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The enzymic reactivation of reduced ribonuclease PN 10021

It is known from the work of ANFINSEN AND HABER[1], and WHITE[2] that bovine pancreatic RNAase (EC 2.7.7.16) is easily reducible by various thiol compounds. The reduced inactive enzyme may be reoxidized by atmospheric oxygen and its original enzymic activity is almost recovered if the oxidation is carried out under well-defined conditions. Recently similar reoxidation and reactivation of reduced insulin[3], lysosyme[4], trypsin[5], Taka-amylase[6] and alkaline phosphatase[7] have been reported.

We found that pigeon and chicken pancreas contain a heat-labile factor which catalyses the reoxidation of reduced RNAase.

Crystalline bovine pancreatic RNAase (prepared in our institute) was reduced with mercaptoethanol in the presence of 8 M urea, according to the method of ANFINSEN AND HABER[1]. The reagents were separated from the reduced protein on Sephadex G 25 column, with 0.1 N acetic acid as eluent. The pooled RNAase-containing fractions could be stored at 2° for about 2 weeks without considerable oxidation. The solution was neutralized with NaOH before use.

In the reoxidation experiments the reduced RNAase was incubated in Tris

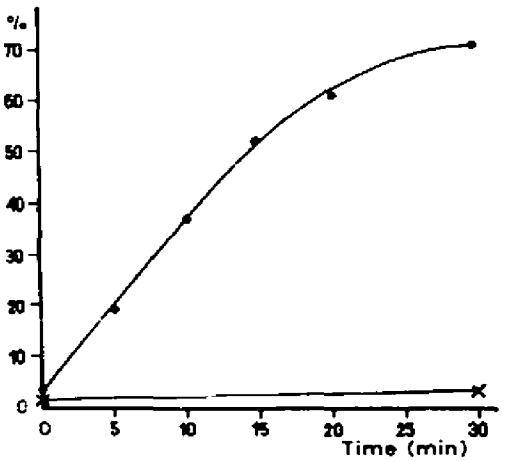

Fig. 1. Time course of the reactivation Incubation mixtures contained: 30 μmoles Tris buffer (pH 7.5) $1.7 \cdot 10^{-2}$ μmoles reduced RNAase, and 0.2 ml pigeon-pancreas extract (containing 9.2 mg protein and RNAase equivalent to $0.4 \cdot 10^{-3}$ μmoles bovine pancreatic RNAase). The control curve × contained the same mixture without pancreatic extract. The final volume was 0.8 ml. RNAase activity is expressed as the percentage of the theoretical maximum (original) activity.

buffer in the presence of pancreatic extract and aerated at 37°. At various intervals 0.1-ml aliquots were taken out and pipetted into 1 ml cold 0.25 N H_2SO_4 (in order to release the bound RNAase of the extract, and to stop the oxidation). After 10 min the solutions were neutralized with 1 N NaOH, and after appropriate dilution RNAase activity was assayed by the method of SCHUCHER AND HOKIN[8].

Fig. 1 shows the results of a typical experiment. In the presence of pancreatic extract (acetone-dry powder of pigeon pancreas was extracted with 10 vol. 0.05 M

TABLE I

DEPENDENCE OF THE REACTIVATION ON TWO COMPONENTS

$7 \cdot 10^{-3}$ μmoles reduced RNAase incubated with 25 μmoles Tris buffer (pH 7.5) and additions as noted. Final volume, 0.5 ml. Dialyzed extract, 0.1 ml (4.2 mg protein) chicken-pancreas extract dialyzed for 48 h against Tris buffer. Initial RNAase in all samples less than $0.1 \cdot 10^{-3}$ μmoles. Boiled extract, 0.05 ml from the supernatant of a boiled and centrifuged pancreatic extract. Yeast extract, 2 mg Difco powdered yeast extract.

No.		RNAase activity increase in 40 min (mμ moles)
1	No addition	0.2
2	Boiled extract alone	0.3
3	Dialyzed extract alone	1.1
4	Dialyzed extract + boiled extract	2.5
5	Dialyzed extract + yeast extract	3.1

Tris–HCl buffer (pH 7.5), more than 70% of the original activity of RNAase was recovered in 30 min. During this time the control showed no appreciable rise in activity.

Further investigations have shown that the reoxidizing activity of the pancreatic extract depends on the presence of a non-dialyzable heat-labile factor and a dialyzable heat-stable factor. This latter could be replaced by the solution of Difco powdered yeast extract (Table I).

We tried to purify the heat-labile factor. The acetone-dry powder of chicken pancreas was extracted by 0.05 M Tris-HCl buffer (pH 7.5). From this extract, by means of $(NH_4)_2SO_4$ precipitation and chromatography on DEAE-cellulose, an approx. 30-fold purification was obtained. When testing the purification procedure, the incubation mixtures were supplemented with 2 mg Difco yeast extract.

We conclude from these results that pigeon and chicken pancreas contain an enzyme which is able to catalyze the sulfhydryl → disulfide transformation in a protein molecule. We assume that this new enzyme might play a role in the last step of protein biosynthesis, in the formation of the properly folded active enzyme molecules.

A detailed report will be published elsewhere.

Note: During the preparation of our report we received through the courtesy of Dr. C. B. ANFINSEN the manuscript of the report of GOLDBERGER *et al.*[9]. These authors found a rat-liver microsomal system which is very similar to the pancreatic enzyme described by us.

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PN 10019

Enzymic formation of adenosine triphosphate with acetyl phosphate as donor in a yeast extract

When studying the polyphosphate synthesis by polyphosphate kinase (ATP: polyphosphate phosphotransferase, EC 2.7.4.1) in a yeast extract using ^{32}P-labelled acetyl phosphate, ADP, and a preparation of bacterial acetate kinase (ATP: acetate phosphotransferase, EC 2.7.2.1) as ATP-forming system (*cf.* MUHAMMED[1]), it was found that polyphosphate synthesis proceeds almost at the same rate when the acetate kinase was omitted from the reaction mixture. This observation seems to imply that our yeast extract contains an enzyme which catalyzes the transfer of a phosphate residue from acetyl phosphate to ADP.

A preparation of this enzyme can be obtained by treating a suspension of 3 g well-washed pressed baker's yeast in 30 ml isotonic phosphate buffer (pH 7.4) containing 0.05 M cysteine, in the cell disintegrator of MERKENSCHLAGER *et al.*[2] for 3 min. The resulting mixture is diluted with the same amount of the buffer solution and then centrifuged at 20 000 × *g* for 1 h at 4°. The supernatant contains the enzyme; it can be dialyzed against the buffer solution containing cysteine without loosing its activity. Its protein content is 15–20 mg/ml, measured according to WARBURG AND CHRISTIAN[3]. An approximately 3-fold concentration of the enzyme can be achieved by saturation with ammonium sulfate to 60% of the solution and removal of the inactive precipitate.

Enzyme activity is measured by incubating a mixture of 10 μmoles labeled acetyl phosphate (prepared according to KORNBERG *et al.*[4]) in 0.1 ml water, 10 μmoles ADP in 0.1 ml water, and 0.3 ml of the buffered enzyme preparation for 15 min. Then the reaction is stopped by adding 1 ml 7% $HClO_4$. For the removal of polyphosphate, formed by the polyphosphate kinase present in the preparation, 1.5 ml of a 0.1% albumin solution are added. After 10 min cooling in ice-water the mixture is centrifuged for 10 min at 6000 × *g* and 4°. To the supernatant 10–20 mg Norite charcoal are added and the mixture is shaken for 30 min. The Norite is then centrifuged off (6000 × *g* at 4°) and washed with distilled water until the activity of the water is negligible.

For routine measurements the activity of the Norite suspension can be measured directly in a liquid β-counter (M2H, 20th Century Electronics Ltd.). For more exact determinations, it is necessary to elute with 10% pyridine; the activity is measured in the eluate.