Antigenicity of Xf Protein Components

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SUMMARY

The antigen-antibody neutralization of filamentous phage Xf showed first-order kinetics within the first 10 minutes. Failure to reverse the neutralization reaction by dilution indicated that a stable combination might be formed between phage and antibody.

Antigenicity of Xf was analysed by agar double diffusion and three distinct precipitin lines were observed. For identifying these three antigenic components, Xf particles were dissolved and separated into A-protein and B-protein. The results of agar double diffusion showed that one precipitin line corresponds to the A-protein and the other two precipitin lines are determined by the B-protein.

Xf is a filamentous phage isolated from Xanthomonas oryzae (Kuo et al. 1969). Polyacrylamide gel electrophoresis of the Xf protein coat has shown that the protein component contains two protein subunits, A-protein and B-protein (Marvin et al. 1974). However, when the antigenicity of Xf was analysed in this laboratory using agar double diffusion and immunoelectrophoresis, three precipitin lines were observed. Since this aspect of filamentous phages has been little studied (Marvin & Hohn, 1969), we were curious to know what is responsible for the presence of the extra antigen in Xf phage.

The inactivation of filamentous phage Xf by anti-Xf sera was investigated by neutralization study (Fig. 1). The inactivation curve was obtained by plotting the logarithm of the fraction of survivors against the time. It can be seen that during the first 10 min, inactivation is a first-order process, showing upward concavity afterward. Further incubation after stopping neutralization at 10 min shows no appreciable increase or decrease in the surviving fraction. Apparently, no reversible neutralization occurred in the reaction mixture after dilution. Similar results were obtained in f2 and φX174 studies (Bowman & Patnode, 1964; Rolfe & Sinsheimer, 1965; Rowlands, 1967; Witte & Slobin, 1972). Witte & Slobin (1972) concluded that there was a single critical site on the surface of f2, probably at the A-protein or in the vicinity of the A protein, responsible for the inactivation of the phage. But the deviations from linearity during the course of the inactivation indicate that the possibility of a multi-hit process cannot be ruled out (Rolfe & Sinsheimer, 1965; Witte & Slobin, 1972).

When the antigenicity of Xf was analysed by modified agar double diffusion (Ouchterlony, 1958) and immunoelectrophoresis, three precipitin lines were observed (Fig. 2a). This finding confirmed the presence of three antigenic determinants in Xf. To identify the three antigenic components, the A- and B-proteins were separated by SDS polyacrylamide gel electrophoresis (Marvin et al. 1974) followed by elution with 0.01 M-tris-buffer, pH 8.0, and tris-buffer containing 1% SDS, respectively. After testing by agar double diffusion, one precipitin line corresponds with A-protein (Fig. 2b), the other two precipitin lines are determined by B-protein (Fig. 2c). Only one precipitin line was reported for f2 (Rowland, 1967) and two precipitin lines in φX174 (Rolfe & Sinsheimer, 1965). The two antigenic determinants carried by the B-protein of Xf may be due to three different regions, acidic region, hydrophobic and basic region, of the B-protein (Marvin & Hohn, 1969). The different antigenic
response may depend on the difference of structure, complexity or even the outside electron cloud shape between different regions of B-protein.

The heat stability of the Xf antigen was examined by incubating phage suspension in a water bath for 10 min at various temperatures. Of the three antigenic determinants, which were designated a, b and c, with antigen a responsible for the precipitin line furthest from the antiserum well, antigenic determinant a was most thermolabile. The corresponding precipitin line was blurring after treatment at 60 °C for 10 min. Antigenic determinants b and c were abolished by treatment at 70 °C.

Trypsin at concentrations of 1 mg/ml or 0.1 mg/ml destroyed the antigenic determinant c. Treatment with chymotrypsin and deoxyribonuclease resulted in no loss of capacity of three determinants to give precipitin lines.

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Fig. 2. Serological reactions on agar double diffusion. (a) S = Xf immune serum; X = Xf phage; C = phosphate buffered saline. (b) S = Xf immune serum; A = A-protein; C = Phosphate buffered saline. (c) S = Xf immune serum; B = B-protein; C = phosphate buffered saline.
REFERENCES


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