Subunits of RNA Polymerase in Function and Structure. 8. Catalytic Properties of Self-Reactivated Core Enzyme†

Tsunao Saitoh and Akira Ishihama*

ABSTRACT: As an attempt to identify the maturation pathway of *Escherichia coli* RNA polymerase, the catalytic properties of core enzyme reactivated in the absence of maturation-promoting factors (σ subunit or DNA) (that is, of self-reactivated core enzyme) were compared with those of native core enzyme. Differences have been found in the intrinsic activities such as in the template specificity, K_m value of DNA template for the polymerase, activation energy for RNA synthesis, and increment of enzyme activity by σ subunit. These observations imply that the transcription initiation by self-reactivated core enzyme is inaccurate and, therefore, more strict conditions including the presence of maturation-promoting factors are required for premature core be activated to the genuine function with the transcription specificity of native core enzyme.

The in vitro assembly of *Escherichia coli* DNA-dependent RNA polymerase (ribonucleoside 5′-triphosphate:RNA nucleotidyltransferase [EC 2.7.7.6]) proceeds stepwisely under the following sequence: $2\alpha \rightarrow \alpha_2 \overset{\beta}{\rightarrow} \alpha_2\beta \overset{\beta'}{\rightarrow} \alpha_2\beta\beta'$ (premature core) → E (active core) (Ishihama & Ito, 1972; Ishihama et al., 1973; Palm et al., 1975; Saitoh & Ishihama, 1976). Evidence has accumulated which indicates that the pathway of RNA polymerase[1] assembly in vivo is identical with that found in vitro (Ito et al., 1975; Taketo & Ishihama, 1976, 1977). The rate-limiting step of the assembly in vitro is the last-step reaction, i.e., temperature-dependent activation of premature core enzyme. Both σ subunit and DNA promote the activation of premature core (enzyme maturation) (Ishihama et al., 1973; Fukuda & Ishihama, 1974). Thus, the enzyme maturation in vitro can be achieved by the following three different ways: self-reactivation in the presence of high concentrations of salt or glycerol, σ subunit promoted reactivation leading to yield holoenzyme, and DNA-promoted reactivation resulting in formation of DNA–core enzyme complex (Saitoh & Ishihama, 1976).

One of the shortcuts to get insight into the maturation in vivo is detailed comparisons of the structure and function of RNA polymerase reconstituted by the three different ways with native holo- or core polymerase. This report describes some of the catalytic properties of core enzyme reconstituted in the absence of promoting factors for enzyme maturation, hence of self-reactivated core enzyme. The results raised the notion that the self-reactivated core enzyme is not completely identical with native core enzyme in the specificity of transcription initiation.

Materials and Methods

Chemicals. Unlabeled ribonucleoside 5′-triphosphates were purchased from P-L Biochemicals and Boehringer Mannheim GmbH (West Germany), while ^{3}H- and ^{14}C-labeled ribonucleoside 5′-triphosphates were from Schwarz/Mann. [γ-^{32}P]ATP was prepared essentially according to the method of Glynn & Chappell (1964). Recrystallized products of urea, sodium dodecyl sulfate ($NaDodSO_4$), and sucrose were obtained from Wako Chemicals and Nakarai Chemicals, Japan.

T7 phage DNA was purified by phenol extraction of phage stocks prepared by CsCl centrifugation. Synthetic deoxy polymers were products of P-L Biochemicals. Phosphocellulose P11 was a product of Whatman; Sepharose 6B and DEAE-Sephadex A-50 were products of Pharmacia, Sweden. DNA-cellulose was prepared as described previously (Fukuda et al., 1974).

RNA Polymerase. RNA polymerase holoenzyme I was purified from *Escherichia coli* K12 W3350 and assayed as described in the previous report (Fukuda et al., 1974). The standard reaction mixture for RNA polymerase contained in 0.25 mL: 120 mM Tris-HCl (pH 7.8 at 37 °C); 5 mM magnesium acetate; 2 mM manganese sulfate; 0.2 mM dithiothreitol; 0.16 mM each of labeled and unlabeled ribonucleoside 5′-triphosphates; and DNA and enzyme.

RNA polymerase core enzyme was purified by repeated chromatography of holoenzyme through phosphocellulose columns in the presence of 5% glycerol. Residual σ subunit in the core enzyme preparations used was less than 0.1%, as analyzed by $NaDodSO_4$–polyacrylamide gel electrophoresis. The σ subunit dissociated was further purified by centrifugation through glycerol gradients.

Self-reactivated core enzyme (core enzyme reconstituted in the absence of maturation-promoting factors) was prepared as described previously (Saitoh & Ishihama, 1976; Ishihama et al., 1979). Enzyme maturation was performed by incubating premature core enzyme for 60 min at 30 °C in the presence of 0.3 M KCl and 20% glycerol.

Sucrose Gradient Centrifugation. Sucrose gradient centrifugation of RNA synthesized in vitro was carried out, after treatment of RNA samples with 0.5% $NaDodSO_4$, in 5–20% sucrose in 0.05 M sodium acetate buffer (pH 5.0) containing 0.5% $NaDodSO_4$ or in 10 mM Tris-HCl (pH 7.4 at 4 °C), 0.1 M NaCl, 1 mM EDTA, and 0.5% $NaDodSO_4$. As a reference marker for estimation of sedimentation coefficient, *E. coli* ribosomal RNA was employed.

Results

Interaction with DNA. Previous studies of the in vitro reactivation of premature core polymerase showed that the recovery of RNA polymerase activity varied significantly depending on what template was used for the assay of reactivated enzyme. As shown in Table I for example, the recovery of enzyme activity is generally better with T7 DNA than with synthetic deoxy polymers, e.g., poly[d(A-T)]·poly[d(A-T)] and poly[d(A)]·poly[d(T)], which are rather

† From the Department of Biochemistry, Institute for Virus Research, Kyoto University, Sakyo-ku, Kyoto 606, Japan. *Received August 17, 1978; revised manuscript received October 25, 1978.* This work was supported in part by grants from the Ministry of Education, Science and Culture, of Japan and the Asahi Press. Paper 7 in this series is Ishihama et al. (1979).

[1] Abbreviations used: RNA polymerase, ribonucleoside 5′-triphosphate:RNA nucleotidyltransferase (DNA-dependent) (EC 2.7.7.6); $NaDodSO_4$, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetate.

0006-2960/79/0418-0979$01.00/0

Table I: Template Specificity of Native and Reactivated Core Enzyme[a]

template	labeled substrate	labeled substrate incorp (nmol) native core	react. core	recovery (%)
T7 DNA	[^{3}H]ATP	0.158	0.098	62.4
poly[d(A-T)]·poly[d(A-T)]	[^{3}H]ATP	0.754	0.363	48.2
poly[d(A)]·poly[d(T)]	[^{3}H]ATP	2.37	0.878	37.0
poly[d(A)]·poly[d(T)]	[^{3}H]UTP	0.197	0.103	52.3

[a] RNA polymerase activity of 3 μg each of native and reactivated core was measured in the standard reaction mixture, as described in Figure 1, using 2.5 μg of T7 DNA or 0.5 μg of synthetic deoxy polymers as the template. In addition to the labeled substrate indicated, the reaction mixture contained unlabeled GTP, CTP, and UTP for the T7 DNA directed reaction or unlabeled UTP for the poly[d(A-T)]·poly[d(A-T)]-directed reaction. RNA synthesis was carried out at 37 °C for 10 min.

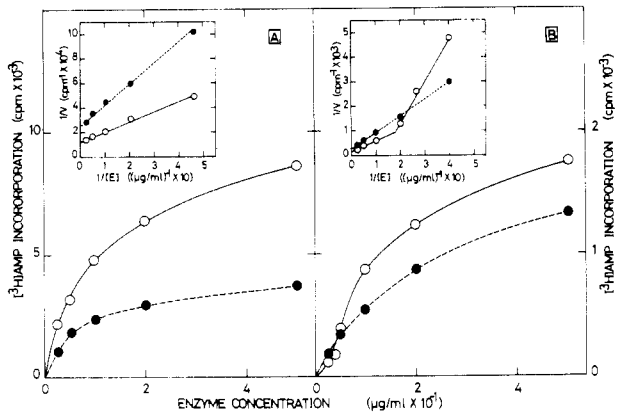

FIGURE 1: Effect of enzyme concentration. RNA polymerase activity of the indicated amounts of native or reactivated core enzyme was determined in the standard reaction mixture containing 0.25 μg of poly[d(A-T)]·poly[d(A-T)] (A) or 2.5 μg of T7 DNA (B). [^{3}H]ATP (specific activity, 11 000 cpm per nmol) was used as the labeled substrate. RNA synthesis was carried out for 10 min at 37 °C. Double-reciprocal plots are shown in the inserts. (O—O) Native core enzyme; (●---●) reactivated core enzyme.

good templates for native core enzyme (Fukuda et al., 1974). In order to further clarify the difference of template specificity between native and reconstituted core enzyme, the enzyme saturation for various templates was examined with the two core enzyme preparations. This type of experiment permits determination of the maximum number of template sites where RNA synthesis can be initiated by the enzyme used.

Figure 1 shows the results obtained with poly[d(A-T)]·poly[d(A-T)] and T7 DNA as the template. Native core enzyme exhibited higher activities than reconstituted enzyme on both templates and at all the enzyme concentrations examined. In agreement with the result noted above, the activity ratio of reconstituted to native enzyme was considerably higher with T7 DNA than poly[d(A-T)]·poly[d(A-T)]. Nevertheless, the amounts of enzyme required to saturate transcription initiation sites on poly[d(A-T)]·poly[d(A-T)] were almost equal between the two enzymes. The double-reciprocal plot gave linear lines with the apparent K_m values of 2.40 μg per 0.25 μg of poly[d(A-T)]·poly[d(A-T)] for both native and reconstituted enzymes (Table II). The result indicates that the affinity of this template to the two enzymes was equal; i.e., the same number of enzymes are required to saturate initiation sites on poly[d(A-T)]·poly[d(A-T)]. In contrast, the enzyme saturation curves for T7 DNA were significantly different with the two enzymes (Figure 1B). The replot figure gave K_m

Table II: Effect of Enzyme Concentration on RNA Synthesis by Native or Reactivated Core Enzyme[a]

DNA		native core	react. core
T7 DNA	K_m for enzyme	4.57	8.13
	V_{max}	0.194	0.159
poly[d(A-T)]·poly[d(A-T)]	K_m for enzyme	2.40	2.40
	V_{max}	0.727	0.355
ratio of V_{max} (T7/poly[d(A-T)]·poly[d(A-T)]		0.267	0.449

[a] K_m for enzyme (μg of enzyme for 2.5 μg of T7 DNA or 0.25 μg of poly[d(A-T)]·poly[d(A-T)]) and V_{max} (nmol of labeled substrate incorporation) were calculated from the data shown in Figure 1.

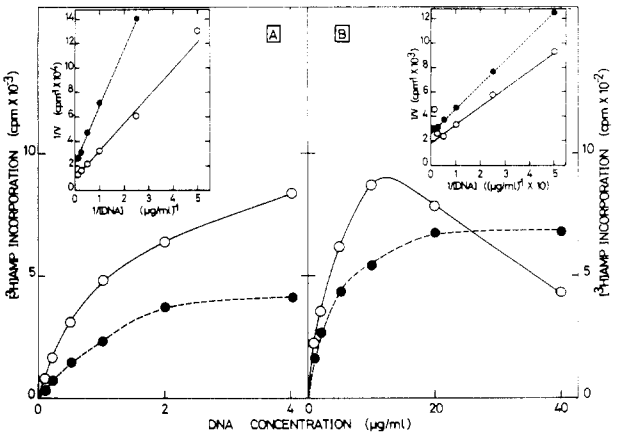

FIGURE 2: Effect of DNA concentration. RNA polymerase activity of 3 μg each of native or reactivated core enzyme was determined in the standard reaction mixture using poly[d(A-T)]·poly[d(A-T)] (A) or T7 DNA (B) as the template. DNA concentration was varied as indicated. RNA synthesis was carried out for 10 min at 37 °C. (O—O) Native core enzyme; (●---●) reactivated core enzyme.

values of 4.57 μg per 2.5 μg of DNA for native enzyme and of 8.13 μg per 2.5 μg of DNA for reconstituted enzyme (Table II), indicating that the affinity of T7 DNA was higher with native enzyme than reconstituted enzyme. In addition, only native enzyme gave a sigmoidal curve, which is characteristic of the positive cooperativity in the T7 DNA directed RNA synthesis. Such a unique profile, however, could also be due to preferential binding of native core enzyme to nonspecific sites on T7 DNA, i.e., outside transcription initiation sites, resulting in repression of RNA synthesizing activity at low enzyme concentrations.

The amounts of active polymerase in the two enzyme preparations could be determined by the template saturation experiment, and one of the typical results is shown in Figure 2. From the double-reciprocal plot, the ratio of V_{max} of reconstituted to that of native enzyme was calculated to be approximately 0.5 for both templates (Table III), which indicates that the amount of active enzyme in reconstituted enzyme preparation used was approximately half of that in native enzyme preparation. This estimation is based on the assumption that the rate of RNA synthesis is identical for the two enzymes.

The K_m value for template DNA, hence the amounts of DNA to be recognized, was almost equal with the two core enzymes: 1.37~1.10 μg of T7 DNA per 3 μg of enzyme and 0.31~0.33 μg of poly[d(A-T)]·poly[d(A-T)] per 3 μg of enzyme. The result implies that enzymatically inactive molecules in the reconstituted enzyme preparation are capable of binding DNA sites where active enzyme initiates RNA synthesis and is in good agreement with the observation that

Table III: Effect of DNA Concentration on RNA Synthesis by Native or Reactivated Core Enzyme[a]

enzyme		T7 DNA	poly-[d(A-T)]·poly-[d(A-T)]
native core enzyme	K_m for DNA	1.37	0.31
	V_{max}	0.120	0.918
react. core enzyme	K_m for DNA	1.10	0.33
	V_{max}	0.067	0.497
ratio of V_{max} (react./native)		0.557	0.526

[a] K_m for DNA (μg of DNA for 3 μg of enzyme) and V_{max} (nmol of labeled substrate incorporation) were calculated from the data shown in Figure 2.

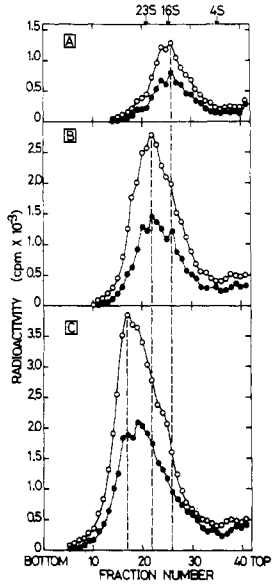

FIGURE 3: Sucrose gradient centrifugation of RNA. RNA synthesis was carried out at 30 °C in the standard reaction mixture (0.25 mL) containing 38 μg of T7 DNA and 30 μg each of native or reconstituted core enzyme. As the labeled substrate, [^{3}H]ATP (specific activity, 330 Ci/mol) and [^{14}C]ATP (specific activity, 51 Ci/mol) were used in the reaction catalyzed by the reconstituted and the native core enzyme, respectively. After 6 (A), 11 (B), and 16 min (C) of incubation, 0.05-mL aliquots were removed and treated with 0.025 mL of 10% $NaDodSO_4$ containing 0.1 M EDTA. Mixtures of ^{14}C- (O—O) and ^{3}H-labeled RNA (●---●) were layered on the top of 5–20% sucrose gradients containing 10 mM Tris-HCl (pH 7.4 at 4 °C), 0.1 M NaCl, 1 mM EDTA, and 0.5% $NaDodSO_4$ and centrifuged in a Spinco SW50.1 rotor for 100 min at 50 000 rpm at 20 °C. The positions of the reference *E. coli* 4S, 16S, and 23S RNA are shown by arrows.

premature core enzyme is fully active in binding DNA (Fukuda & Ishihama, 1974).

Rate of RNA Chain Elongation. The rate of RNA chain elongation by reconstituted enzyme was determined by measuring the growth of RNA chains after centrifugation of RNA products through sucrose gradients. As shown in Figure 3, the size distribution of RNA synthesized on T7 DNA template was virtually identical at least within the reaction periods examined. From the increase of sedimentation coefficients of the peaks, the rate of RNA chain growth at 30 °C was calculated to be 7–8 nucleotides per second, which reasonably coincided with the value initiated by holopolymerase under the same reaction conditions (Naito & Ishihama, 1974).

Products directed by poly[d(A-T)]·poly[d(A-T)] also gave essentially the same patterns with the two enzyme preparations (data not shown). It should be worthwhile to note that product

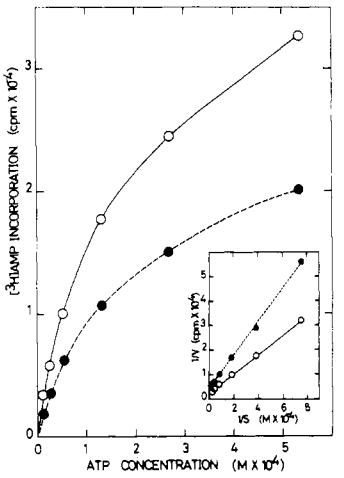

FIGURE 4: Effect of ATP concentration. RNA polymerase activity of 4 μg each of native or reconstituted core enzyme was determined as described in Figure 1 except that 1 μg of poly[d(A)]·poly[d(T)] was used as the template and [^{3}H]ATP (specific activity, 11 000 cpm per nmol) was varied as indicated. The data are replotted to double-reciprocal forms. (O—O) Native enzyme; (●---●) reconstituted enzyme.

poly(A-U) was larger than template poly[d(A-T)] in size, presumably because the enzymes continued chain elongation at the end of poly[d(A-T)] by transcribing the opposite strand.

The above conclusion was also confirmed by double-label experiments with [^{3}H]ATP and [γ-^{32}P]ATP. The incorporation ratio of ^{3}H to ^{32}P, which is indicative of the size of RNA, was virtually the same with the two core enzymes and at various times of the reaction (data not shown).

Effect of Substrate Concentration. As an attempt to compare activities of the two core enzymes to carry out the discrete steps of RNA synthesis, the effect of substrate concentrations was analyzed in the reaction directed by poly[d(A-T)]·poly[d(A-T)]. When ATP concentration was varied in the presence of saturation concentration of UTP, the half-saturation concentration for ATP was found to be approximately 0.14 mM with both native and reconstituted polymerase (Figure 4). The two core enzymes also gave essentially the same K_m value for UTP (data not shown). Thus, the affinity of reconstituted enzyme to substrates is equal with that of native core enzyme. The result is consistent with the observation that premature core is as active in binding substrates as native core enzyme (Ishihama, unpublished observation).

Interaction with σ Subunit. Core enzyme initiates RNA synthesis mainly at termini or single-stranded regions of DNA (Vogt, 1969; Ishihama et al., 1971). Core enzyme associated with σ subunit regains the ability to initiate RNA synthesis from double-stranded promoters. Since the functional difference between the two core enzymes has so far been found only in the K_m value for T7 DNA, it appears that initiation specificity of the reconstituted enzyme used is different from that of native enzyme. To test this possibility, stimulation by σ subunit was compared between the two core enzyme preparations. RNA polymerase activity with equal amounts of the two core enzymes was examined after addition of various amounts of σ subunit (Figure 5). Both core enzymes were stimulated to the same extent in the presence of excess σ subunit. It is, however, noteworthy that stimulation by low concentrations of σ subunit was higher for reconstituted core than native core enzyme. In consequence, stimulation of native core enzyme exhibited an S-shape curve.

Stable association of σ subunit with self-reactivated core enzyme but not with premature core enzyme has been directly

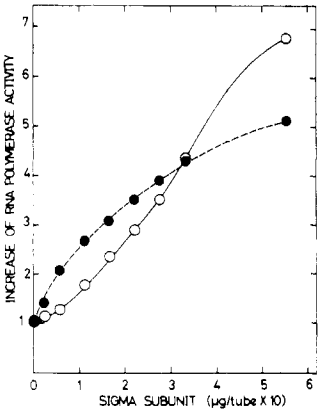

FIGURE 5: Stimulation by σ subunit. RNA polymerase activity of 3 μg each of native or reconstituted core enzyme was determined as described in Figure 1 except that 5 μg of T7 DNA was used as the template and the indicated amounts of subunit were added. The activity without σ-subunit addition was 1530 cpm for native enzyme and 900 cpm for reconstituted enzyme, respectively, and the relative increase is plotted. (O—O) Native enzyme; (●---●) reconstituted enzyme.

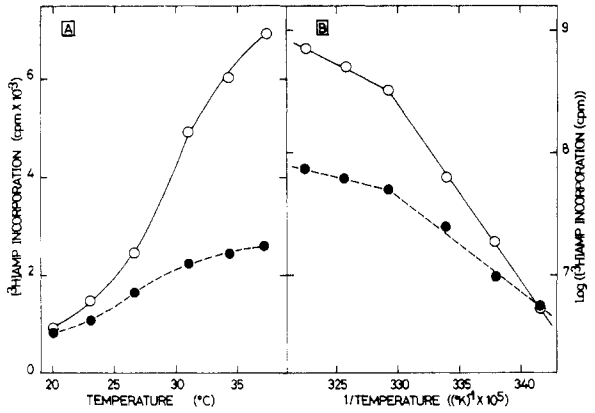

FIGURE 6: Effect of temperature. RNA polymerase activity of 4 μg each of native or reconstituted core enzyme was determined as described in Figure 1 except that 1 μg of σ subunit was added and 10 μg of T7 DNA was used as the template. (A) RNA synthesis was carried out at the temperature indicated and the amounts of [^{3}H]AMP incorporated in the initial 5 min are plotted. (B) The data are converted to Arrhenius plots. (O—O) Native enzyme; (●---●) reconstituted enzyme.

demonstrated by glycerol gradient centrifugation (Fukuda & Ishihama, 1974; Saitoh & Ishihama, 1976).

Effect of Temperature. Enzymatic synthesis of RNA exhibits unique profiles of temperature dependence. A hypothesis has been proposed that the initiation of RNA synthesis involves temperature-dependent partial separation of double-stranded DNA at the promoter sites, which has not, however, been supported by experimental evidences (Chamberlin, 1976). Nevertheless, examination of the influence of reaction temperature provides some information on the nature of transcription initiation by the enzyme and DNA used. RNA polymerase activity of the two core enzymes supplemented with σ subunit was therefore measured with T7 DNA as the template and at various temperatures. As shown in Figure 6, the activity of native core enzyme supplemented with σ subunit prior to RNA synthesis increased by more than sevenfold upon the increase of temperature above 30 °C, analogous to that found with native holoenzyme (Saitoh and Ishihama, unpublished results). In contrast, the activity increase of reconstituted enzyme accompanied with the temperature shift was less significant (2.5–3-fold increase). The apparent activation energy of native core enzyme supplemented with σ subunit, hence of holoenzyme, was about 25 kcal per mol below 30 °C and about 10 kcal per mol above 30 °C, whereas that of reconstituted core enzyme fortified with σ subunit was about 17 kcal per mol and 6 kcal per mol, respectively.

Table IV: Activities of RNA Polymerase Proteins

protein	activity: substrate binding	DNA binding	rifampicin binding	σ-subunit binding	RNA synthesis
α subunit	–	–	–	–	–
β subunit	–	–	–	–	–
β' subunit	–	+[a]	–	+[a]	–
$\alpha_2\beta$ complex	–	+[a]	+[b,c]	+[a]	–
$\alpha_2\beta\beta'$ complex (premature core)	+[c]	+[a]	+[b,c]	+[a]	–
self-reactivated core enzyme	+	+	+	+[d]	+[d-f]

[a] Fukuda & Ishihama (1974). [b] Harding & Beychok (1976). [c] A. Ishihama, unpublished observation. [d] Saitoh & Ishihama (1976). [e] This report. [f] Inaccurate initiation.

Discussion

During the assembly of *E. coli* RNA polymerase, the gross conformation is established in the premature core enzyme (Ishihama et al., 1979). It is, however, virtually inactive in the catalysis of RNA synthesis but retains activities to bind substrates, template DNA and rifampicin, a potent inhibitor of RNA polymerase (Talbe IV). Close examination revealed that premature core is different from native core in the structure observed by near-ultraviolet circular dichroism spectra, hydrogen–tritium exchange rates, and sensitivity to proteases and in addition that the distances among α, β, and β' subunits in premature core differ from those in native core enzyme, measured by the rate and extent of intersubunit cross-linking with bifunctional reagents (Ishihama et al., 1979). In accordance with these observations as well as the sedimentation behavior, the premature assembly is readily dissociated into $\alpha_2\beta$ complex and free β' subunit at low protein concentrations or by passing through the phosphocellulose column (Ishihama et al., 1979). These findings raised the concept that there exist minor and presumably local differences in the structure between native and premature core enzymes and that the proper maturation be accompanied by the change in the structure mentioned above. Systematic comparison on the structure and catalytic properties of enzymes reactivated under the three different pathways, which have been observed in in vitro reconstitution systems (Saitoh & Ishihama, 1976), may lead to reveal which of the maturation pathways operates in vivo.

As described in this report, core enzyme reactivated in the absence of promoting factors for enzyme maturation (self-reactivated enzyme) is as active in RNA synthesis as native core enzyme but differs in the following ways: (i) activity ratios of reactivated enzyme directed by varieties of DNA template are not identical with those of native enzyme; (ii) the K_m value of T7 DNA, but not of poly[d(A-T)]·poly[d(A-T)], for reactivated enzyme is higher than that for native enzyme; (iii) activation energy of RNA synthesis catalyzed by reactivated enzyme is lower than that of native enzyme; (iv) increment of RNA synthesis upon temperature shift is smaller with reactivated enzyme than with native enzyme; (v) RNA synthesis by reactivated enzyme is stimulated by σ subunit at lower concentration of the factor than those required

for native enzyme. Since these characteristics are originated in the initiation among discrete steps of RNA synthesis and since the rate of RNA chain elongation is indistinguishable between the reactions catalyzed by native and reconstituted enzymes, it appears that self-reactivated core enzyme is different from native enzyme in the specificity of transcription initiation (Table IV).

In concert with the difference in the transcription specificity, self-reactivated core enzyme gives a different shape of near-ultraviolet circular dichroism spectrum from that of native core enzyme (Ishihama et al., 1979). In a consequence of the present observations, it remains to be determined that such a partially reactivated enzyme is an obligatory intermediate in the process of active enzyme formation, because structured species not on the renaturation pathway may be formed during the renaturation of some enzymes (Ikai & Tanford, 1971; Teipel & Koshland, 1971). These observations, together with the previous finding that self-reactivation proceeds only at nonphysiological conditions, e.g., in the presence of high concentrations of salt or glycerol (Saitoh & Ishihama, 1976), might suggest that RNA polymerase whose structure and function are completely identical with that of native enzyme, can be formed via one or both of the other two maturation pathways, i.e., σ subunit promoted or DNA-promoted maturation. Study of the properties of the enzymes formed in the presence of these maturation-promoting factors should provide a critical test of the above hypothesis.

Acknowledgments

The authors wish to thank Drs. R. Fukuda and M. Taketo for their discussions and help in preparation of RNA polymerase and DNA.

References

Chamberlin, M. (1976) in *RNA Polymerase* (Losick, R., & Chamberlin, M., Eds.) pp 159–192, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Fukuda, R., & Ishihama, A. (1974) *J. Mol. Biol. 87*, 523–540.
Glynn, I. M., & Chappell, J. B. (1964) *Biochem. J. 90*, 147–158.
Harding, J. D., & Beychok, S. (1976) in *RNA Polymerase* (Losick, R., & Chamberlin, M., Eds.) pp 355–367, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Ikai, A., & Tanford, C. (1971) *Nature (London) 230*, 100–105.
Ishihama, A., & Ito, K. (1972) *J. Mol. Biol. 59*, 111–123.
Ishihama, A., Murakami, S., Fukuda, R., Matsukage, A., & Kameyama, T. (1971) *Mol. Gen. Genet. 111*, 66–77.
Ishihama, A., Fukuda, R., & Ito, K. (1973) *J. Mol. Biol. 79*, 127–136.
Ishihama, A., Aiba, H., Saitoh, T., & Takahashi, S. (1979) *Biochemistry 18* (preceding paper in this issue).
Ito, K., Iwakura, Y., & Ishihama, A. (1975) *J. Mol. Biol. 96*, 257–271.
Naito, S., & Ishihama, A. (1975) *Biochim. Biophys. Acta 402*, 88–104.
Palm, P., Heil, A., Boyd, D., Grampp, B., & Zillig, W. (1975) *Eur. J. Biochem. 53*, 283–291.
Saitoh, T., & Ishihama, A. (1976) *J. Mol. Biol. 104*, 621–635.
Taketo, M., & Ishihama, A. (1976) *J. Mol. Biol. 102*, 297–310.
Taketo, M., & Ishihama, A. (1977) *J. Mol. Biol. 112*, 65–74.
Teipel, J. W., & Koshland, D. E., Jr. (1971) *Biochemistry 10*, 798–803.
Vogt, V. (1969) *Nature (London) 233*, 854–855.

Nuclear Protein Modification and Chromatin Substructure. 3. Relationship between Poly(adenosine diphosphate) Ribosylation and Different Functional Forms of Chromatin†

Donald B. Jump, Tauseef R. Butt, and Mark Smulson*

ABSTRACT: The relationship between poly(adenosine diphosphate) ribosylation of nuclear proteins and functionally different forms of chromatin from mid-S-phase HeLa nuclei was investigated. The major observations emerging from this study were that unique nonhistone proteins were modified in mid-S-phase HeLa nuclei. The major acceptor for poly(adenosine diphosphate-ribose) [poly(ADP-Rib)] was an internucleosomal nonhistone protein (protein C; 125 000 molecular weight). Histones H3, H1, H2b, and H2a but not H4 were ADP-ribosylated in S-phase nuclei. Chromatin fragments preferentially released by micrococcal nuclease were enriched in nonhistone proteins, poly(ADP)-ribosylated nuclear proteins, poly(ADP-Rib) polymerase activity and nascent DNA from the DNA replicating fork. In extended forms of chromatin, contiguous to the DNA replicating fork, poly(ADP-Rib) polymerase was maximally active. However, in chromatin distal to the replicating fork (i.e., more condensed structures), nucleosomal histones and histone H1 were not significantly ADP-ribosylated, and poly(ADP-Rib) polymerase activity was depressed two- to threefold. The data suggest that a subset of nucleosomes in extended regions of chromatin is subject to extensive ADP ribosylation.

The objective of the present study was to ascertain the relationship between nuclear protein modification and different functional forms of chromatin, as selectively released during digestion by micrococcal nuclease. Recent advances in understanding the structure of chromatin have revealed that its basic subunit nature is stabilized by cooperative interactions between DNA and protein (Kornberg, 1977; Elgin & Weintraub, 1975; Felsenfeld, 1978). The higher ordered packaging of nucleosomes into condensed chromosomal structures is thought to involve protein–protein and pro-

† From the Department of Biochemistry, Schools of Medicine and Dentistry, Georgetown University, Washington, D.C. 20007. *Received August 29, 1978; revised manuscript received December 6, 1978.* Supported by National Institutes of Health Grant CA13195. Submitted by D.B.J. to the Department of Biochemistry in partial fulfillment of the requirements for the Ph.D. degree.

0006-2960/79/0418-0983$01.00/0