COMPARATIVE EFFECTS ON RNA POLYMERASE OF THE WHOLE GAR HISTONE AND ITS PEPTIDES CONTAINING CLUSTERS OF BASIC AMINO ACIDS*

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Although the GAR histone itself inhibited the in vitro RNA polymerase system, clusters of basic amino acids isolated from thermolysin digest of calf thymus GAR histone slightly inhibited or stimulated M. leutus and nucleolar RNA polymerase systems. These data indicate that the inhibition of RNA polymerase systems in vitro by the GAR histone is not simply due to a neutralization of the negative charges of the DNA by the positive charges of the histone but may be related to precipitation of rigid, hydrophobic DNA-histone complexes from solution.

INTRODUCTION

In vitro studies which have shown that histones block DNA-primed RNA synthesis (1-3) have been interpreted as showing that histones may be involved in the regulation of gene expression. Although this effect may be due to specific binding of histone to DNA, which prevents binding of RNA polymerase (2,3), deoxynucleohistone is insoluble in dilute salt solutions (4). Accordingly, the inhibition by histones of DNA primed RNA synthesis in vitro could be due to a non-specific precipitation of the primer from solution (1,5,6).

The recent elucidation of the amino acid sequence of the glycine and arginine rich (GAR) histone has indicated it contains four clusters of basic amino acids (7-9) as shown in Table I.

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TABLE I

STRUCTURE OF THE BASIC CLUSTERS OF THE GAR HISTONE (7-9)

Cluster 1	16 Lys(Ac)-Arg-His-Arg-Lys(Me)	20
Cluster 2	35 Arg-Arg-Leu-Ala-Arg-Arg	40
Cluster 3	75 His-Ala-Lys-Arg-Lys	79
Cluster 4	91 Lys-Arg-Gln-Gly-Arg	95

After digestion of the GAR histone by thermolysin and chromatography of the products on carboxymethyl cellulose, three peptides containing basic clusters (Table II) were isolated in quantities sufficient for enzymatic studies.

TABLE II

STRUCTURE OF THE THERMOLYSIN PRODUCED PEPTIDES

	Structure	
10	Leu-Gly-Lys-Gly-Gly-Ala-Lys(Ac)-Arg-His-Arg-Lys(Me)-Val	21
37	Leu-Ala-Arg-Arg-Gly-Gly-Val-Lys-Arg	45
90	Leu-Lys-Arg-Gln-Gly-Arg-Thr.	96

Thermolysin peptide Th_8 contains cluster 1 and thermolysin peptide Th_2 contains cluster 2. Cluster 4 is the thermolytic cleavage product containing amino acids 90-96. Since these basic clusters are likely DNA binding sites (9), they were added to systems containing soluble RNA polymerase from Micrococcus leutus and the aggregate RNA polymerase of the Novikoff hepatoma nucleoli.

MATERIALS AND METHODS

Isolation of nucleoli - Nucleoli were isolated from Novikoff hepatoma cells (9,10) and stored at -20° in 0.05 M

potassium phosphate buffer, pH 7.6 containing 50% glycerol, 0.003 M 2-mercaptoethanol and 0.001 M EDTA. Under these conditions, 60% of the initial activity could be maintained for up to one month.

DNA - Calf Thymus DNA (Worthington Biochemical Corp., Freehold, N. J.) was refined by passage through a carboxymethyl cellulose column in a buffer of 0.01 M Tris-HCl pH 7.1 containing 0.01 M NaCl. After precipitation with ethanol, the solution was then diluted to a concentration of 1.0 mg/ml with 0.01 M Tris-NaCl buffer and stored at -20° until used. Once thawed, the solution was kept at 0-4° for up to two weeks.

Preparation of the basic clusters of the GAR histone - The protein was dissolved in 0.002 M $CaCl_2$ at a concentration of 1 µm/ml. The pH was adjusted and maintained at pH 8.0 with a pH stat. Thermolysin (courtesy of Dr. Matsabara, Space Science Laboratory, Univ. of California, Berkeley, California) dissolved in 0.05 M Tris pH 8.0 containing 0.002 M $CaCl_2$ was added to a final concentration of 1% to 2% (w/w). After 3 hours at 40°, the reaction was stopped by the addition of glacial acetic acid to pH 2.0. The basic peptides were isolated by chromatography on carboxymethyl cellulose as described previously (9,12).

Assays - RNA polymerase activity was determined by the incorporation of UMP-2-C^{14} into acid insoluble product retained on a Millipore filter. The reaction mixture for the soluble M. leutus enzyme was essentially that of Nakamoto et al (13) and contained in a final volume of 0.12 ml, 12.5 µmoles Tris-HCl buffer, pH 7.6, 0.1 µmoles ATP, GTP and CTP, 0.31 µmoles $MnCl_2$, 5 µg calf thymus DNA, 1 unit Micrococcus leutus enzyme (Miles and Sigma). Controls contained no enzyme and enzyme added at 0 time.

The mixture was preincubated at 30°C for 3 minutes and the reaction started by the addition of 0.12 μmoles of UTP-2-C^{14} (specific activity 2.7 x 10^6 cpm/μmole, Schwarz). The reaction mixtures were incubated for 10 minutes at 30° and then chilled in ice. After bovine serum albumin (0.35 mg) was added, the reaction was stopped by addition of 3 ml of ice cold 5% TCA containing 0.1 M sodium pyrophosphate. After standing at 0°C for 15-30 minutes, the reaction mixture was filtered through AP 20, 22 mm Millipore glass filters. The filters were washed three times with ice cold 10% TCA, dried under a heat lamp and transferred to liquid scintillation spectrophotometer vials containing 10 ml of scintillation solution containing per liter, 5 grams of 2,5-diphenyloxazole (PPO), 0.25 grams of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene (dimethyl POPOP) (Packard Instrument Sales Corp., Downers Grove, Ill.) and counted in a Packard Tri-Carb liquid scintillation spectrophotometer.

The aggregate nucleolar RNA polymerase activity was determined in a similar manner. The reaction mixture (14,15) contained in a final volume of 0.12 ml, 1.1 μmoles phosphoenolpyruvate, 0.8 enzyme units of pyruvate kinase (Sigma Chemical Co., St. Louis, Missouri), 0.25 μmoles ATP, 0.03 μmoles GTP and CTP, 0.6 μmoles $MgCl_2$, 0.25 μmoles $MnCl_2$, 5 μmoles dithiothreitol, 6.5 μmoles Tris-HCl buffer, pH 8.3, 12.5 μg of DNA, varying amounts of GAR histone or thermolysin produced peptide and 0.20 mg of Novikoff nucleolar protein. The order of addition was DNA, GAR histone or peptide and enzyme. After preincubation for 3 minutes at 30°C, the reaction was started by the addition of 0.009 μmoles of UTP-2-C^{14} (specific activity 5.5 x 10^6 cpm/μmole , Schwarz, Orangeburg, N. Y.). The reaction mixture was incubated at 37° for 6 minutes and subsequently treated as described above.

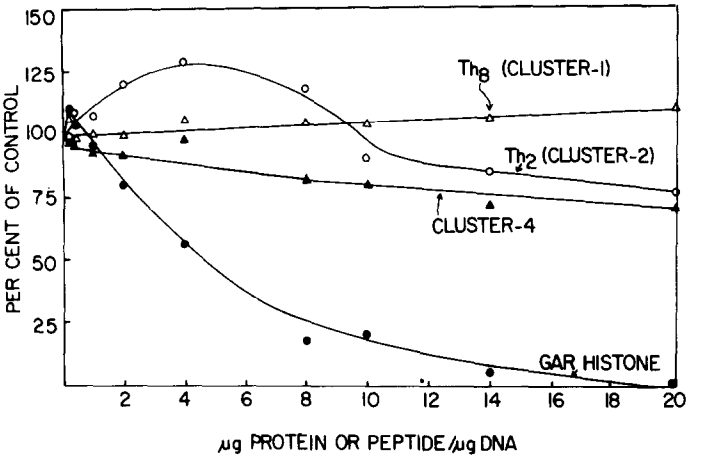

Figure 1. The effect of calf thymus GAR histone and peptides containing clusters of basic amino acids on the calf thymus DNA primed M. leutus RNA polymerase reaction. The reaction mixture (13) contained in a final volume of 0.12 ml, 12.5 μmoles Tris-HCl buffer, pH 7.6; 0.1 μmoles ATP, GTP and CTP; 0.31 μmoles $MnCl_2$, 5 μg calf thymus DNA, 1 unit of M. leutus enzyme, 0.12 μmoles UTP-2-C^{14} (specific activity 2.7 x 10^6 cpm/μmole) and indicated amounts of GAR histone or thermolysin peptide. ●—● GAR histone; △—△ Th_8 (Cluster 1); ○—○ Th_2 (Cluster 2); ▲—▲ Cluster 4.

RESULTS

All experiments were performed at saturating concentrations of primer. The effect of GAR histone and the basic clusters on the M. leutus and nucleolar systems are indicated in Fig. 1 and 2. The whole GAR histone inhibited RNA polymerase in both systems, i.e., 50% inhibition was obtained with approximately 5 μg of histone/μg DNA. At concentrations greater than 10μg of GAR histone/μg DNA, DNP fibers were visible (16). The thermolysin peptide containing cluster 2 stimulated the reaction at low concentrations but inhibited the reaction in the M. leutus system at higher concentrations. The peptide containing basic cluster 4 had little effect in the nucleolar system (Fig. 2) but inhibited the reaction slightly in the M. leutus system. The peptide containing cluster 1 had a slight stimulatory effect.

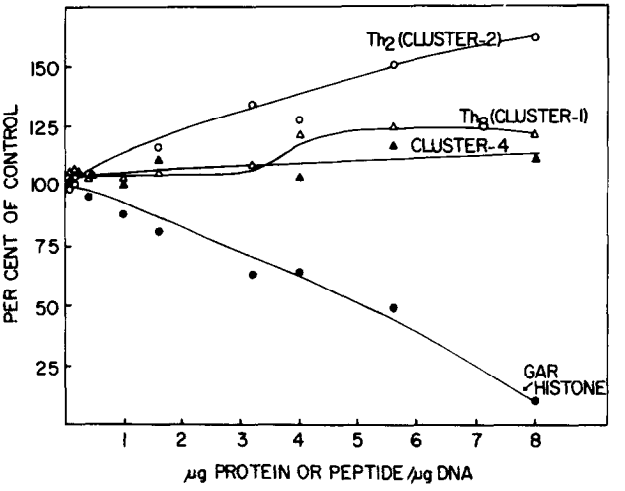

Figure 2. The effect of calf thymus GAR histone and peptides containing clusters of basic amino acids on Novikoff hepatoma nucleolar RNA polymerase. The reaction mixture contained in a final volume of 0.12 ml (14), 6.5 µmoles Tris-HCl buffer, pH 8.3, 1.1 µmoles phosphoenoyl pyruvate, 0.8 enzyme units of pyruvate kinase, 0.25 µmoles ATP, 0.03 µmoles GTP and CTP, 0.009 µmoles UTP-2-C^{14} (specific activity 5.5 x 10^6 cpm/ µmole), 0.25 µmoles $MnCl_2$, 5 µmoles dithiothreitol, 12.5 µg calf thymus DNA, 0.20 mg of Novikoff hepatoma nucleolar protein and indicated amounts of GAR histone or thermolysin peptide. ●—● GAR histone; △—△ Th_8 (Cluster 1); ○—○ Th_2 (Cluster 2); ▲—▲ (Cluster 4.

DISCUSSION

The mechanism of the inhibition by histones of the RNA polymerase system in vitro has not been adequately defined (1-6). These experiments indicate that none of the isolated peptides containing clusters of basic amino acids are as effective in inhibiting the DNA-primed synthesis of RNA as the whole histone; in fact, cluster 1 and cluster 2 mildly stimulate the reaction. Thus, since these isolated clusters probably represent the ionic binding sites of the GAR histone and DNA, this binding alone does not produce inhibition of the RNA polymerase reaction.

These studies support the idea (1,6) that inhibition of

RNA synthesis *in vitro* by histones is probably due to precipitation of the primer from solution (4,16). Questions involving the requisite length of the peptides required for inhibition by the histones remain unresolved. Space-filling models of DNA-histone structure indicate that there is a tight packing of the protein and nucleic acid chains. The DNA-histone structure has a much greater rigidity than either DNA or histone alone. Moreover, the hydrophobic regions in the carboxyl half of the GAR histone (7-9) may decrease the water solubility of the DNA-histone complex.

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