

Variation of the SH Content of BSA by Electrolysis

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The reduction of the disulfide bond of protein by electrolysis was investigated using bovine serum albumin, Fraction V (BSA). A BSA aqueous solution was electrolyzed using platinum plate as electrode up to 5 hr at a current density of 5.5 mA/cm², and the total SH (T-SH) and the SH on the surface of the BSA molecule (R-SH) were measured during electrolysis. The R-SH increased with the time duration of the electrolysis, but it did not when the BSA solution was maintained at a constant pH during electrolysis. As R-SH of the BSA increased only when the BSA solution was in a high pH solution, the increase in R-SH during electrolysis was found to be caused by an increase in pH value, not by the reduction of the SS bond.

1 Introduction

The disulfide bond (SS bond) of protein can be reduced to a sulfhydryl group (SH group) either by reagents such as thiols,¹⁻³⁾ NaBH₄⁴⁾ and sulfite,^{5,6)} or by electrolysis.^{7,8)} As a result of the reduction of the SS bond, biological activities such as enzyme activity decrease or become inactive in many cases.⁸⁾ In a few cases, however, the food processing properties are reported to be improved.⁹⁾ To improve the processing properties of food proteins, using a chemical process rather than an electrolytic process creates the concerns for a wholesomeness of foods due to the residual chemicals left in the foods. On the other hand, the electrolysis process is considered to have greater safety.

In order to investigate the reduction by electrolysis of the SS bond of protein, we measured variation of SH content during electrolysis by use of bovine serum albumin (BSA) at neutral pH.

2 Materials and Methods

2.1 Sample BSA and Preparation of a Sample Solution

In order to study the reduction of the SS bond using electrolysis, proteins of less SH and more SS contents in the molecule were considered to be the most suitable. Thus we chose BSA of 0.7 mol SH and 17 mol SS in a molecule⁶⁾ and BSA, Fraction V (Wako Pure Chemicals Co.) as the most suitable samples and employed for the ex-

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The following abbreviations were used for the chemicals in this paper: 2-ME, 2-mercaptethanol; DTNB, 5,5'-dithiobis-2-nitrobenzoic acid; PCMB, *p*-chloromercuribenzoate; 2-PDS, 2,2'-dithiopyridine; 4-PDS, 4,4'-dithiopyridine; 6-TNA, 6,6'-dithiodinitrocinic acid.

periment. BSA was dissolved in a concentration of 4.5 mg BSA/ml of a $\text{Na}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$ buffer solution (ion strength, 0.1; pH, 7.0). In the case of experiments performed at a high pH value, a $\text{NaOH-KH}_2\text{PO}_4$ buffer solution was employed. All the experiments were conducted at 15°C .

2.2 Electrolysis

A BSA solution (4.5 mg BSA/ml) of 175 ml was prepared in a 200 ml beaker to perform electrolysis as shown in Fig.1. The solution and the 0.4 M NaCl were used as a cathode and an anode, respectively, and a direct current was applied after they were connected with a salt bridge (2% agar/0.4 M NaCl). Electrolysis was carried out up to 5 hr at 5.5 mA/cm^2 . Platinum plates were used for the electrolysis.

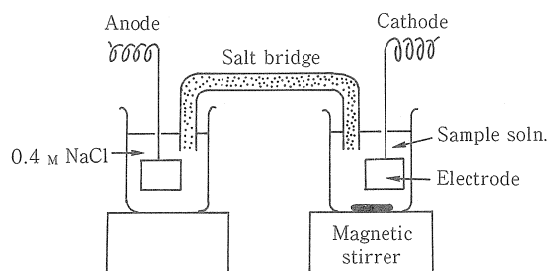


Fig. 1. Apparatus for the electrolysis of BSA.

2.3 Measurement of Reactive SH Content

Reactive SH content (R-SH), defined here as the SH group being exposed on the surface of BSA, was determined according to the Ellman's Method.¹⁰⁾ A solution of 10 mM DTNB (0.03 ml) was added to 4.5 ml of the solution and the mixture was allowed to stand for 1 hr at 5°C . The absorbance was measured at 412 nm and R-SH was calculated as the molar absorption coefficient of the TNB anion as being 1.36×10^4 .¹⁰⁾

2.4 Measurement of Total SH Content

The total SH content of the protein (T-SH) was measured according to the Buttkus' Method.¹¹⁾ A

solution of 10 mM DTNB (0.03 ml) was added to 4.5 ml of the sample solution containing 6M urea and the mixture was heated for 15 min at 40°C . The absorbance was measured at 412 nm and T-SH was calculated as the same manner as R-SH.

2.5 Determination of Protein

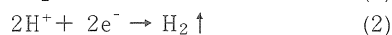
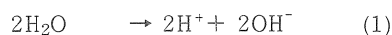
The proteins of the sample solution were measured according to the Biuret Method.¹²⁾

3 Results and Discussion

3.1 Variation of pH and SH Content during Electrolysis

The results of pH and SH content during electrolysis are shown in Fig.2.

The pH value increased slowly during the initial 3 hr of electrolysis, and rose swiftly subsequently. The increase of pH value of the BSA solution (cathode) is explained by the concentration of OH^- in the solution as shown in the following equations:



The H_2O of the BSA solution was slightly ionized into H^+ and OH^- (1), and H^+ turned to hydrogen gas at the cathode (2). As hydrogen gas escaped into the atmosphere, OH^- increased in the solution. Accordingly OH^- was concentrated in the BSA solution, and the pH value increased during electrolysis at the cathode.

R-SH, $4.3 \text{ mol}/10^6\text{g}$ BSA before electrolysis, increased as electrolysis progressed, and became $8.9 \text{ mol}/10^6\text{g}$ after 5 hr. The T-SH, on the contrary, was almost constant ($9.1 \text{ mol}/10^6\text{g}$) for the initial 4 hr, and increased to $10.4 \text{ mol}/10^6\text{g}$ at 5 hr. Both R-SH and T-SH of the control BSA solution indicated almost constant value of 4.1 mol and $9.4 \text{ mol}/10^6\text{g}$, respectively, during standing for 5 hr. The temperature of the BSA solution did not change during electrolysis.

According to the fact that R-SH increased, but

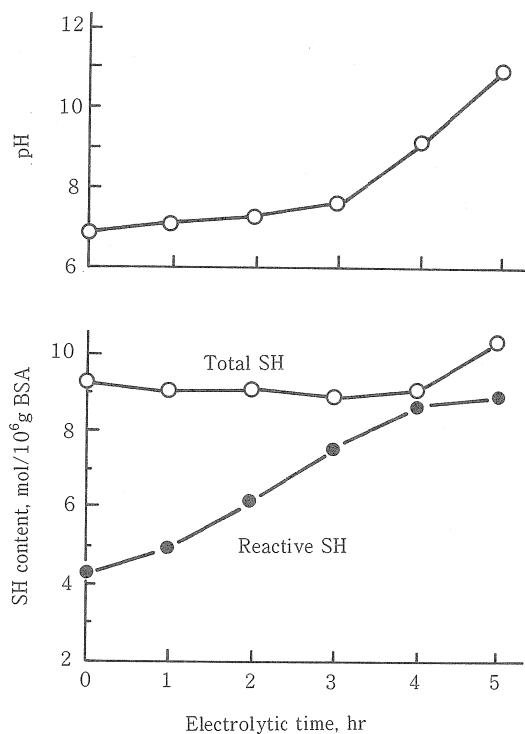


Fig. 2. Variation of pH and SH contents with electrolytic time.

Conc. of BSA, 4.5mg BSA/ml; electric current density, 5.5 mA/cm².

T-SH was nearly constant during electrolysis, the SH group in the BSA molecule became gradually exposed onto the surface of the molecule, and almost all the SH group (95%) was found to be exposed on the surface of BSA after 4 hr of electrolysis. It was believed that the three-dimensional structure of the BSA molecule changed to a completely spread-out state. The reason why T-SH increased after 5 hr of electrolysis is assumed to be due to the reduction of SS to SH, but a detailed reason could not be clearly established from the results of this experiment alone.

The T-SH value during 4 hr electrolysis, which is shown in Fig.2 (9.1 mol/10⁶g BSA), was calculated to be 0.63 mol/molecule. This value was slightly less than those values (0.66¹³ and 0.65-0.69)^{14,15} obtained using PCMB,¹³

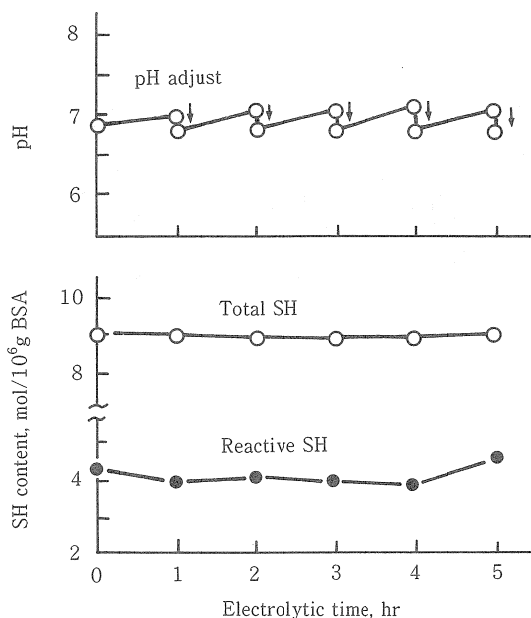


Fig. 3. Variation of SH contents of BSA with electrolytic time under a constant pH value.

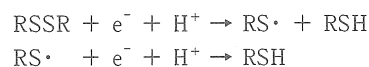
Conc. of BSA, 4.5mg BSA/ml.

2PDS, 4PDS and 6TNA,^{14,15} respectively. The reason might be due to the impurity of the BSA sample used.

3.2 Electrolysis of a BSA Solution under a Constant pH Value

In the previous section, almost all the SH group of the molecule was assumed to be exposed onto the surface of the molecule by electrolysis within the initial 4 hr. In this section we will discuss the reason why R-SH increased.

The reasons for the increase in R-SH by electrolysis are assumed to be dependent on two factors; one factor is the rise in pH of the BSA solution as described, and the other factor is the reduction of the SS bond to the SH group as shown in the following equations:



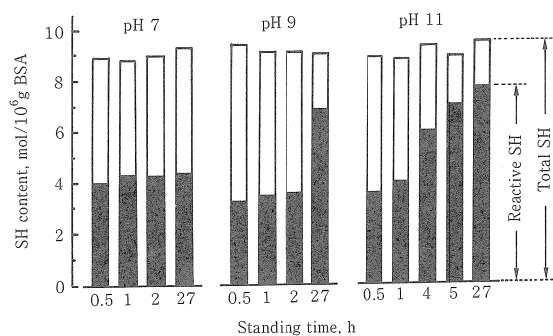


Fig. 4. Variations of SH contents of BSA with elapsed time at different pH values.

The variation of the SH content of BSA by electrolysis was measured while the pH value was kept constant. Namely, R-SH and T-SH were measured, adjusting the pH to the initial value using 1 N HCl at every hr during electrolysis, and the results are summarized in Fig.3.

As shown in the results, the R-SH and T-SH were nearly constant during electrolysis under a constant pH value (pH 6.8–7.1). From these results, the increase in R-SH shown in Fig.2 was found to be caused by the increase in pH of the BSA solution rather than the reduction of the SS bond.

3.3 Variation of both R-SH and T-SH under a high pH Value

We described the reason for the increase in R-SH during electrolysis was due to the rise in pH. In order to prove this assumption, the variations of the SH contents were measured under higher pH values. BSA was dissolved in three different pH values (7, 9 and 11), and T-SH together with R-SH were measured up to 27 hr. The results are shown in Fig.4.

Both R-SH and T-SH were nearly the same, even after 27 hr at pH 7, but they changed greatly in accordance with the standing time at both pH 9 and 11. At pH 9, T-SH was nearly constant, whereas R-SH increased to 75% of T-SH after 27 hr. At pH 11, R-SH increased with

the standing time and was 80% of T-SH after 27 hr. The fact that R-SH increased as the time progressed when BSA was in a high pH solution (Fig.3) suggests the increase in R-SH shown in Fig.2 was found to be caused by the increase in pH value, not by the reduction of the SS bond.

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電気分解による BSA・SH 基含量の変動

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タンパク質のジスルフィド結合 (SS 結合) が電気分解 (電解) によってスルフィド基 (SH 基) に還元されるかどうかを検討するために、牛血清アルブミン (BSA) を用いて実験を行った。白金板を電極として $5.5\text{mA}/\text{cm}^2$ で最大 5 時間通電し、全 SH 基含量 (T-SH) と分子表面の SH 基含量 (R-SH) を測定した。R-SH は電解時間に伴い次第に増加したが、一定 pH 下で電解した場合には変動しなかった。一方、R-SH は BSA を高 pH 下にさらただけで増加した。したがって、電解によって R-SH が増加した理由は、水の電解によって陰極側における BSA 溶液の pH が高くなったためであり、白金電極板上では BSA の SS 結合は SH 基に還元されないことを明らかにした。