditions for methionine

a) Effect of treatment temperature of Al–Ni alloy with NaOH on the desulfurization reaction: After treatment of Al–Ni alloy (100 mg) with 5 N NaOH at various temperature for 90 min, 10 to 100 μmol of methionine was subjected to the desulfurization as described under “Experimental”, and ABA formed was determined by gas chromatography. The result is shown in Fig. 1-a, indicating that the temperature of 60 °C was optimum for the desulfurization.

b) Washing effect of Raney nickel after NaOH treatment: After treatment of the alloy (100 mg) with NaOH, the effect of washing of the nickel with water on the desulfurization of methionine (100 μmol)
was studied. As shown in Fig. 1-b, the repeated washing weakens the desulfurizing ability of nickel.

c) Effect of pH on the desulfurization of methionine: After treatment of the alloy with NaOH, pH of the reaction mixture was adjusted to various pH by addition of NaOH, ammonia, potassium phosphate buffer, acetic acid, or HCl, and the desulfurization reaction was performed using methionine (100 μmol). It turned out that the desulfurization ability was not affected in the range from 7 to 13, but fell steeply below 7.

d) Effect of reaction temperature on the desulfurization of methionine and time course of the reaction: The desulfurization of methionine (100 μmol) with the Raney nickel from the alloy (100 mg) was studied under various reaction temperature and different times. As shown in Fig. 2-a, the reaction temperature of 60 °C, and the reaction time of 30 min are optimum for the desulfurization of methionine.

e) Effect of the amount of methionine on the desulfurization: Methionine in the range from 10 to 200 μmol was reacted with the Raney nickel from Al–Ni alloy (100 mg), and ABA formed was determined. Fig. 2-b depicts that the nickel from 100 mg of the alloy desulfurizes methionine up to 50 μmol quantitatively. It was noteworthy that 100 mg of the alloy itself could desulfurize 100 μmol of methionine quantitatively. It will be discussed later.

f) Effect of the amount of the Raney nickel on the desulfurization of methionine: The nickel from the alloy in the range from 25 to 100 mg was reacted with 100 μmol of methionine, and ABA formed was determined. As shown in Fig. 2-c, a linear relationship is observed between the formation of ABA and the amount of the nickel.

Desulfurization of other sulfur-containing amino acids

The desulfurization of other sulfur-containing amino acids was studied under the condition as described under “Experimental”. When methionine sulfoxide, homocysteine sulfenic acid, homocysteine, and homocysteine were reacted with the Raney nickel, ABA was formed, and methion, cysteine, cystine, cysteine sulfenic acid, and CPC formed alanine. Homocysteic acid, cysteic acid, and methionine sulfone were not desulfurized.

Desulfurization of methionine by modified Raney nickels

In order to suppose the reaction mechanism, the Raney nickel was modified by H2S, H2O2, or subjected to combustion prior to the desulfurization of methionine (100 μmol). Fig. 3 shows that the pretreatment with H2S gas, or H2O2, and the combustion of dried nickel abolished the desulfurization of methionine. An attempt was made to regenerate the desulfurization activity of Raney nickel. Fig. 3 shows that the cleavage of methionine by the Raney nickel stopped at 30 min under the conditions tested. At 60 min after the start of the desulfurization reaction, H2 gas was introduced into the reaction mixture at 20 °C, and N2 gas was used as the control. No further cleavage was, however, observed as shown in Fig. 3.

When untreated Raney nickel was treated with H2S, vigorous evolution of hydrogen was observed, which stopped soon after. As shown in Fig. 2-b and 3, it is particularly interesting that Al–Ni alloy
Fig. 3. Time course of the desulfurization reaction of methionine by various kind of Raney nickel.

Methionine (100 μmol) was desulfurized at 60 °C for various time by the Raney nickel from 100 mg of the alloy (○-○), and 100 mg of the alloy itself (○-○-○), or by Raney nickel previously treated with H₂S (▲-▲), H₂O₂ (△-△) and by Raney nickel subjected to dryness and combustion (×-×).

Itself has 2.5 to 3 times as active as the Raney nickel from the same amount of the alloy.

Discussion

It is well known that amino acids and peptides containing thiol and disulfide such as cysteine, cystine, and glutathione are much more subject to the desulfurization catalyzed by usual Raney nickels than those having sulfide. In this study, we mainly used methionine as model compound to know the desulfurization reaction. As shown in Fig. 2-b and 3, Al-Ni alloy itself was the most active reagent for the desulfurization of the amino acids. Theoretically one molecule of methionine is desulfurized by two atoms of hydrogen adsorbed on the Raney nickel, but in the case of the use of the alloy itself about 8 mols of the alloy was needed in the desulfurization of 1 mol of methionine (Fig. 2-b). Therefore, assuming that one atom of Ni adsorbs one atom of hydrogen, it may be calculated that methionine is desulfurized in a 25% yield based on adsorbed hydrogen atom. On the other hand, the Raney nickel prepared from 100 mg of the alloy desulfurized 40 μmol of methionine, indicating that 21 atoms of the nickel desulfurized 1 mol of methionine. In this case, the reaction occurred in a 10% yield based on the assumed quantity of adsorbed hydrogen atom. The yield is reasonable taking into account that only hydrogen on the surface of the nickel granule participate in the reaction. Needless to say, the nickel alloy itself is used as desulfurizing agent with the highest activity, but it should be noted that bulky aluminum hydroxide formed disturbed subsequent reaction. The use of the alloy itself is not desirable especially when protein and peptide were subjected to the desulfurization reaction.

Since the desulfurization of sulfides is believed to proceed through free radical mechanisms [3], there is the possibility that other amino acid, i.e. 2,7-diaminoocanadieioic acid than ABA may be found in the reaction mixture after the desulfurization of methionine and homocysteine. However, no such amino acid was detected in the reaction mixture by amino acid analyzer.

Usually, Raney nickel W-2 is washed by water until washings are neutral to litmus, and about 40 washings are required to remove alkali completely [12]. The preparation has been used for the desulfurization. This must be a reason why usual Raney nickel did not cleave thioether bond of amino acids (see Fig. 1-b).

As the preparation and conditions of Raney nickel described in this paper are very effective in the desulfurization of naturally occurring thiols, thioethers, sulfinic acid, and sulfoxides, we are applying them to the determination of some naturally occurring glutathione derivatives.

The quantitative desulfurization of methionine by Raney nickel in an ingenious way has been reported by Keil and Sorm in 1962 [18]. They made a column packed with Raney nickel, and applied methionine solution to the column. However, the analysis of methionine and its reaction product was performed by paper chromatography, and characterization of Raney nickel and conditions for the desulfurization reaction were not reported.

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