The Effects of Cyanate in Urea Solutions on the Gel Electrophoresis of the Subunits of Soybean 11S Globulin

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In ultracentrifugal studies, 11S globulin, which is one of the major components of soybean protein, has been shown to be capable of dissociating into subunits¹⁾ in the presence of urea and β -mercaptoethanol (β -ME). The subunits could be fractionated by DEAE-cellulose column chromatography in the presence of urea at room temperature.²⁾

However, it was observed that the migration bands of the fractionated subunits were not as sharp as that of the control. The vague bands not only made it dif-

ficult to discriminate the subunits, but also indicated that some chemical reaction might be taking place in the protein side chains during column chromatography. Indeed, aqueous solutions of urea develop appreciable concentrations of cyanate on standing³⁾ and reactions of cyanate with proteins have been reported, such as carbamylation. The present study is concerned with carbamylation of the 11S-subunits on gel electrophoresis with urea.

RESULTS AND DISCUSSION

Figure 1-A shows the migration patterns of dissociated CIF⁴⁾ incubated in 0.04 M Tris-citrate buffer (pH 8.7) containing 8 M urea and 0.02 M β -ME for 1 to 120 hr at 25°C. It can be seen that the migration bands incubated for longer than 24 hr become vague, and as regards anodic migration, one band B₁ gradually disappeared, while another new band B₃ gradually appeared as the incubating time increased. Similar migration changes in the presence of urea were reported on carbamylation of globin⁵⁾ and α -crystallin.⁶⁾

Figure 1-B shows the migration patterns of dissociated CIF incubated in the above buffer at 25°C for 1 hr as a control and at 7 to 50°C for 72 hr. It is

FIG. 1-A (left) and FIG. 1-B (right).

The effects of incubating time and incubating temperature on the electrophoretic patterns of CIF incubated in 0.04 M Tris-citrate buffer (pH 8.7) containing 8 M urea and 0.02 M β -ME at 25°C in Fig. 1-A and for 72 hr in Fig. 1-B.

Gel buffer; 0.04 M tris-citrate (pH 8.7) containing 7 M urea and 0.02 M β -ME.
Constant voltage; 250 V, about 20 mA. Time; about 5 hr.

Temperature; 7°C.

* CIF incubated in the above buffer for 1 hr at 25°C.

FIG. 2. The Accumulation of Cyanate in Aqueous Solutions of 8 M Urea with 0.04 M Tris-citrate Buffer (pH 8.7) ○---○ and without 0.04 M Tris-citrate Buffer ●—●. I, 7; II, 25; III, 37; IV, 50°C.

remarkable that the incubating temperature very strongly affects the migration patterns.

Figure 2 shows the strong dependence of concentrations of cyanate developed from solutions of 8 M urea upon the temperature at which they are left to stand. The quantitative analytical method used for cyanate was the colorimetric method of Werner.⁷⁾ The apparent concentrations of cyanate developed from solutions of urea with Tris buffer are less than those in the absence of Tris buffer, because cyanate is consumed by chemical reaction with the Tris amino group. It is notable that concentrations of cyanate developed at 7°C are at a very low level.

Figure 3 shows the migration patterns of 11S globulin⁸⁾ incubated in the above buffer for 1 hr at 25°C as a control and in the above buffer with added KOCN up to concentrations from 0.005 to 0.10 M for 2 hr at 25°C. It can be seen that higher concentrations of cyanate lead to a gradual broadening of the cationic bands and to the appearance of anodic bands. Moreover, the migration changes in Fig. 3 are quite similar to those in Fig. 1-A.

From the above results, it can be concluded that carbamylation of the 11S-subunits takes place on incubation in solutions of urea with Tris buffer. Carbamylation seriously affects tryptic digestion of proteins, because lysine residues in proteins are easily attacked by cyanate to form stable homocitrulline residues.⁹⁾ Thus, it is necessary to watch for the appearance of artifact bands during gel electrophoresis in the presence of urea. Fortunately, cyanate concentration can be kept at a low level at low temperatures

FIG. 3. Electrophoretic Patterns, Showing the Effects of Incubation of 11S Globulin in Urea Solutions with Added KOCN up to the Concentrations shown.

Urea solution; 8 M urea in 0.04 M Tris-citrate buffer (pH 8.7) containing 0.02 M β -ME.

Conditions; incubating for 2 hr at 25°C.

* 11S Globulin incubated in urea solution for 1 hr at 25°C.

or at low pH, and therefore it is preferable to fractionate subunits at low temperature with DEAE-cellulose, or at low pH with an anion exchange cellulose.

In fact, at 5°C we succeeded in fractionating the 11S-subunits by DEAE-cellulose column chromatography. The results of further studies will be presented in a forthcoming paper.

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