Dissociation of RNA-Histone Complexes by DNA

Histones have been shown to form complexes with RNA[1-3] as well as with acidic proteins[2-7] and DNA[1,2,8,9]. Acidic proteins can interact and form complexes with free histones to prevent histone inhibition of the in vitro DNA-dependent RNA synthesis[2,3,6]. However, exogenous RNA, which also can form complexes with free histones, failed to prevent the histone inhibition of the in vitro RNA synthesis[3]. This report describes initial results of an investigation into the cause of the failure of RNA to prevent the histone suppression of the DNA template.

Materials and methods. Millipore filters (HA 0.45 μ pore size from the Millipore Corp., Bedford, Mass.) were used to analyze the complexes between histone and RNA and between histone and DNA. Whole unfractionated histone was prepared by acid extraction from saline-washed calf thymus nucleoprotein[10]. This histone preparation was found to contain very little if any nuclease activity. Highly polymerized calf thymus DNA (Sigma Biochem. Corp., St. Louis, Missouri) used in the experiments was tested for RNA and protein contamination, as well as protease and RNase activity, and was found to be essentially pure. The RNA (total yeast RNA, Mann Research Lab., 14% nitrogen and 8.5% phosphorous on a dry weight basis) was also found to be essentially free of protein and of nuclease and protease activity. Native DNA was diluted to 0.5 mg/ml and was denatured by either heating for 10 min at 100 °C followed by quick cooling to 4 °C in an ice bath or by increasing the pH to 12.5 with NaOH for 10 min at room temperature and then neutralizing with HCl and buffer 0.1 × SSC (0.015 *M* NaCl + 0.0015 *M* Na citrate, pH 7.0). The degree of denaturation was measured by hyperchromicity at 260 nm and by retention by Millipore filters of the DNA (totally denatured DNA is retained on these filters whereas native DNA passes through the filters).

Single-stranded DNA was prepared from heat-denatured calf thymus DNA by the method of McCALLUM and WALKER[11] in which the heat-denatured DNA is passed through an hydroxyapatite column at 60 °C in phosphate buffer. The proof of single-stranded DNA was obtained with melting studies on a Zeiss spectrophotometer. The single-stranded fraction showed no hyperchromicity when heated up to 95 °C and displayed a thermal denaturation pattern similar to that of the random coil polynucleotides.

Results and discussion. Both DNA and RNA can be selectively extracted from Millipore filters quantitatively using 0.5 *N* $HClO_4$. Incubation of the filters at 50 °C for 10 min in 0.5 *N* $HClO_4$ removes all the RNA; DNA is extracted by increasing the temperature to 100 °C (10 min extraction). The selectivity of this procedure is shown in Figure 1. In this experiment the filters were spotted with known amounts of nucleic acids and dried. The extracted RNA or DNA was determined by reaction with orcinol or diphenylamine respectively. Extreme care must be taken that fragments of the filters are not present in the aliquots used for orcinol reaction because they will interfere with the color reaction.

In RNA-histone retention experiments, the mixtures of histone and RNA were filtered through Millipore filters and each filter was washed with 20 ml of 0.030 *M* NaCl + 0.0030 *M* Na_3 citrate buffer, pH 7.0. Using a constant amount of RNA, a linear increase in RNA is retained on the filters as the ratio of histone to RNA is increased

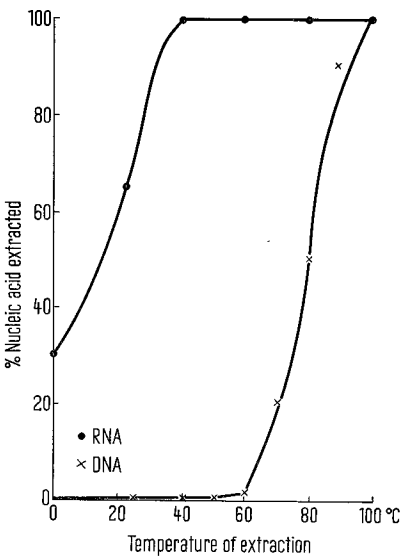

Fig. 1. Extraction of RNA and DNA from Millipore filters (Type HA, 0.45 μ pore size) by 0.5 *N* $HClO_4$. Total yeast RNA (100 μg) or calf thymus DNA (100 μg) were dried on a series of filters. The filters were placed in 2.0 ml of 0.5 *N* $HClO_4$ for 10 min at designated temperatures. The solutions were assayed for RNA by orcinol and DNA by diphenylamine. (Due to the hydrolytic products of the filters under more severe conditions, care must be taken that fragments of the filters are not included in the aliquots for the orcinol reactions.)

The effect of RNA on DNA-histone complexes

μg RNA added	μg DNA/filter	μg RNA/filter
0	94	0
25	91	0
50	90	0
75	93	0
100	93	0
125	90	0
150	92	0
175	93	0
200	93	0

Histone (100 μg) was mixed with DNA (100 μg) in a series of tubes in 0.1 × SSC. RNA (total yeast) was added in increasing amounts and the solutions passed through Millipore filters (see legends of Figures 2 and 3). The DNA is native calf thymus.

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(Figure 2). Histone was also found to be increasingly retained on the filters under these same conditions. Hence, the extent of complex formation between histones and RNA can be readily measured by this technique. The quantities of RNA and DNA in the 0.5 N $HClO_4$ extract of the filters can be measured by its absorbancy at 260 nm or by the reaction with orcinol and diphenylamine respectively. The latter 2 colorimetric tests were used to measure the quantities of nucleic acids. Using more harsh conditions, e.g. stronger acids, longer hydrolysis periods, or higher temperatures, to remove the nucleic acids from the filters, causes excessive interference with the color reactions for DNA and especially for RNA. This was found to be caused by the hydrolytic products of the filters alone. This is also the reason for not allowing any fragments of the filters in the aliquots for the orcinol reactions.

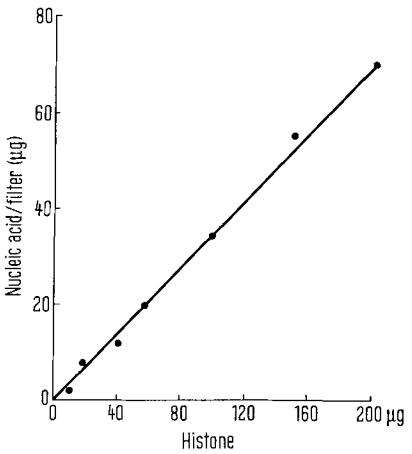

Fig. 2. Retention of RNA on filters by histone. In a series of reaction mixtures, total yeast RNA (100 µg) was mixed with increasing amounts of histone in 0.1 × SSC. The solutions were passed through Millipore filters (0.45 µ), the filters were washed with 20 ml each of 0.2 × SSC, dried, and placed in 0.5 N $HClO_4$ and hydrolyzed at 50 °C for 10 min to extract the RNA. RNA was analyzed by orcinol.

In another experiment, a constant amount of RNA and histone was used followed by the addition of increasing amounts of DNA, either native or denatured, to the interaction mixtures. Figure 3 shows that as the DNA is added to the mixtures containing complexes of histone and RNA, the amount of RNA retained on the filters is reduced while the amount of DNA is increased. These results can be interpreted that the histone-RNA complex is dissociated in the presence of the DNA. This explains the failure of the RNA to prevent the histone inhibition of the in vitro DNA-dependent RNA synthesis[3]. Further studies of the RNA-histone complexes showed that these complexes are weak as compared to histone-DNA complexes; the histone-RNA complexes dissociate at 0.15 M NaCl and higher whereas histone-DNA complexes were stable up to 0.4 M NaCl. To test whether RNA is capable of dissociating complexes of DNA and histone, 100 µg of DNA and 100 µg of histone were mixed followed by increasing amounts of RNA. Data shown in Table I demonstrate that RNA, even at quantities twice that of the DNA, does not dissociate complexes between DNA and histone. These results indicate that complexes between DNA and histone are more stable than complexes between RNA and histone.

It can be speculated that native DNA can dissociate the histone-RNA complex due to the higher affinity of histones for double-stranded DNA than for single-stranded DNA[9]. However, the fact that denatured DNA (heat or alkali denatured) is also able to dissociate the histone-RNA complex contradicts this possibility. Experiments using single-stranded DNA prepared from hydroxyapatite column chromatography showed similar results (Figure 4). If the histones bind to nucleic acids mainly through the phosphate groups, the dissociation of the histone-RNA complex by native, denatured, or isolated

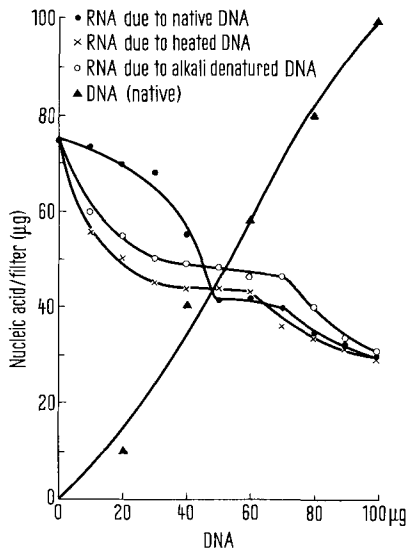

Fig. 3. The effects of native and (alkali or heat) denatured DNA on RNA-histone complexes. To a series of tubes was added 100 µg of total yeast RNA and 200 µg histone in 0.1 × SSC. Then native or denatured DNA was added in increasing amounts to the series of mixtures. The solutions were passed through filters and the filters treated as described in the legend of Figure 2. DNA (extracted by 0.5 N $HClO_4$, 10 min at 100 °C) was analyzed by diphenylamine.

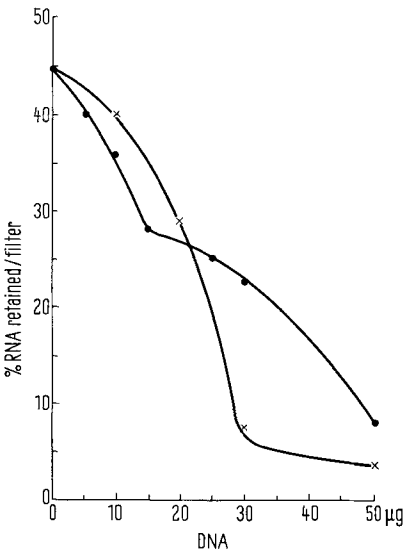

Fig. 4. The effect of single-stranded DNA on the histone-RNA complex. Histone (100 µg) was mixed with 50 µg of total yeast RNA in a series of tubes. Increasing amounts of (1) ●—●, native double-stranded DNA, or (2) ×—×, single-stranded DNA from the hydroxyapatite column were added to the mixtures. The samples were treated as indicated in the legends of Figures 2 and 3 and 'Methods'.

single-stranded DNA indicates much weaker binding of histones by the phosphate groups of RNA. The existence of histone-RNA complexes in cell nuclei is still uncertain; the in vitro dissociation of these complexes by low ionic strength (0.15 *M* NaCl and higher) and by DNA suggests that such complexes based on electrostatic bonding, may not exist under in vivo conditions. In addition, the failure of RNA to prevent the histone inhibition of the in vitro synthesis of RNA is probably due to the dissociation of histone-RNA complexes by the template DNA[12].

Zusammenfassung. Eine Bestimmungsmethode für die Interaktion zwischen Histon und Nukleinsäuren (RNS oder DNA) wurde beschrieben. Beide Nukleinsäuren komplexieren mit Histon; RNS-Histonkomplexe sind schwach und dissozieren in Gegenwart von DNS. Damit wird die Hemmung der RNS-Synthese in vitro mit Histon, die unabhängig von der Gegenwart der RNS im Reaktionsgemisch ist, erklärt.

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The University of Texas at Houston, M. D. Anderson Hospital and Tumor Institute, Department of Biochemistry, Houston (Texas 77025, USA), 21 May 1969.

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3-Methoxytyramine, a Catecholamine Catabolite regularly present in Human Urine[1]

In 1958 AXELROD, SENOH and WITKOP[2] could demonstrate by paperchromatography that rats given i.p. injections of dopamine excrete 3-methoxytyramine in the urine. This observation suggested that dopamine can serve as substrate for the catecholamine-*O*-methyltransferase not only in vitro as shown previously by AXELROD[3], but also in vivo. Since subsequently 3-methoxytyramine was found by fluorimetric methods to be present in brain homogenates of various mammals[4–6], no doubt remained that part of the dopamine formed in the organism is *O*-methylated under physiological conditions.

In view of the fact that the methoxyderivatives of norepinephrine and epinephrine, i.e. normetanephrine and metanephrine, are always excreted in certain amounts in the urine of normal individuals, we wondered whether this was not true also for 3-methoxytyramine, which, up to now, was rarely assessed and not always found in the urine[7–11]. Assuming that chromatographic techniques might not be sensitive enough and that the procedure of CARLSSON and WALDECK[13] for the determination of 3-methoxytyramine in tissue homogenates was inappropriate for our purposes, we developed a fluorimetric method[12] for quantitative measurements of 3-methoxytyramine in urine and used this procedure for the estimation of 3-methoxytyramine excretion in healthy individuals.

Materials and method. To 20 ml of a 24 h urine specimen 1 ml of 0.2 *M* EDTA (ethylene diamine tetraacetate · $2H_2O$) solution, 0.25 ml of a 2% ascorbic acid solution and 1 ml of 0.1 *M* phosphate buffer pH 6.5 (Sörensen) are added. After titration to pH 6.5 the urine is passed at 25 °C through a thermostatized column with an inner diameter of 4 mm containing Dowex AG 50 W × 8, 200–400 mesh, in Na^+-form, up to a height of 35 mm. This column adsorbs the catecholamines and their methoxyderivatives and, after rinsing with a mixture consisting of 10 ml of phosphate buffer, 1 ml of ascorbic acid solution and 40 ml water, norepinephrine and epinephrine can be eluted with 7 ml 1 *N* HCl (flow-rate 7–9 drops/min). If thereafter 25 ml of 5 *N* HCl are used for elution at the same flow-rate, a fraction containing dopamine and 3-methoxytyramine is obtained. To 5 ml of this fraction 3 ml of 0.5 *M* citrate-boric acid buffer pH 6.5 and 3 ml of 10 *N* NaOH are added. By heating this alkaline solution to 50 °C during 20 min dopamine but not 3-methoxytyramine will be destroyed quantitatively. The same is then adjusted to pH 6.5 with HCl (= solution for oxidation). 3 ml from this solution are then oxidized according to the scheme given in Table I, the 3-methoxytyramine being converted thereby into a fluorescent indole. Another sample of the aforementioned solution for oxidation is used as blank and processed as shown in Table I.

If the intensity of fluorescence is determined as described above in a sample of plain urine ($F_1 - B_1$) as well as in a second sample of the same urine to which a known amount of 3-methoxytyramine (Q) was added ($F_2 - B_2$), the quantity of 3-methoxytyramine initially present can be calculated according to

$$\text{3-methoxytyramine } (\mu g/24\text{ h}) = \frac{(F_1 - B_1)}{(F_2 - B_2) - (F_1 - B_1)} \cdot Q \cdot \frac{V}{20}$$

where: F_1 = fluorescence units of the urine to be analyzed; B_1 = fluorescence units of the corresponding blank; F_2 = fluorescence units of the same urine to which 3-methoxytyramine was added; B_2 = fluorescence units of the corresponding blank; Q = amount of 3-methoxytyramine added (μg); V = 24 h urine volume (ml).

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