INHIBITION OF ENZYME-ANTIENZYME INTERACTION BY TRYPTIC DIGESTS OF ENZYME

S. MINATOGAWA AND P. J. RUSSELL

Department of Biochemistry, University of Kansas Medical Center, Kansas City, Kansas 66103

Received February 28, 1968

This paper presents evidence that peptides of tryptic digests of adenylate kinases (EC 2.7.4.3) from yeast (Chiu et al., 1967) and rabbit muscle (Noda and Kuby, 1957) are capable of inhibiting the rate of interaction of these enzymes with their respective antisera (Minatogawa et al., 1967). The inhibitions appeared specific and affected only the rate of interaction and not the final quantity which interacted. The findings suggest that a kinetic approach to the search for active peptide fragments is a sensitive method which permits the detection of antigen fragments which reversibly combine with antibody.

METHODS AND MATERIALS

Trypsin, in a ratio of 1:10 by weight, was incubated with the enzymes or bovine serum albumin (10 to 40 mg) at 25° in 10 mM $CaCl_2$, maintained at pH 9.0 by a pH-stat apparatus. The reactions were complete in 1-2 hr, at which time no enzymic activity of adenylate kinase was detectable at 1000-fold concentration greater than that used to assay untreated enzyme. Two mg of soybean trypsin inhibitor per mg of trypsin were then added to the tryptic digests to insure inactivation of trypsin. It was ascertained that 10 mg of soybean inhibitor or inactivated trypsin had no effect on adenylate kinase activity and no effect on the rate of inactivation with antiserum.

Enzyme-antienzyme interactions. Interactions were in 0.05 M potassium phosphate buffer, pH 7.0 at 25° containing equal volumes of antiserum (untreated or previously incubated for 2 hr with an equal volume of tryptic digest) and about 6 enzyme units (IUB) of adenylate kinase per ml. Portions of such mixtures were removed at time intervals for assay at 25°. The enzyme was assayed by the reverse reaction of adenylate kinase, AMP +ATP = 2 ADP (Su et al., 1967).

Assay for antisera. The enzyme inactivating capacity of an antiserum is expressed as the number of enzyme units inactivated per ml of serum after incubation for 2 hr at 25° followed by 16 hr at 4°. No further inactivations were detected after this procedure.

Antisera. Antiserum was obtained by a single injection of 2 mg adenylate kinase emulsified with Freund's complete adjuvent into adult guinea pigs. Collections of serum were made weekly. Sera were never pooled. All controls contained normal serum which was without effect upon the adenylate kinases. Homologous enzyme-antienzyme systems were capable of complete inhibition of enzyme activity within a few seconds.

Enzymes. Yeast adenylate kinase was the homogeneous F 2 fraction of Chiu et al., (1967) and myokinase was a purified commercial preparation (Sigma Co., Type III).

RESULTS

As shown in Figure 1, tryptic digests of myokinase and yeast adenylate kinase inhibited the rate of formation of the enzyme-antienzyme complex. In Figure 1(A), antimyokinase reacted with myokinase at a greatly reduced rate when the tryptic digests of myokinase were pre-incubated with the antiserum. Similarly, in Figure 1(B) the tryptic digests of yeast adenylate kinase inhib-

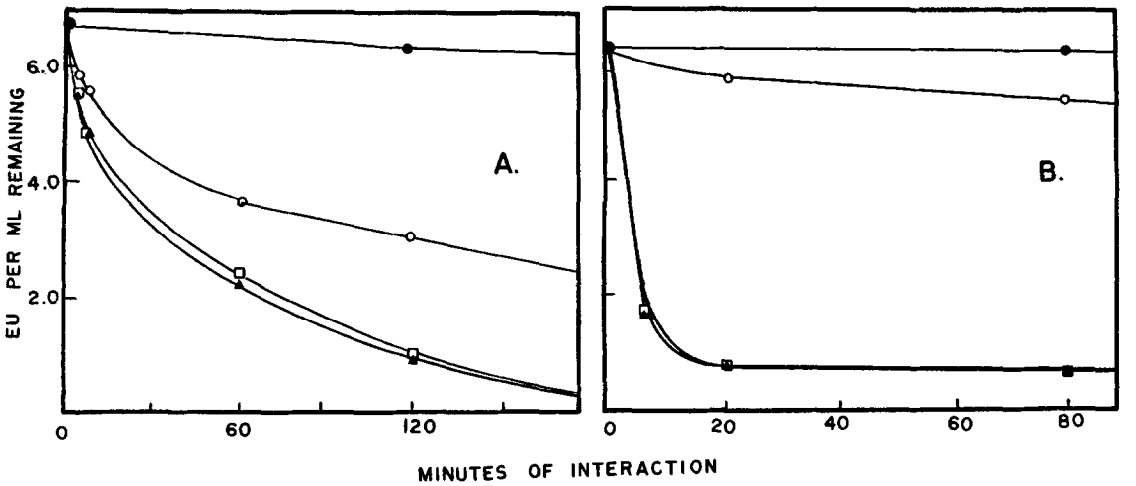

Figure 1. Inhibition of the rates of interactions of the adenylate kinases with their antienzymes by tryptic digests of the enzymes.
A. The antimyokinase inactivated 20 enzyme units (EU) of myokinase per ml of antiserum. Final antiserum dilution during the inactivation was 1:3.
B. The anti-yeast adenylate kinase inactivated 23 EU of yeast adenylate kinase per ml of antiserum. Final antiserum dilution during the interaction was 1:4.

The symbols are as follows: (●——●), interaction with normal serum; (o——o), interaction with antiserum previously incubated with tryptic digests of enzyme; (▲——▲), interaction with antiserum alone; and (□——□), interaction with antiserum previously incubated with tryptic digests of bovine serum albumin.

---

ited the rate of interaction of yeast adenylate kinase with its antiserum. In both instances, tryptic digests of bovine serum albumin (BSA) had no effect on these reaction rates. Though not illustrated here, the tryptic digests of myokinase had no effect on the rate of interaction of the homologous enzyme-antienzyme reactions of the yeast system, and the tryptic digests of yeast enzyme had no effect on the myokinase system.

It is to be emphasized that only the rate of the reaction with antiserum was affected by the enzyme fragments, not the final quantity reacted. Incubation of the systems shown in Figure 1 for 24 hr at 4° resulted in the same quantity of enzyme

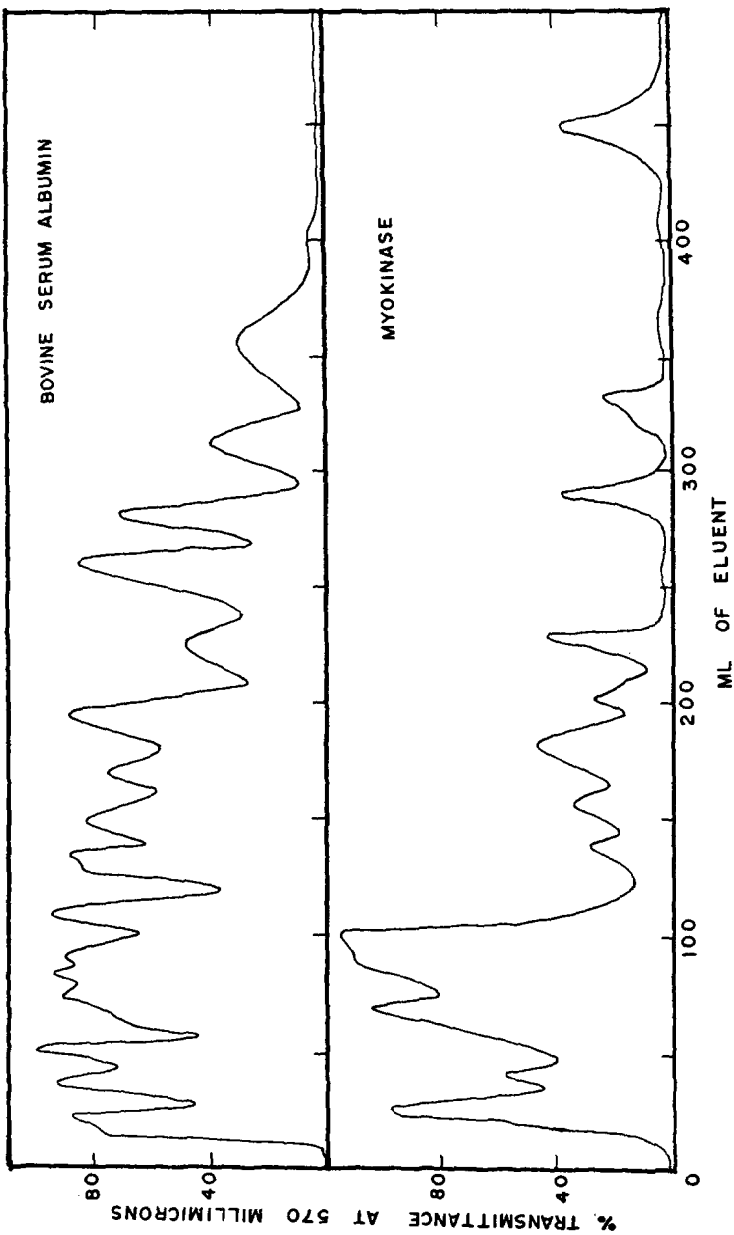

Figure 2. Peptide patterns of soluble tryptic peptides from bovine serum albumin and myokinase obtained by automatic chromatography (Jones, 1964). The trypsin digestion procedures are given in the text.

inhibited by antiserum, whether or not tryptic fragments were present. Under the same conditions, incubation with normal serum showed no inhibition.

The tryptic digests of myokinase and albumin have been subjected to automatic column chromatography by the method of Jones, (1964). Figure 2 shows the tryptic digest peptide patterns of BSA and myokinase. The individual peptide fractions are presently being prepared for assay of their ability to inhibit the rate of interaction of myokinase with its antienzyme. Similarly, the yeast enzyme fragments are being prepared for analysis. In the preparation of tryptic digests of myokinase for column chromatography, acidification of the digests to pH 3 or lower resulted in some precipitation. The precipitate was removed, brought into solution by neutralization and had no effect on the rate of the myokinase-antimyokinase interaction. Tryptic digests of BSA behaved similarly, but the acid insoluble precipitate was less.

DISCUSSION

It has been shown that tryptic digests of yeast adenylate kinase and myokinase inhibited the rate of interaction of the enzymes with their respective antienzymes, but not the final quantity of enzyme inhibited. The specificity of the inhibition was indicated by the lack of effect by tryptic digests of bovine serum albumin and the lack of effect by tryptic digests of one system upon another.

The peptide fragments which react with the antibody were probably displaced by the complete antigen (enzyme) since the quantity of enzyme ultimately complexed was not affected. These enzyme-antienzyme complexes are not readily reversed (Russell *et al.*, 1966; Minatogawa *et al.*, 1967). The presence of an essentially irreversible system in a reversible system would thus proceed

to "completion", but at a diminished rate.

In our search for active peptides, the effects of such fragments on the rates of interaction of antigen-antibody systems was found to be very sensitive. It is feasible that the application of kinetic parameters to detect peptide fragments might also be adapted for precipitin reactions (Landsteiner, 1942; Porter,1959). In such cases, the rate of the precipitin reaction rather than the quantity precipitated becomes the parameter. It is considered that these inhibiting peptide fragments contain the whole or part of the antigenic sites on the antigen. When isolated, such inhibiting peptides may then be analyzed for amino acid content (Lapresle and Webb, 1965).

ACKNOWLEDGEMENT

The authors wish to express their gratitude to Dr. J. Kimmel and Mrs. B. Sammons for the fractionation of the tryptic digests.

REFERENCES

Chiu, C. S., Su, S. and Russell, P. J., Biochim. Biophys. Acta, 132, 361 (1967)
Jones, R. T., Cold Spring Harbor Sympos. Quant. Biol., 29, 297 (1964)
Landsteiner, K., J. Exp. Med., 75, 269 (1942)
Lapresle, C. and Webb, T., Biochem. J., 95, 245 (1965)
Minatogawa, S., Russell, P. J. and Mira, O. J., Bacteriol. Proc., 67, 81 (1967)
Noda, L. and Kuby, S. A., J. Biol. Chem., 226, 541 (1957)
Porter, R. R., Biochem. J., 66, 667 (1959)
Russell, P. J., Mira, O. J. and Chiu, C. S., Federation Proc., 25, 247 (1967)
Su, S., Chiu, C. S. and Russell, P. J., Biochim. Biophys. Acta, 132, 370 (1967)