Inhibition of RNA Polymerase Activity by Arginine-Rich Histones

Although histones have been shown to inhibit the DNA-dependent RNA synthesis when complexed with the DNA template, the exact mechanism of the inhibition is not clear. Experiments reported in this paper investigate the possible interactions of histones with the enzyme DNA-dependent RNA polymerase and their effect on the DNA-dependent RNA synthesis *in vitro*.

Partially purified preparations of the enzyme were prepared from *Micrococcus lysodeikticus* (spray dried from Miles Chemical Corp.) by the method of NAKAMOTO et al.[1]. The specific activity used in these experiments ranged from 600–800 U/mg of enzyme. Highly purified enzyme (specific activity 1550 U/mg), purchased from Miles Chemical Corporation, was also employed. The standard reaction mixture for assay of RNA synthesis in vitro was essentially that of NAKAMOTO et al.[1] except that the quantity of each component and final volume were halved. Generally, reactions were incubated at 37 °C for 10 min with 30 µg of enzyme and 50 µg DNA. The incorporation was determined by the method of BOLLUM[2]. All interactions of histones with the DNA or with the enzyme were performed in 0.005*M* *Tris*-HCl, pH 7.5, and water was added to assure equal volumes when necessary.

Whole, unfractionated sample of calf thymus histone was prepared by acid extraction from saline-washed calf thymus nucleoproteins. The various fractions (F1, F2a, F2b, F3) were obtained from calf thymus nucleoprotein by extraction with ethanolic-HCl and with 0.2*N* HCl[3]. Further fractionation was achieved by chromatography on carboxymethyl cellulose[4] and by gel filtration on Sephadex G-75[5]. Histone fractions F2c (chicken erythrocytes) and H-1 and H-3 (sperm) were obtained from chicken erythrocytes and from the sperm of sea urchin *Strongylocentrotus purpuratus*, respectively, by similar methods. To insure the identity of the fractions, all samples were analyzed for the amino acid and N-terminal amino acid composition; the purity of each of the fractions was determined by electrophoresis in starch[6] or polyacrylamide[7] gels. Protamine sulfate (Mann Research Laboratories), polylysine (M.W. 100,000 Mann Research Laboratories), and polyarginine (M.W. 15–50,000, Miles Laboratories) were used in place of histones in some assays.

Initial studies using unfractionated calf thymus histone demonstrated that the extent of inhibition of the DNA-dependent RNA synthesis in vitro depends greatly on whether the histones initially were allowed to interact with the polymerase enzyme, the nucleotide triphosphates, or the DNA template. Table I shows the amino acid analysis and source of the various histone fractions used in further assays. The effects of these various histone fractions on the rate of RNA synthesis when

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Table I. The amino acid composition of the various histone fractions and their source

Fraction CODE	Total histone CTH	F1	F2a1	F2a	F2b	F3	H-1	H-3	F2c	Poly-arginine	Poly-lysine	Culpeine
Source	Calf thymus	Calf thymus	Calf thymus	Calf thymus	Calf thymus	Calf thymus	Sea urchin		Chicken erythro-cyte	Synthetic		Herring
Amino acid												
Lysine	*14.4*	*27.7*	*10.0*	*11.4*	*16.0*	*10.3*	*20.2*	*11.9*	*24.9*	–	*100*	–
Histidine	1.7	–	1.9	2.1	2.4	2.4	1.7	2.3	1.8	–	–	–
Arginine	*8.3*	*1.8*	*13.9*	*11.6*	*7.8*	*13.6*	*19.0*	*20.1*	*11.4*	*100*	–	*74*
Aspartic acid	4.8	2.1	5.1	5.2	5.1	4.5	3.4	4.6	1.7	–	–	–
Threonine	5.6	5.4	6.7	5.9	5.8	6.5	4.8	6.3	3.1	–	–	2.1
Serine	6.1	6.8	2.5	4.2	9.2	3.9	7.6	11.2	13.1	–	–	4.6
Glutamic acid	8.3	3.7	6.4	7.6	7.9	10.6	4.2	5.0	3.7	–	–	–
Proline	5.4	10.0	1.4	3.0	3.4	4.4	2.8	2.0	6.9	–	–	7.5
Glycine	8.5	6.9	15.9	12.1	7.0	6.0	4.2	10.0	4.7	–	–	–
Alanine	13.8	25.1	7.6	10.4	10.8	13.4	18.9	7.8	15.2	–	–	6.6
Half cystine	–	–	–	–	–	0.1	–	–	–	–	–	–
Valine	6.0	4.3	8.0	7.0	7.1	4.8	4.5	5.8	4.0	–	–	3.7
Methionine	1.0	–	1.0	1.0	1.0	1.5	1.3	1.2	0.5	–	–	–
Isoleucine	4.0	0.9	5.6	5.4	5.5	4.9	2.5	4.2	3.0	–	–	1.2
Leucine	7.7	4.3	8.1	8.4	5.8	9.2	2.7	4.0	4.0	–	–	–
Tyrosine	2.5	0.5	3.5	3.0	3.6	2.1	1.1	2.3	1.4	–	–	–
Phenylalanine	1.9	0.5	2.3	1.7	1.6	2.0	0.8	1.1	0.5	–	–	–
Arginine/lysine	0.58	0.06	1.39	1.02	0.48	1.32	0.92	1.69	0.46	–	–	–

All serine values are corrected (10%) for hydrolytic losses. All values are expressed as % of total moles of amino acids recovered.

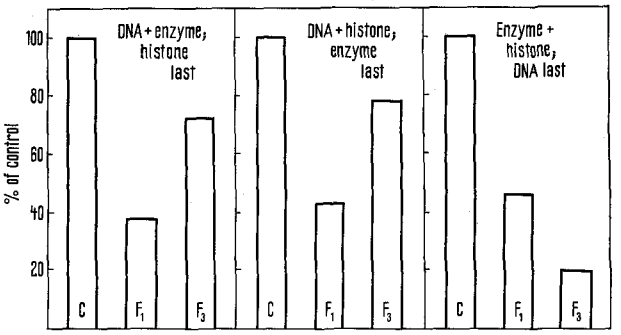
Table II. Comparison of the inhibition of RNA synthesis, expressed as % of control (no histone), of each histone fraction after interaction with DNA or with enzyme

Histone: DNA = 0.7			Histone: DNA = 0.3		
Fraction	% of control		Fraction	% of control	
	with DNA	with enzyme		with DNA	with enzyme
CTH	54	30	CTH	74	36
F1	20	30	F1	66	76
F2a	61	24	F2a	82	34
F2a1	42	36	F2a1	59	55
F2b	77	29	F2b	94	35
F3	67	29	F3	91	25
H-1	44	33	H-1	77	61
H-3	40	16	H-3	88	27
F2c	40	40	F2c	77	69
Protamine sulfate	65	59	Protamine sulfate	83	31
Polyarginine[a]	–	–	Polyarginine[a]	88	77
Polylysine	10	30	Polylysine	50	90

[a] Dissolved in 0.05 *N* HCl; control for this fraction included the addition of an equivalent amount of 0.05 *N* HCl to reaction mixtures.

allowed to interact initially with the DNA template or with the polymerase enzyme are shown in Table II. In each of these assays, the DNA (50 µg) and enzyme (30 µg) levels were constant, while 2 levels of each of the histone fractions were used. All of the lysine-rich fractions (F1, F2b, H-1, and F2c), except the F2b fraction, exhibited little variation in the inhibition of RNA synthesis when preincubated either with the DNA template or with the enzyme. More arginine-rich fractions (F2a, F3, F2a1, H-3, and protamine sulfate), however, demonstrated a 2-to 4-fold greater inhibition of RNA synthesis when allowed to interact initially with the enzyme than with the DNA template. Fraction F2a1 was an exception in this case. These results indicate that the increase in inhibition of RNA synthesis caused by the interaction of the total calf thymus histone with the enzyme instead of the DNA template, was largely due to the more arginine-rich histones of that fraction. The synthetic polycations (polyarginine or polylysine) failed to show any correlation of arginine-lysine content to the change in inhibition of RNA synthesis by the preincubation. In general, the lysine-rich histones exhibited a greater inhibition of RNA synthesis than the arginine-rich histones when allowed to interact first with the DNA template. However, the arginine-rich histones showed the greater inhibition when the histones were allowed to interact initially with the polymerase enzyme. This arginine-rich histone-enzyme interaction was found with histones from several species of organisms.

To facilitate the size of further experiments, only the F1 (very lysine-rich) and F3 (relatively arginine-rich) histone fractions were compared. Both of these fractions are common to all eucaryotic organisms and can be obtained in considerable purity. When the RNA polymerase was allowed to interact initially with the DNA, later additions of histones resulted in inhibitions similar to that where histones were allowed to interact with the DNA (Figure). Here, the lysine-rich histones exhibited greater inhibition than the arginine-rich fractions. It has been shown that the RNA polymerase enzyme binds readily to the DNA template. This binding appears, therefore, to prevent inactivation of the enzyme by



The effect on the rate of RNA synthesis caused by interacting the lysine-rich (F1) or the arginine-rich (F3) histones with either DNA template, the polymerase enzyme, or the enzyme-DNA complex. Enzyme, 30 µg/rx; DNA, 50 µg/rx; histone, 20 µg/rx; C, control (no histone).

arginine-rich histones. Whether the DNA-bound enzyme is unable to interact with the arginine-rich histones or whether the arginine-rich histones have a higher affinity for the unmasked parts of the DNA than for the enzyme remains to be determined. Based on histone-DNA interaction studies reported by AKINRIMISI and BONNER[8], the second alternative appears more probable.

It can be concluded that the sequence of addition of the reaction components of the in vitro DNA-dependent RNA synthesis should be considered when using histones as inhibitors. The level of inhibition caused by the arginine-rich histones can be increased 2-to 4-fold when these histones are allowed to interact first with the polymerase enzyme or with the free nucleotides instead of the DNA template. These findings may help to explain the different results obtained by various laboratories on the relative efficiencies of the lysine-rich and arginine-rich histones in inhibiting the DNA-dependent RNA synthesis[9]. It is necessary to stress that similar experiments undertaken with the enzyme DNA polymerase have shown that no such interaction between arginine-rich or lysine-rich histone takes place in the case of the DNA polymerase enzyme[10].

Zusammenfassung. Im Komplex mit der DNS-Matrize zeigt die lysinreiche Fraktion F1 eine doppelt so grosse Hemmung der RNS-Synthese in vitro als das argininreiche Histon F3. Demgegenüber erwies sich das argininreiche Histon F3, mit RNS-Polymerase komplexiert, wirksamer als das lysinreiche Histon F1, das heisst, dass die Inhibition mit dem argininreichen Histon F3 fünffache Steigerung und damit doppelte Wirksamkeit gegenüber F1 ergibt.

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