A NEW POLYPEPTIDE ASSOCIATED WITH RNA POLYMERASE FROM BACILLUS SUBTILIS DURING LATE STAGES OF VEGETATIVE GROWTH[1]

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DNA-dependent RNA polymerase has been purified from Bacillus subtilis at various stages of vegetative cell growth. Polymerase isolated from cultures approaching the end of the logarithmic growth phase was associated with a 60,000-dalton polypeptide and was only 10-20% as active as polymerase isolated from rapidly growing cells. Appearance of this new polypeptide and the change in template activity occur prior to stage 0 of sporulation.

INTRODUCTION

Involvement of RNA polymerase in the "switchover" from vegetative to spore-specific transcription in Bacillus subtilis was initially suggested by the observation that polymerase looses its ability to transcribe bacteriophage DNA early during sporulation (1). Analysis of the subunit structure of RNA polymerase isolated from sporulating cells showed that one of the large molecular weight beta subunits of the vegetative enzyme was degraded to a 110,000-dalton polypeptide (2). More importantly this new form of polymerase could not be stimulated by sigma subunit which was shown to be required for activity on bacteriophage DNA as well as rRNA cistrons (3).

Recently it has been reported that the ability of RNA polymerase isolated from B. subtilis to transcribe bacteriophage DNA is lost very early during sporulation and prior to measurable degradation of the large molecular weight beta subunit (4). The authors suggest that additional molecular changes must

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occur to cause the loss of vegetative template activity.

In this communication we report an analysis of the subunit structure and properties of RNA polymerase isolated from two strains of B. subtilis at different stages of vegetative cell growth. B. subtilis 168M is a spore-forming strain and B. subtilis 12A is an asporogenous mutant which is blocked at stage 0 of sporulation and contains very low amounts of serine protease (5). A new polypeptide associated with RNA polymerase during late stages of vegetative growth is described. We also present evidence that the loss of vegetative template activity also occurs at these stages of vegetative cell growth.

MATERIALS AND METHODS

Cultures of B. subtilis 168M and 12A were grown at 37°C in 100 liter batches in SDM medium ($4x10^{-2}$M K_2HPO_4, $2.2x10^{-2}$M KH_2PO_4, $1.7x10^{-3}$M sodium citrate, $6x10^{-4}$M $MgSO_4$, $7.6x10^{-3}$M $(NH_4)_2SO_4$, $1x10^{-5}$M L-tryptophan, 0.2% glucose, 0.1% casamino acids, and 1% peptone). Growth was stopped by addition of sodium azide (0.01M, final concentration). Logarithmic and late logarithmic cells were harvested at OD_{600nm}= 0.55 and 1.1, respectively. Stationary cultures had an OD_{600nm} = 1.4. B. subtilis 12A was a gift from J. Spizizen. High molecular weight ø e DNA was extracted with phosphate-buffered phenol at 0°C as described previously (6).

RNA polymerase was prepared by phase extraction, ammonium sulfate fractionation, DEAE-sephadex chromatography, Sepharose 6B chromatography, DNA-cellulose chromatography and glycerol gradient ultracentrifugation as presiously described (7). Core RNA polymerase was prepared by phosphocellulose chromatography after the glycerol gradient step according to Burgess (8). Sodium dodecyl sulfate polyacrylamide gels (7.5%) were run according to Laemmli (9) and stained with Coomassie blue.

RNA polymerase activity was measured in reaction mixtures containing: 0.08µCi 8-C^{14}-ATP (30mCi/mM), 0.8mM ATP, GTP, UTP and CTP, 0.05M Tris buffer (pH 7.9), 10mM $MgCl_2$, 1mM EDTA, 1mM β-mercaptoethanol and 12.5µg øe DNA in a total volume of 0.5ml. Assays were incubated at 37°C. Specific activity is defined as mµmoles of AMP incorporated/mg protein/10min.

TABLE I: PURIFICATION SCHEME FOR RNA POLYMERASE FROM LOGARITHMIC AND LATE LOGARITHMIC CELLS OF B. SUBTILIS 168M AND 12A.

	12A (log)			168M (log)		
	Total units	Specific activity	Yield	Total units	Specific activity	Yield
30-70% $(NH_4)_2SO_4$	4,640	47	100	3,320	36	100
DEAE Sephadex	2,280	173	49	1,500	121	45
Sepharose 6B	1,740	328	37	1,320	290	40
DNA-Cellulose	1,677	1,240	36	1,250	960	38
Glycerol Gradient	1,560	1,460	34	1,040	1,140	31
Phosphocellulose				41	45	1
	12A (late log)			168M (late log)		
	Total units	Specific activity	Yield	Total units	Specific activity	Yield
30-70% $(NH_4)_2SO_4$	1,120	14	100	960	6	100
DEAE Sephadex	685	46	57	610	28	63
Sepharose 6B	620	83	51	535	52	56
DNA-Cellulose	574	340	48	502	142	52
Glycerol Gradient	517	370	43	450	160	47

RESULTS AND DISCUSSION

A summary of the purification of RNA polymerase from logarithmic and late logarithmic cultures of B. subtilis 168M and 12A is shown in Table I. High specific activity polymerase was obtained in good yield from logarithmic cultures of both strains of B. subtilis. The specific activity of polymerase obtained from late logarithmic cells was, on the other hand, only 10-20% that obtained from logarithmic cells. This reduction in activity is similar to that described for polymerase from sporulating B. subtilis (1) and is not restricted to ϕe DNA template since proportionate decreases in activity were observed when DNAs from other B. subtilis phages(ϕ29, SP82, β22 and SP02c$_1$) were used as templates for the enzyme (unpublished observations).

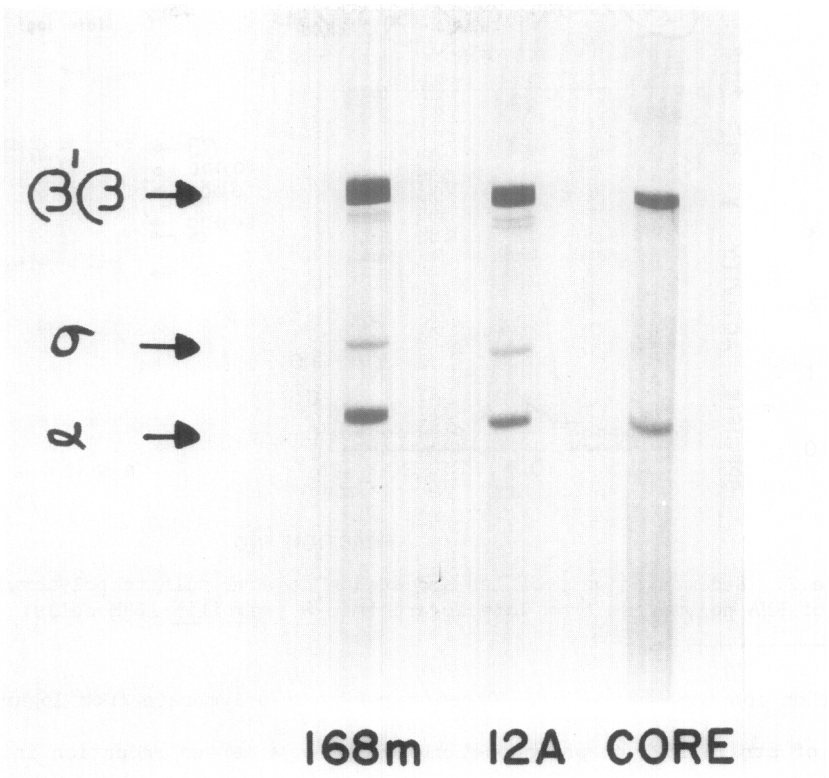

Figure 1. Sodium dodecyl sulfate polyacrylamide gels of RNA polymerase from logarithmic cells of B. subtilis 168M and 12A.

We did not observe any decrease in the yield of polymerase activity from late logarithmic cells relative to logarithmic cells at any step of the purification procedure. This result suggests that extensive proteolysis of polymerase does not occur during the preparation since proteolysis lowers the activity of polymerase on bacteriophage DNA (3). Failure to use phenylmethylsulfonylfluoride in our preparations results in almost complete loss of polymerase activity from late logarithmic cultures and increased degradation of the large molecular weight beta subunit of the enzyme.

The subunit structure of polymerase from logarithmically growing cells, Figure 1, is very similar to that reported for polymerase from B. subtilis 168 (10). The molecular weights of the ββ', σ and α, estimated from the sodium dodecyl sulfate polyacrylamide gels were 165,000, 52,000 and 40,000, respectively. Polymerase obtained from logarithmic phase cells had a sedi-

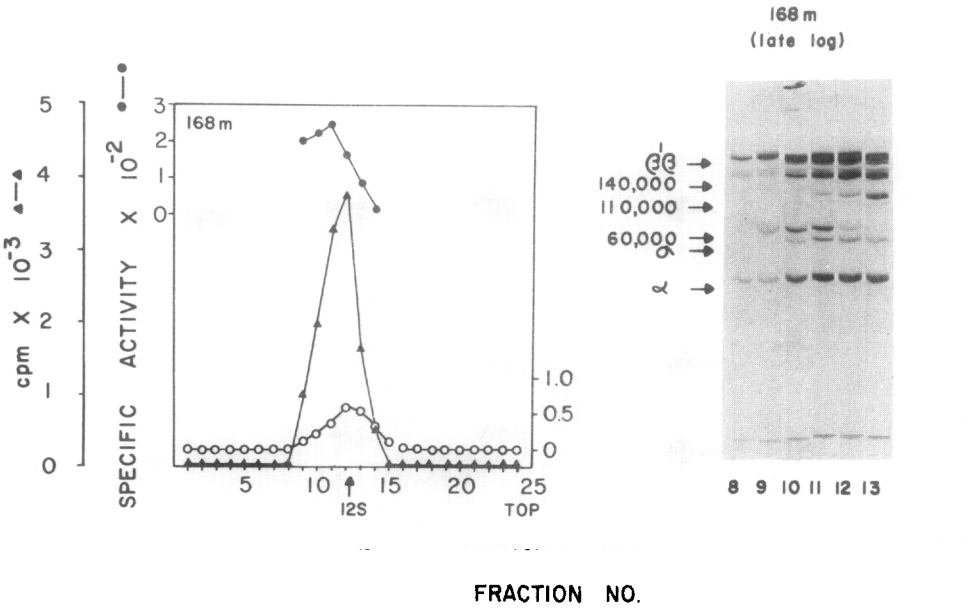

Figure 2. Sedimentation profiles and sodium dodecyl sulfate polyacrylamide gels of RNA polymerase from late logarithmic B. subtilis 168M cells.

mentation constant of 12-13S. Chromatography of polymerase from logarithmic cells of strain 168M on phosphocellulose caused a marked reduction in specific activity (Table I) and eliminated the sigma subunit (Figure 1). Core enzyme had a sedimentation constant of 9-10S.

In contrast to polymerase isolated from logarithmic cells, additional polypeptides were associated with polymerase isolated from late logarithmic B. subtilis 168M (Figure 2). The most rapidly sedimenting fractions of the glycerol gradient contained a 60,000-dalton polypeptide and the more slowly sedimenting fractions contained 140,000 and 110,000-dalton polypeptides. The 60,000-dalton polypeptide appeared to be bound to RNA polymerase, since fractions which contained large amounts of this polypeptide and no sigma subunit (fractions 9 and 10) sedimented more rapidly than "core" polymerase. The specific activity of the fractions containing the 60,000-dalton polypeptide (150-300) was only 15-20% of that obtained with polymerase from logarithmic cells (1,000-1,500). This new form of the enzyme was, however, more active than "core" polymerase(30-100).

A similar analysis of RNA polymerase from late logarithmic cells of B. subtilis 12A is shown in Figure 3. The specific activities and patterns of

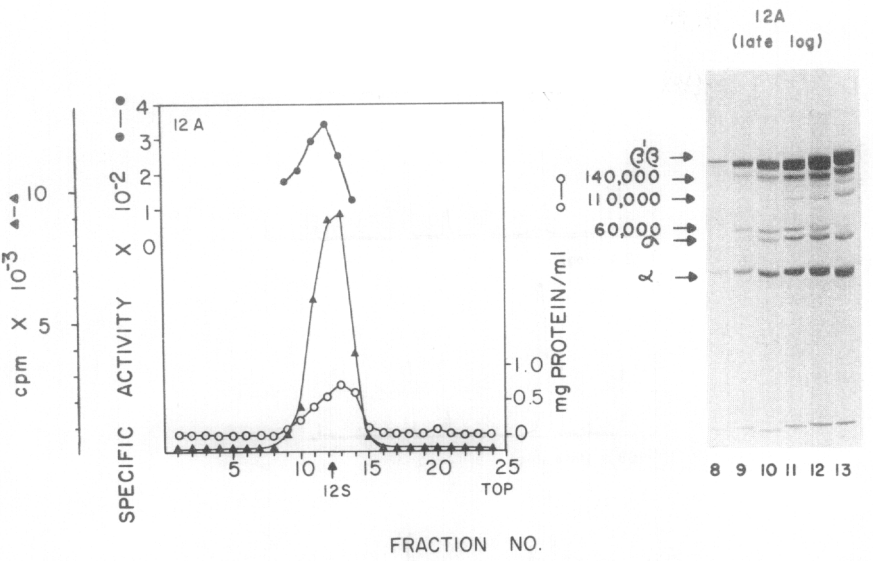

Figure 3. Sedimentation profiles and sodium dodecyl sulfate polyacrylamide gels of RNA polymerase from late logarithmic B. subtilis 12A cells.

polypeptides on polyacrylamide gels were similar to that obtained from late logarithmic cells of B. subtilis 168M.

Densitometer tracings of stained sodium dodecyl sulfate polyacrylamide gels of polymerase from logarithmic and late logarithmic cells of B. subtilis 168M and 12A are shown in Figure 4. Polymerase isolated from late logarithmic cell of B. subtilis 168M and 12A does not contain a full complement of the high molecular weight beta subunit. Instead polymerase is associated with a 110,000-dalton polypeptide and a 140,000-dalton polypeptide. The 110,000-dalton polypeptide has been shown to be derived from the beta subunit of polymerase by proteolytic cleavage (11). The 140,000-dalton polypeptide has not been described previously and probably represents an intermediate degradation product of the beta subunit. Polymerase from late logarithmic cells of strain 12A, a strain which has significantly reduced amounts of serine protease(s), contained substantially higher amounts of the high molecular weight vegetative beta subunit than did the enzyme from strain 168M. Despite this large difference in the extent of beta subunit degradation, polymerase isolated from late logarithmic cells of both strains had the same reduced specific activity on

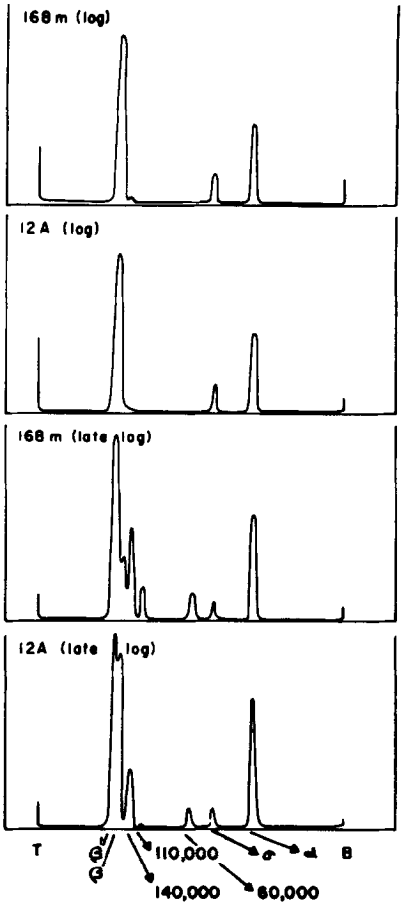

Figure 4. Densitometer traces of sodium dodecyl sulfate polyacrylamide gels of RNA polymerase from logarithmic and late logarithmic B. subtilis 168M and 12A.

bacteriophage DNA templates and was associated with similar amounts of the 60,000-dalton polypeptide. These findings are consistant with the results of Linn et al. (4), which suggest that the reduced activity of polymerase is independent of degradation of the beta subunit.

All the modification reported for the spore-forming B. subtilis strain 168M have been observed in the early-blocked asporogenous mutant strain 12A, indicating that the alterations in polymerase subunit structure and template activity commence prior to stage 0 of sporulation. We have also observed the 60,000-dalton polypeptide associated with polymerase from late logarithmic cells of B. subtilis SB11 (a spore-forming derivative of the non-transformable

strain W23) and two additional asporogenous mutants (unpublished observation): B. subtilis SR22 (12) and B. subtilis RB163 (13). We are currently investigating the possibility that loss of template activity on bacteriophage DNA is related to association of the 60,000-dalton polypeptide with vegetative RNA polymerase.

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