INSULIN-CATALYZED PROTEOLYSIS

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Previous studies from this laboratory (Rieser and Rieser, 1964) have shown that trypsin and chymotrypsin can act like insulin with regard to several anabolic activities in rat diaphragm muscle. The catalytically inactive diisopropylfluorophosphate-derivatives of these enzymes tend to lose their insulin-like action. The apparent hormonal activity of these two proteases thus depends, at least for the most part, on their integrity as enzymes. This raises the question whether the converse is also true, that is, whether insulin might be endowed with enzymatic properties. The present study was undertaken to test insulin for possible proteolytic activity.

MATERIALS AND METHODS

Crystalline beef insulin, lot numbers PJ-4609 and 795372 (Lilly) was dissolved in N/300 hydrochloric acid. Hemoglobin and casein were purchased from Fisher Scientific Company, horse heart myoglobin from Nutritional Biochemicals Corporation, elastin and ribonuclease from Worthington Biochemical Corporation. Protein solutions were incubated with insulin at pH 7.5 and 37 degrees Centigrade. Proteolytic activity was assayed at different time intervals of incubation by means of the Folin phenol reagent or the colorimetric ninhydrin method (Moore and Stein, 1948) applied to the trichloroacetic acid-soluble fraction of the incubation mixture. Elastase activity was assayed by the method of Sachar et al (1955) and carboxypeptidase activity according to Snoke and Neurath (1950). Activity toward

N-benzoyl-L-arginine ethyl ester was determined according to Schwert and Takenaka (1955) and activity toward p-toluenesulphonyl-L-arginine methyl ester and N-benzoyl-L-tyrosine ethyl ester according to Hummel (1955). Dipeptidase activity was determined with the colorimetric ninhydrin method, and activity toward benzoyl DL-arginine p-nitroanilide with the method of Erlanger et al (1961).

RESULTS AND DISCUSSION

Figures 1 and 2 show that insulin does indeed possess proteolytic activity: it is active toward hemoglobin and myoglobin. In figure 1, the amount of proteolysis of a one percent solution of urea-denatured hemoglobin is expressed as the number of micrograms of tyrosine released. The weight ratio of insulin

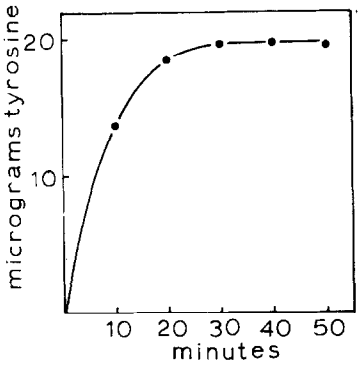

fig.1

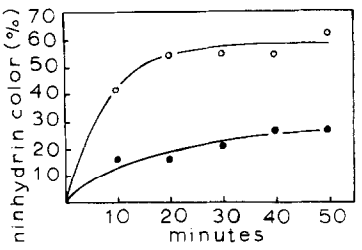

fig.2

to hemoglobin was 1 : 125. In figure 2, proteolysis of one percent myoglobin is expressed as relative increase in ninhydrin color value. The ratio of insulin to myoglobin was 1 : 125 (open circles) and 1 : 1250 (filled circles). In addition, crystalline beef insulin caused a 12.5 percent increase in ninhydrin color value after ten minutes in the trichloroacetic acid-soluble fraction of incubation with myosin-free acetone powder of rabbit skeletal muscle. This preparation contained actin, tropomyosin, and several other proteins. However, insulin did

not show hydrolytic activity toward a purified actin preparation. Thus the muscle fraction toward which insulin is active, in addition to myoglobin, is not yet known. Insulin does not catalyze the hydrolysis of elastin, casein, or ribonuclease. It is similarly inactive toward the dipeptides glycyl glycine, glycyl-L-tyrosine, and DL-leucyl-DL-tyrosine, toward N-benzoyl-L-tyrosine p-nitroanilide, and toward all other synthetic substrates tested except for p-nitrophenyl acetate (see below).

The possibility existed that the insulin preparations used in this work were contaminated with pancreatic proteases. A contamination with trypsin was especially suspected, since the insulin preparations we received had been treated with trypsin in order to remove glucagon. But contamination by the major pancreatic proteases, including trypsin, cannot account for the observed results, for insulin, even at a concentration of one milligram per milliliter, failed to hydrolyze the specific trypsin substrate p-toluenesulphonyl-L-arginine methyl ester as well as the chymotrypsin substrates benzoyl-L-tyrosine ethyl ester and acetyl-L-tyrosine ethyl ester. Similarly, insulin preparations did not exhibit any carboxypeptidase activity. Furthermore, insulin which had not been treated with trypsin (Iletin, Lilly), will cause an 11.3 percent increase in ninhydrin color value of trichloroacetic acid-soluble supernate from an incubation with hemoglobin (ratio of insulin to hemoglobin, 1 : 1250).

We must therefore conclude that the insulin molecule is itself endowed with the ability to catalyze the hydrolysis of proteins. This property of the hormone is not surprising, since it has already been shown by Hartley and Kilby (1954) that insulin can catalyze the hydrolysis of p-nitrophenyl acetate to yield p-nitrophenol and acetic acid. Whether the newly found proteolytic capability of insulin has anything to do with the normal

functioning of the hormone is, of course, an open question at this time. It is perhaps significant that insulin should have among its "substrates" proteins from muscle, one of its target organs. Work is now in progress in this laboratory to attempt to answer some of the many questions raised by the results of the present study.

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