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Influence of Added Amounts of Succinic Anhydride on the Succinylation Level for some Proteins

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The relationship between the added amount of succinic anhydride (SA) and the succinylation level of five sample proteins was examined to clarify some effective functions to control the succinylation level. The sample proteins used were casein, bovine serum albumin, egg white albumin, two fish proteins, sarcoplasmic proteins (Sp-P) and myofibrillar proteins(Mf-P) prepared from ordinary dorsal muscle of fish. No linear relationship was obtained between SA/Protein (w/w) and the succinylation level, whereas a very smooth S-shaped relationship was obtained between log SA/Protein and the succinylation level (succinylation curve). Succinylation curves of the proteins, except for Sp-P, virtually coincided only in the lower regions of log SA/Protein. The reasons for the inadequate coincidence among succinylation curves were thought to be dependent mainly on the spatial structure of the microenvironment around the ε- amino group of Lys by different proteins.

1 Introduction

Many fundamental and applied investigations of acylation of proteins, especially succinylation and acetylation, have been carried out in order to improve their chemical, physical, nutritional and processing properties, and thermal stabilities.[1-23] By succinylation, it has reported that the proteins are improved in their reactivity in Maillard reaction, surface active and rheological properties[4-6,17,22,23] and solubility and dispersing properties against water.[5]

From the nutritional viewpoint, complete succinylation of proteins, however, is undesirable because of the shortage of effective Lys content,[4,13] and consequently it will be needed to control the succinylation level in order to balance the merits and the demerits accompanying succinylation. Up to date, few papers have been reported on the control of succinylation level. Here, we describe the influence of added amount of SA on the succinylation level for some proteins.

2 Materials and Methods

2.1 Protein samples

The proteins used in the experiments were three commercially available proteins, casein (Merk),

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The following abbreviations were used for the chemicals and sample proteins in this paper : SA, succinic anhydride; BSA, bovine serum albumin ; EWA, egg white albumin ; Mf-P, fish myofibrillar proteins ; Sp-P, fish sarcoplasmic proteins.

BSA and EWA (Wako Pure Chemicals), and two fish proteins, Mf-P and Sp-P, were extracted from the ordinary dorsal muscle of rudder-fish *Girella punctata*. The Mf-P and Sp-P were prepared in our laboratory according to the methods shown in Fig.1, and all the procedures were carried out at 5 ℃.

2.2 Methods of succinylation

Four sample proteins, casein, BSA, EWA and Sp-P were individually dissolved in M/15 phosphate buffer solution (pH 7.0) at concentration of 4 mg protein/ml, whereas Mf-P was dissolved in 0.6M KCl/phosphate buffer(pH 7.0). The proteins were separately succinylated according to the method of Groninger[4] at pH 8.5 with increasing of SA. Thus the succinylated proteins were allowed to stand overnight at 5℃, and followed by the measurement of succinylation level.

2.3 Calculation of succinylation level

The Lys contents of the sample proteins were measured by the method of Kakade and Liener,[24] and succinylation level was calculated. Whereas protein content was measured by the micro-biuret method.[25] Extinction coefficients of the sample proteins were 22.1, 17.4, 19.8 for BSA, casein and EWA, respectively. Those of Mf-P and Sp-P were 20.4, which were obtained in our preliminary experiments by the Kjeldahl and the micro-biuret methods.

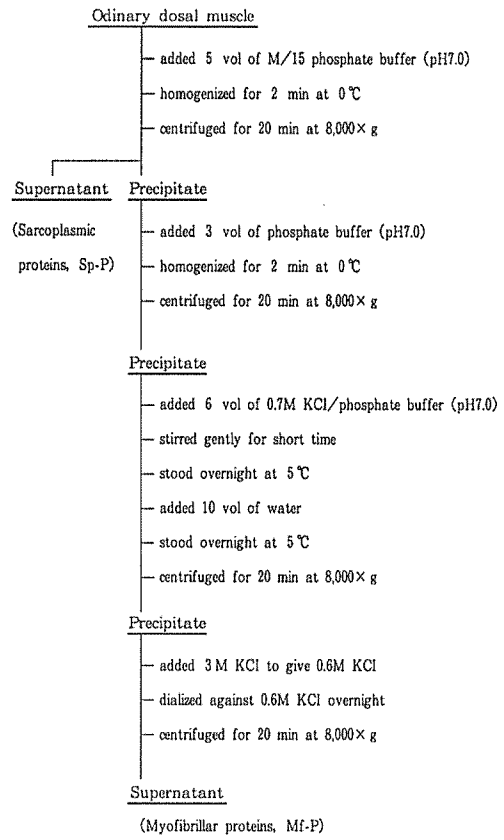

Fig. 1. Preparation methods for sarcoplasmic proteins (Sp-p) and myofibrillar proteins (Mf-P) from ordinary dorsal muscle of rudder fish, *Girella punctata*.

3 Results and Discussions

3.1 Influences of added amounts of SA on the succinylation level for BSA

Succinylation of protein is mainly caused by the reaction of ε-amino group of Lys residues with added SA, though SA also react with other functional group on the protein, i.e.tyrosine, histidine, and aliphatic hydroxy amino acids.[12] And accordingly, succinylation level would be influenced by the added amount of SA. Therefore, the succinylation level of BSA as a function of increasing the weight ratio of SA (SA/BSA) in the reaction mixture was investigated. This relation was termed as succinylation curve. The succinylation curve of BSA is shown in Fig.2.

As can be seen in this curve, succinylation level increased linearly with increasing the weight ratio of SA up to 0.5, whereas it moderately increased at higher regions up to 1.0. As apparent in these results, a linear correlation could not be obtained between SA/BSA(w/w) and succinylation level, and

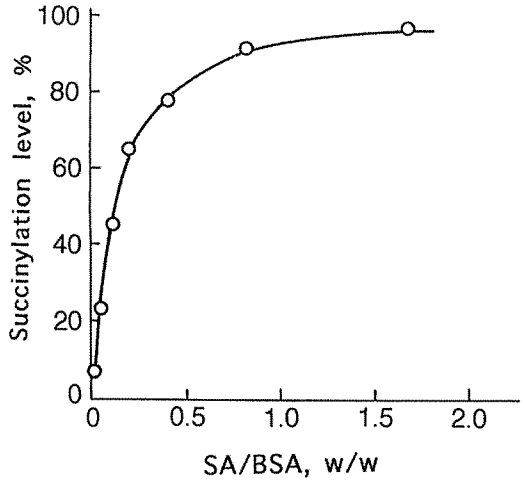

Fig. 2. Relationship between the weight ratio SA/BSA and the succinylation level.
Succinylation reaction was carried out by dissolving powdered succinic anhydride to BSA solution in 4mg/ml at pH 8.5.

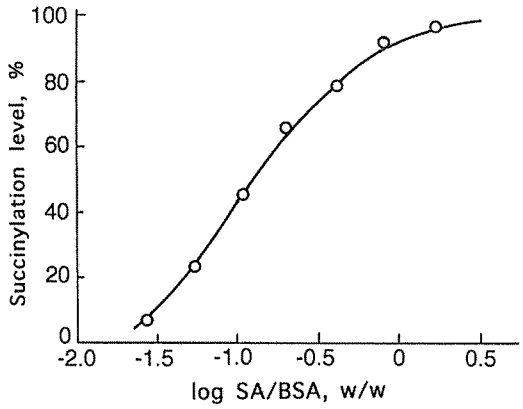

Fig. 3. Relationship between logarithmic weight ratio of SA/BSA and succinilation level.
The data of Fig.2 were plotted in logarithmic scale.

accordingly SA/BSA was not an appropriate function to control succinylation level. On the other hand, the logarithm of SA/BSA was thought to be more linear against succinylation level, from the variation pattern of the succinylation curve, and the relation of log SA/BSA against succinylation level was obtained as illustrated in Fig.3.

As shown in Fig.3, a very smooth S-shaped relation was obtained between log SA/BSA and succinylation level, and log SA/BSA is thought to be an appropriate function as to control the succinylation level. From this relation, we can easily calculate the prerequisites of SA to obtain a desirable succinylation level.

3.2 Comparison of the succinylation curves among different sample proteins

The succinylation curve of BSA was compared with those of four other sample proteins, and the succinylation-receiving property was compared among sample proteins. The succinylation curves of five sample proteins are shown in Fig.4.

As shown in Fig.4, the succinylation curves other than Sp-P coincided virtually at lower ratio regions of log SA/protein. One of the reasons why the succinylation curves did not coincide with each other at higher regions of log SA/proteins was thought depending on the difference in Lys content by sample proteins. That is, it would be more appropriate to think of the molar ratio of SA and Lys, because the succinylation is mainly the reaction of added SA and ε-amino group of Lys at the same molar ratio. And accordingly, the molar ratio of SA against Lys content was calculated on the same results shown in Fig.4, and succinylation level was plotted against log SA/Lys as shown in Fig.5. The Lys contents determined by the method of Kakade and Liener[24] were 11.77g Lys/100g protein (%) for BSA, 6.62% for casein, 5.68% for EWA, 7.52% for Mf-P, and 8.95% for Sp-P. These Lys content were somewhat lower than those of Tristram.[26]

As seen in Fig.5, all the succinylation curves with the exception of Sp-P, cannot be superposed upon each other, and therefore the three dimensional structure around ε-amino group of Lys residues, tyrosine, histidine, and aliphatic hydroxy amino

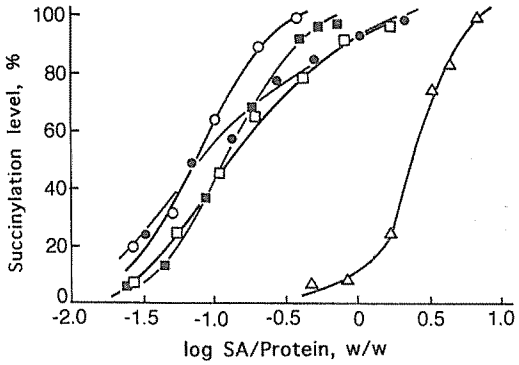

Fig. 4. Comparison of the succinylation curves, the relation between log SA/Protein (w/w) and succinylation level, among five sample proteins. −○−, Casein; −●−, EWA; −□−, BSA; −■−,Mf-P; −△−, Sp-P.

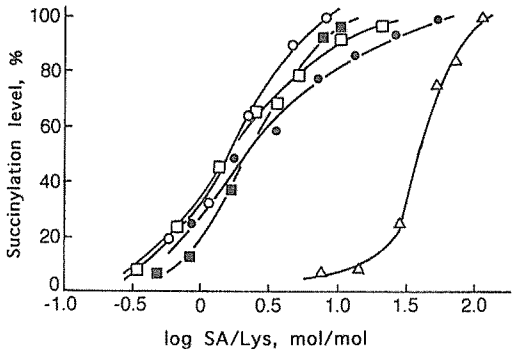

Fig. 5. Comparison of the succinylation curves, the relation between log SA/Lys and succinylation level, among five sample proteins.

acids[12] may be somewhat different among sample proteins. The reasons for the great deviation of Sp-P, on the other hand, could not be clarified in this experiment.

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タンパク質のサクシニル化率におよぼす無水コハク酸の添加量の影響

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牛血清アルブミン，卵白アルブミン，Casein，魚（メジナ）の筋原線維タンパク質および筋形質タンパク質を試料として，無水コハク酸（SA）の添加量がサクシニル化率におよぼす影響について検討した。SAとタンパク質の重量比（SA/Protein，W/W）とサクシニル化率の間には直線関係は見られなかったが，その対数値（log SA/Protein）とサクシニル化率との間には直線に近いゆるやかなS字の関係（サクシニル化曲線）が得られた。タンパク質の違いによるサクシニル化曲線はサクシニル化率が低い領域ではほぼ一致したが，高い領域では一致しなかった。しかし，SAとリジンのモル比（log SA/Lys，mol/mol）とサクシニル率の関係はより一層一致した。タンパク質の違いによる不一致の理由は，タンパク質によってリジンのアミノ基周囲の荷電状態に違いがあるためと推測された。