proline in penultimate position. Recently peptides which block the conversion of angiotensin I and the inactivation of bradykinin were purified from snake venoms[4-6] and subsequently synthetized. The C-terminal end of most of the potent ones is prolyproline. That makes them resistant to hydrolysis by DH. Because the N-terminal region of bradykinin also contains a prolyproline sequence (Arg[1]-Pro[2]-Pro[3]-), we tested this tripeptide derivative of bradykinin as an inhibitor of DH.

Materials and methods. Homogenous DH was obtained by purifying the enzyme from hog lung[7]. The activity was determined in a Cary 15 recording UV spectrophotometer at 254 nm in a 0.1 *M Tris* buffer of pH 7.4 containing 0.1 NaCl[2,3]. The temperature was kept at 37 °C. The substrate was hippuryl-glycyl-glycine (HGG). In routine assay, a 1×10^{-3} *M* concentration was used. The inhibitor was preincubated for 5 min with the enzyme before adding the substrate.

Results and discussion. HGG was hydrolyzed by 1 mg of DH at a rate of 13 µmole per min. When the reciprocal values of velocity were plotted against the reciprocal concentrations of the substrate, and average K_m of 1.2×10^{-3} *M* was obtained (Figure 1).

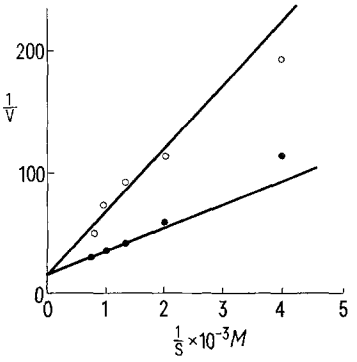

Fig. 1. Lineweaver-Burk plot of the hydrolysis of hippuryl-glycyl-glycine in presence (●—●) and absence (○—○) of 2.10^{-5} *M* Arg-Pro-Pro. The angiotensin I converting enzyme was purified from hog lung.

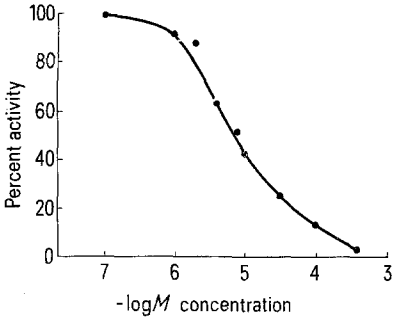

Fig. 2. Arg-Pro-Pro inhibits the hydrolysis of hippuryl-glycyl-glycine by purified angiotensin I converting enzyme of the lung.

The tripeptide Arg-Pro-Pro inhibited the reaction, giving a mean I_{50} value of 1×10^{-5} *M* (Figure 2). Using the same Lineweaver-Burk equation, the plotted 1/v values of the inhibited and uninhibited reactions intercepted on the ordinate. Thus, the tripeptide was a competitive inhibitor. The K_i of Arg-Pro-Pro was calculated to be 6×10^{-6} *M* using standard equation for estimation of K_i of competitive inhibitors.

Besides the two vasoactive peptides, DH cleaves a variety of substrates, such as the B chain of insulin[3] and several shorter optically active peptide substrates[2,3,5,7]. It was shown, however, that the ratio of the rates of cleavage of a short peptide substrate and angiotensin I stayed constant during the purification of the enzyme from lung[8,9]. Various agents inhibit DH, among them are the split products of the hydrolysis of bradykinin by DH (Phe-Arg) or that of angiotensin I (His-Leu)[1,2]. Competitive substrates such as the B chain of insulin or bradykinin inhibit the conversion of angiotensin I in the perfused lung in situ[3] and also in vitro[10].

Our experiments have shown that a fragment of bradykinin, with the same sequence as an active portion of the inhibitors derived from snake venoms, competitively inhibits DH. Thus, in addition to bradykinin which has a very short half-life in the body, its enzymatic degradation product as well, may block the conversion of angiotensin I.

Zusammenfassung. Nachweis, dass Arg-Pro-Pro, das N-terminale Tripeptid von Bradykinin, für die Hemmung der Konversion von Angiotensin I zu Angiotensin II in der Lunge verantwortlich ist.

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Ribonuclease Activity of Rat Thymus Chromatin Proteins

Today it is known that a substantial part of newly synthesized RNA in the cells of higher organisms is degraded in the nucleus[1,2]. Hybridization experiments show only about 20% of all RNA types synthesized in the nuclei come out into the cytoplasm[3,4]. Thus in the nuclei there must be an enzymatic system responsible for the strictly regulated specific degradation of certain RNA. Some data suggest that the degradation of nuclear RNA may take place directly in chromatin[5].

Many authors have tried to determine the ribonuclease activity of chromatin, mainly in order to assay the role of ribonucleases in the study of chromatin template activity

in RNA polymerase systems[6–8]. More detailed work[9] has shown the presence of ribonucleases in the chromatin of uterus mucosa in mice and rats, and a change of chromatin ribonuclease activity after administration of estrogens to animals.

The present study contains data on ribonuclease activity of rat thymus chromatin and the non-histone proteins (NHP) isolated from rats.

Methods. Isolation of chromatin. Chromatin was isolated from Wistar rat (150–180 g) thymus. The tissue was homogenized in 0.25 *M* sucrose, 3 m*M* $MgCl_2$, 0.01 *M* *Tris*-HCl (pH 7.2) in a glass-teflon homogenizer. The nuclie were separated by centrifugation (10 min at $700 \times g$) and then washed twice by the homogenization medium. The nuclei were washed consecutively with 0.14 *M* NaCl– 0.01 *M* *Tris*-HCl (pH7. 2), 0.075 *M* NaCl– 0.24 *M* EDTA (pH 8.0) and 3 times with 0.01 *M* *Tris*-HCl (pH 8.2). After the last centrifugation, the chromatin precipitate was suspended in 0.01 *M* *Tris*-HCl (pH 7.5). The protein/DNA ratio in chromatin was usually 1.4–1.45.

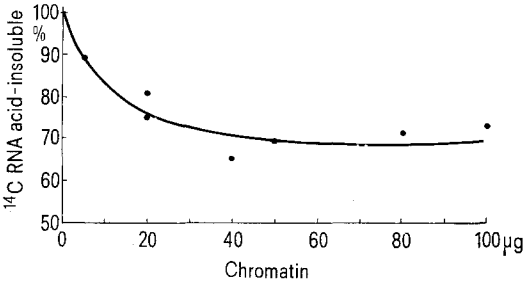

Fig. 1. Ribonuclease activity of rat thymus chromatin at pH 7.5 (see Methods).

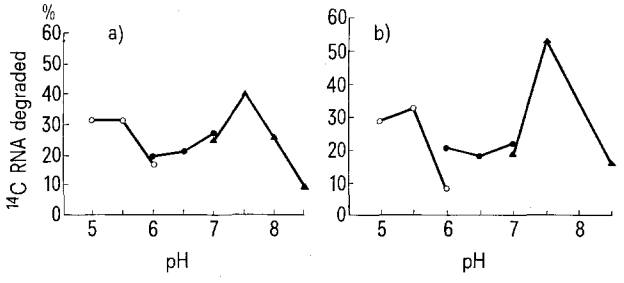

Fig. 2. Ribonuclease activity of rat thymus chromatin (a) and non-histone proteins (b) at different pH values. Each sample contained 50 µg of chromatin (a) or non-histone proteins (b). ○, 0.05 *M* acetate buffer; ●, 0.05 *M* sodium phosphate buffer; ▲, 0.05 *M* *Tris*-HCl buffer.

Isolation of non-histone protein. NHP was isolated from chromatin as is described earlier[10], with some modifications. Chromatin was suspended in 2.5 *M* NaCl, 0.01 *M* *Tris*-HCl (pH 8.2) and DNA was separated by ultracentrifugation (20 h at $170,000 \times g$) The supernatant was dialyzed against 0.5 *M* NaCl, 0.01 *M* sodiumphosphate buffer (pH 6.8) and passed through an Amberlite column CG-50. Histones were adsorbed by the column and NHP obtained from the column were dialyzed against 0.01 *M* *Tris*-HCl (pH 7.5). In some cases NHP was applied to the DEAE cellulose column in 0.01 *M* *Tris*-HCl (pH 7.5) and eluted either by 1 *M* NaCl in the same buffer (to obtain a concentrated protein solution), or by NaCl gradient from 0 to 0.35 *M* in *Tris*-HCl (pH 7.5).

Isolation of labelled RNA. 4 hours before killing, each rat was given 200 µCi of orotic acid. Nuclear RNA was isolated from rat liver by the phenol-detergent method[11]. The specific activity of nuclear RNA obtained by this method was 6×10^5 cpm/mg.

Estimation of ribonuclease activity. The 0.6 ml sample contained (except for cases which will be specified): 3µg nuclear [C^{14}]-RNA, 5 m*M* Mg Cl_2, 0.1 *M* NaCl, 0.05 *M* *Tris*-HCl (pH 7.5) and chromatin or NHP in various concentrations. The control sample did not contain any chromatin or NHP. The incubation was caried out for 2 h at 37 °C. The sample was rapidly cooled in an ice bath and 100 µg of RNA carrier and 0.7 ml of 10% TCA were added; 10–15 min later the precipitate was collected on membrane filters. Radioactivity was measured in a gas-flow counter. The ribonuclease activity of chromatin was judged by the decrease of the acid-insoluble fraction.

Results and discussion. The data given in Figure 1 on RNA hydrolysis by increasing chromatin concentrations indicate the presence of ribonuclease activity in the rat thymus chromatin preparation. It should be noted that the amount of acid-soluble products formed first goes up, as the concentration of chromatin increases, then the curve levels off. Figure 2a presents data on ribonuclease activity of chromatin at different pH values. It can be

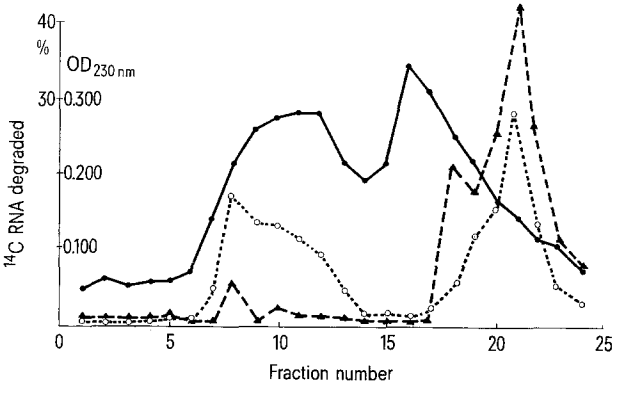

Fig. 3. Fractionation of NHP on DEAE cellulose column. NHP were eluted by NaCl gradient from 0 to 0.35 *M* in 0.01 *M* *Tris*-HCl (pH 7.5). 3 ml fractions were collected and optical density at 230 nm was measured. 0.2 ml aliquots of each fraction were used for measurement of ribonuclease activity. —●—, optical density; ---○---, RNAse activity (pH 7.2); ---▲---, RNAse activity (pH 5.2).

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seen that RNA degradation occurs at all pH values studied but there are 2 peaks on the curve – in the acid and weakly alkaline areas of pH. Acidic but not alkaline ribonuclease activity can be partially extracted by the 0.6 *M* and 1.0 *M* NaCl treatment. The acid ribonuclease is more effectively removed at pH 8.0 than at pH 6.0; pH does not influence the extractability of the alkaline ribonuclease. It thus appears that ribonucleases are chromatin components, as proteins adsorbed on chromatin are usually removed already at 0.35 *M* NaCl treatment[12].

Table I. NHP effect on the yield of RNA in an RNA-polymerase system

Template	Concentration of NHP (μg)	cpm	Inhibition (%)
Rat thymus DNA	—	3260	—
	21	2560	21.4
	42	1740	46.5
	63	1380	57.6
Rat liver chromatin	—	1700	—
	35	1276	25.5
Rat spleen chromatin	—	1500	—
	43	1120	25.2
	64.5	726	51.5
	86	640	57.5
Rat thymus chromatin	—	2030	—
	30	1625	20.0
	45	1245	38.7

RNA-polymerase was isolated from *E. coli* B. by the method of BABINET[19] with small modifications[20] to A-II fraction. Assay mixtures of 0.25 ml volume containing 50 m*M* *Tris*-HCl (pH 7.8), 10 m*M* $MgCl_2$, 0.15 *M* KCl, 1 m*M* β-mercaptoethanol, ATP, GTP, CTP and [^{14}C]-UTP 0.1 m*M* each, 12 μg of DNA or 25 μg of chromatin 3.1 μg of RNA polymerase and varying amounts of NHP, were incubated for 15 min at 37 °C. The tubes were then chilled in ice and 400 μg of bovine serum albumin in 2 ml of 0.01 *M* EDTA were added, followed by TCA to 5%. After 10 min the precipitates were collected on Millipore filters, washed with 20 ml of 5% TCA and dried. Radioactivity was measured on a Nuclear Chicago liquid scintillation spectrometer. Data of experiments with different templates cannot be compared because different RNA-polymerase preparations and ^{14}C-UTP were used.

Table II. Effect of NHP added after beginning of RNA synthesis on the RNA yield in RNA-polymerase system

Tube number	Incubation time	Addition	cpm
1	8	—	570
2	12 (8 + 4)	NHP	400
3	12 (8 + 4)	Water	620

Tube I. Assay mixture of 0.075 m*M* volume, containing 165 m*M* *Tris*-HCl (pH 7.8), 16.5 m*M* $MgCl_2$, 3.3 m*M* β-mercaptoethanol, ATP, GTP, CTP and [^{14}C]-UTP 0.3 m*M* each, 12 μg of DNA and 3.1 μg of RNA polymerase was incubated for 8 min chilled and treated as described in the legend to Table I. Tube 2. The same as Tube I but after 8 min incubation 0.175 ml (67 μg) of NHP were added and sample was incubated for additional 4 min. Tube 3. The same as Tube 2 but 0.175 ml of water were added instead of NHP. KCl was omitted to avoid reinitiation. Each value is the mean of 3 determinations.

In spite of the fact that a number of authors have found ribonuclease activity in different histone fractions[13–15], the suggestion that ribonucleases are constituent parts of NHP and the ribonuclease activity of histones is associated with NHP admixture, seems to be most likely[16]. The data in Figure 2b indicate that ribonuclease activity is really found in NHP preparation and that pH optima are the same as in chromatin. The two ribonuclease activities can be separated by NHP fractionation on DEAE cellulose (Figure 3).

The data of the Table I show that the yield of RNA in RNA-polymerase system (both for chromatin or DNA as templates) is reduced on addition of NHP. Reduction of DNA transcription by NHP was shown by SPELSBERG and HNILICA[17,18], and these data were interpreted as inhibition of DNA template activity with NHP. However, in our experiments this effect seems to be accounted for by RNase activity of NHP. Thus it is shown in Table II that the yield of RNA in RNA-polymerase system (with DNA as a template)for 8 min incubation period is higher than that for 12 min when NHP were added 8 min after beginning of RNA synthesis.

The data presented show that the effect of chromatin RNases on the RNA yield in RNA-polymerase system is greater than usually assumed. The ribonuclease activity of chromatin should be considered in studies of chromatin template activity and of NHP influence on the template activity of DNA or chromatin in the RNA polymerase system.

ВЫВОДЫ. Хроматин тимуса крыс обладает рибонуклеазной активностью с двумя оптимумами pH – в кислой и слабо-щелочной областях. Обе рибонуклеазы входят в состав негистоновых белков хроматина и могут быть разделены хроматографией на ДЕАЕ-целлюлозе.

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