PN 10023

Reactivation of cortisone-induced liver tryptophan pyrrolase by boiled liver cell sap and by cyclic adenosine 3',5'-phosphate

The lability of the preparations of the liver tryptophan pyrrolase is known[1]. Homogenates[2] prepared from the liver of rats after induction of the tryptophan pyrrolase activity by injection of cortisone showed a loss of activity if they were stored at 2° or incubated at 38°. The addition of tryptophan during the homogenization of the liver lowered the loss of activity[2]. Recently it was demonstrated that the tryptophan pyrrolase activity in the liver cell sap of normal[3] and cortisone[4]-treated rats is stimulated by the addition of hematin. Addition of boiled cell sap of liver or other organs has an activating effect on this system in the liver cell sap[5,6].

The present paper reports new findings showing the ability of the boiled liver cell sap and of the cyclic adenosine 3',5'-phosphate to reactivate the liver tryptophan pyrrolase system. We were able to confirm that the tryptophan pyrrolase activity in the liver cell sap from rats 4–5 h after cortisone injection falls after repeated freezing (−25°) and thawing or if the enzyme preparations were stored at 2° or incubated at 38°. However, if after 60 min of preincubation of the liver cell sap at 38° a sample of boiled liver cell sap was added to the preparation during the enzyme assay, there was a marked rise of enzyme activity (Table I). The rise was higher than after addition of hematin. The simultaneous addition of hematin with boiled liver cell sap brought about the highest activity.

Very similar results were obtained when synthetic[6] cyclic adenosine 3',5'-phosphate was added to the preincubated liver cell sap (Table II). The sample was

TABLE I

REACTIVATION OF THE TRYPTOPHAN PYRROLASE SYSTEM IN THE LIVER CELL SAP BY BOILED LIVER CELL SAP

Livers of adult female Wistar rats (180–250 g) 4–5 h after injection of 5 mg cortisone acetate per 100 g body wt. were homogenized in 7 vol. 0.14 M KCl containing 25 mM NaOH and 0.3 mM DL-tryptophan under cooling. The homogenate was centrifugated at 75 000 × *g* for 60 min at 0° and the cell sap diluted, 1 : 1, with 0.14 M KCl. The preparation was preincubated at 38° with shaking. 1 ml was used for the estimation of tryptophan pyrrolase activity in the medium described by KNOX[7]. The formylkynurenine and kynurenine formed was estimated spectrophotometrically at 321 and 365 mμ. The activity of the tryptophan pyrrolase system is expressed as a sum of μmoles of formylkynurenine and kynurenine liberated per 1 h by 1 g originally homogenized liver using corresponding extinction coeficients. Boiled liver cell sap[8] was prepared by immersing the cell sap in a flask in a boiling-water bath for 20 min. The clear supernatant after centrifuging was used.

Time of preincubation of the liver cell sap (min)	*Additions to the assay medium*[*]	*Tryptophan pyrrolase activity (μmoles/h/g liver)*
0	None	2.74
30	None	1.16
60	None	0.91
60	Hematin ($4 \cdot 10^{-3}$ μmoles)	1.96
60	Boiled liver cell sap (1 ml)	3.05
60	Hematin ($4 \cdot 10^{-3}$ μmoles) + Boiled liver cell sap (1 ml)	3.79

[*] Total volume, 4 ml.

electrophoretically pure. The addition of this cyclic nucleotide to the preincubated liver cell sap stimulated the activity especially when hematin was also added.

The results presented show that the loss of activity of the tryptophan pyrrolase system during incubation of the liver cell sap is at least in part accompanied by the formation of an inactive enzyme. The inactive enzyme can be reactivated in the presence of boiled liver cell sap or cyclic adenosine 3′,5′-phosphate. Hematin, which behaves as a dissociable cofactor[9] of this enzyme, is probably not directly involved in the transformation of the inactive enzyme to an active enzyme.

TABLE I

REACTIVATION OF THE TRYPTOPHAN PYRROLASE SYSTEM IN THE LIVER CELL SAP BY CYCLIC ADENOSINE 3′,5′-PHOSPHATE

Conditions as in Table I.

Time of preincubation of the liver cell sap (min)	*Additions to the assay medium**	*Tryptophan pyrrolase activity (μmoles/h/g liver)*
0	None	4.24
30	None	2.46
60	None	1.02
60	Hematin ($4 \cdot 10^{-2}$ μmoles)	1.05
60	Cyclic adenosine 3′,5′-phosphate (1 μmole)	2.71
60	Hematin ($4 \cdot 10^{-2}$ μmoles) + Cyclic adenosine 3′,5′-phosphate (1 μmole)	5.23

* Total volume, 4 ml.

The question arises whether *in vivo* there are some inactive molecules of the tryptophan pyrrolase system, as in light of the presented results any preparation of this enzyme could be regarded as partially inactivated, especially if the preparation is made without cooling and in the absence of extra tryptophan. Experiments directed towards confirmation of the identity of the factor present in the boiled liver cell sap with cyclic adenosine 3′,5′-phosphate and elucidation of the mechanism of reactivation of the tryptophan pyrrolase activity by this cyclic nucleotide which is known to be involved in regulation of the liver phosphorylase[10] are in progress.

The authors are indebted to Dr. J. SMRT from the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, for the generous gift of a synthetic sample of the cyclic adenosine 3′,5′-phosphate.

Czechoslovak Academy of Sciences,
Institute of Physiology,
Salmovská, Prague 2 (Czechoslovakia)

F. CHYTIL
J. SKŘIVANOVÁ

1 T. TANAKA AND W. E. KNOX, *J. Biol. Chem.*, 234 (1958) 1162.
2 J. W. DUBNOFF AND M. DIMICK, *Biochim. Biophys. Acta*, 31 (1959) 541.
3 P. FEIGELSON AND O. GREENGARD, *J. Biol. Chem.*, 236 (1961) 153.
4 P. FEIGELSON, M. FEIGELSON AND O. GREENGARD, *Recent Progr. Hormone Res.*, 18 (1962) 491.
5 F. CHYTIL, *Biochim. Biophys. Acta*, 48 (1961) 217.
6 F. CHYTIL, *Collection Czechoslov. Chem. Commun.*, 27 (1962) 1487.

[7] W. E. KNOX, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 2, Academic Press, New York, 1955, p. 242.
[8] M. SMITH, G. I. DRUMMOND AND H. G. KHORANA, *J. Am. Chem. Soc.*, 83 (1961) 698.
[9] P. FRIGELSON AND O. GREENGARD, *Biochim. Biophys. Acta*, 50 (1961) 200.
[10] T. W. RALL AND E. W. SUTHERLAND, *Cold Spring Harbor Symp. Quant. Biol.*, 26 (1961) 347.

Received November 5th, 1962

Biochim. Biophys. Acta, 67 (1963) 164–166

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

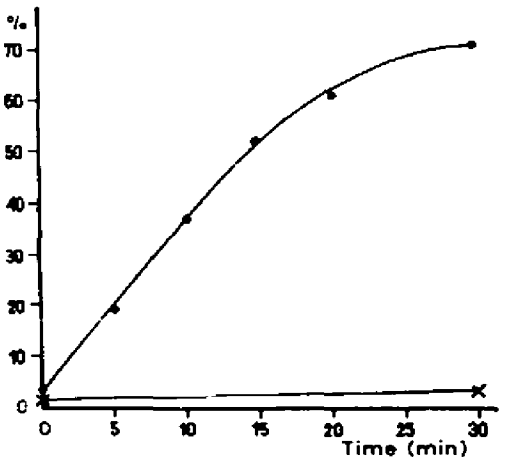

Fig. 1. Time course of the reactivation Incubation mixtures contained: 30 μmoles Tris buffer (pH 7.5) $1.7 \cdot 10^{-2}$ μmoles reduced RNAase, and 0.2 ml pigeon-pancreas extract (containing 9.2 mg protein and RNAase equivalent to $0.4 \cdot 10^{-3}$ μmoles bovine pancreatic RNAase). The control curve × 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.