Note

Thermally Induced Gelation of Sesame 13S Globulin Acylated with Maleic and Citraconic Anhydrides

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Although chemical modification has been widely used as a tool for improving the chemical and physical properties of food proteins, problems concerning the safety for eating remain to be solved. In previous reports, we have shown that thermally induced gelation of sesame 13S globulin can be brought about under alkaline conditions, but not under acidic conditions.^{1~3)} However, under the alkaline conditions, artificial intramolecular crosslinkages such as lysinoalanine may be formed.⁴⁾ In this report, the effects of reversibly converting amino groups to carboxylate anions on the gelation of 13S globulin were investigated. We found that the globulin modified with maleic or citraconic anhydrides can form gels by thermal treatment under acidic conditions. In addition, the carboxylate anions in a modified globulin were found to be reversed to original free amino groups during the thermal treatment for gelation.

The 13S globulin (extract II) was prepared from sesame seeds as described previously.¹⁾ Acylation was carried out essentially as described by Butler and Hartley.⁵⁾ To the protein solution (1.6 g per 80 ml of 1 M NaCl), 40 mg or 400 mg of maleic anhydride, or 400 mg of citraconic anhydride were slowly added. The solutions were maintained at pH 8.5~9.0 and pH 8.0~8.5 during modification by the maleic and citraconic anhydrides, respectively. After the solutions had been incubated for 30 min at 0°C, they were dialyzed against distilled water, and then lyophilized. The two globulin samples modified with maleic anhydride are termed Mal₁- and Mal₂-globulin, and the globulin sample modified with citraconic anhydride is termed Cit-globulin. The proteins were dissolved in distilled water in test tubes, and the solutions were adjusted to 8% concentration and pH 3.0 at 0°C by adding dilute HCl solution. The samples were treated at 60°C or 70°C for 20 min, and then rapidly cooled in a water bath containing ice. The extent of gelation was macroscopically estimated by inverting the test tubes to check whether

the contents were fixed.

Table I shows that no gel was formed by incubating the native 13S globulin solution at either 60°C or 70°C. In contrast, hard gels were formed when the Mal₁-, Mal₂- and Cit-globulin solutions were incubated at 70°C. Thermally induced gels were also formed at 60°C, although they were less hard. Therefore, modification with maleic and citraconic anhydrides appears to improve the gelation properties of sesame 13S globulin.

The number of free amino groups in the native and modified globulins was estimated in 5 mM Na-borate buffer, pH 9.5, containing 0.5 mM sodium dodecyl sulfate both before and after gelation treatment by the technique using trinitrobenzene sulfonate.⁶⁾ After the thermal treatment, the gels were dissolved in 0.19 M sodium dodecyl sulfate. The samples were diluted with 10 mM Na-borate buffer, pH 9.5, and the free amino groups determined. As shown in Table I, before the gelation treatment, 51%, 81%

TABLE I. GELATION OF SESAME 13S GLOBULIN

Protein	Treatment at pH 3	Gelation ^a	TNBS-reactive amino group (%) ^b
Native globulin	N.T. ^c		100
	60°C	—	106
	70°C	—	108
Mal ₁ -globulin	N.T.		49
	60°C	+	73
	70°C	++	85
Mal ₂ -globulin	N.T.		19
	60°C	+	33
	70°C	++	62
Cit-globulin	N.T.		44
	60°C	+	101
	70°C	++	100
dMal ₁ -globulin	N.T.		102
	60°C	—	101
	70°C	—	102
dCit-globulin	N.T.		101
	60°C	—	96
	70°C	—	92

^a The minus, one plus, and two plusses represent no gel formation, soft gel formation and hard gel formation, respectively.

^b The number of trinitrobenzene sulfonate-reactive amino groups in native globulin without thermal treatment is expressed as 100%.

^c N.T. represents no thermal treatment.

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and 56% of the amino groups were acylated in Mal₁-, Mal₂- and Cit-globulin, respectively. After the gelation treatment, the number of free amino groups were partially reversed in Mal₁- and Mal₂-globulins, and completely in Cit-globulin. These observations are consistent with the previous data showing that amino groups acylated with maleic or citraconic anhydride can be reversed by incubating in acidic conditions.⁷⁾

It was tested whether the gelation of acylated globulins depends on some secondary effects including protein denaturation. The Mal₁- and Cit-globulins were deacylated by dialyzing modified protein solutions against 10 mM acetate buffer at pH 2.0 for 42 hr and 4 hr, respectively (the deacylated proteins are respectively termed dMal₁- and dCit-globulin). The deacylated proteins were tested for their ability to form thermally induced gels in the same way. The solutions containing the deacylated globulins were more viscous than native globulin solution both before and after thermal treatment, suggesting that some denaturation may have been accompanied by acylation and subsequent deacylation. However, as shown in Table I, no gel formation was seen when the dMal₁- and dCit-globulins were incubated at 60°C or 70°C. The lack of gelation in the deacylated globulins indicates that during the gelation process under acidic conditions, some of the amino groups may need to be acylated. In the case of dCit-globulin, deacylation may have been completed after the important processes for gelation were finished.

In conclusion, the thermal gelation of 13S globulin could be induced under acidic conditions by its acylation

with maleic and citraconic anhydrides. In Cit-globulin at least, the modified protein was completely deacylated during gelation treatments. If the released citraconic acid has been removed by dialysis, sesame 13S globulin gels can be made safely edible.

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