JOURNAL OF BACTERIOLOGY, June 1977, p. 1091-1097
Vol. 130, No. 3

Printed in U.S.A.

In Vivo Aminoacylation of Transfer Ribonucleic Acid in *Bacillus subtilis* and Evidence for Differential Utilization of Lysine-Isoaccepting Transfer Ribonucleic Acid Species

JUDITH TOCKMAN[1] AND BARBARA S. VOLD[2]*

Department of Microbiology, Scripps Clinic and Research Foundation, La Jolla, California 92037

Received for publication 7 February 1977

The presence or absence of certain amino acids has different effects on the ability of *Bacillus subtilis* to sporulate, and the intracellular pool size of amino acids has been reported to vary during sporulation. The idea that these variations might exert a regulatory effect through aminoacylation of transfer ribonucleic acid (tRNA) was investigated by studying the levels of aminoacylation in vivo in the logarithmic or stationary phase of growth. Both the periodate oxidation method and the amino acid analyzer were used to evaluate in vivo aminoacylation. The results indicated that in general the level of aminoacylation of tRNA's remained constant through stage III of sporulation, although there were detectable variations for specific amino acid groups. Our studies also showed that periodate oxidation damaged certain tRNA's; therefore, the results obtained by such a method should be interpreted with caution. Because the damage can affect certain isoaccepting species specifically, the periodate oxidation method cannot be used to establish which isoaccepting species are acylated in vivo. We also investigated the possibility of preferential use of particular tRNA species by polyribosomes. These results demonstrated a preferential use of lysyl-tRNA's at different growth stages. Control mechanisms operating during the early stages of sporulation, therefore, do not affect the overall level of aminoacylation. However, there is an effect on the levels of aminoacylation of specific amino acids and on which isoaccepting species are utilized by the polyribosome system.

Amino acid requirements for sporulation were shown by Doering and Bott to have differential effects on the ability of *Bacillus subtilis* 168 to sporulate (2). Large variations in the intracellular pool of amino acids have been reported in *B. licheniformis* (1), including a rapid decrease after the end of growth. Since certain regulatory effects of amino acids have been shown to be mediated by transfer ribonucleic acid (tRNA), we set out to examine the state of aminoacylation of tRNA's of *B. subtilis* 168 during logarithmic growth and the stationary phase. We were also interested in ascertaining whether particular isoaccepting tRNA species were preferentially aminoacylated, or preferentially utilized by polyribosomes, at particular stages of growth. Precedence for the idea of preferential utilization of isoaccepting species come from the work of Sueoka and Kano-Sueoka, who reported changes in the pattern of binding of leucine tRNA isoaccepting species to polyribosomes after T2 bacteriophage infection of *Escherichia coli* (16), and from Holmes et al., who noted differential utilization of leucyl-tRNA's in *E. coli* (W. M. Holmes, E. Goldman, T. A. Miner, and G. W. Hatfield, unpublished data).

Our experimental results indicate that in *B. subtilis*, as in *E. coli* (19), major changes in the levels of intracellular aminoacylation do not occur as cells progress from logarithmic to stationary growth. This parameter was measured by the periodate method, by the amino acid analyzer, and by chromatography on dihydroxybory (DBAE)-cellulose. However, we did observe a difference in which tRNA species were utilized by polyribosomes. We present evidence that one of the three species of lysyl-tRNA is preferentially bound to polyribosomes in stationary-phase cells, whereas utilization of another species is depressed. A slight tendency toward preferential binding of the former species also occurs in logarithmic cells; however, utilization of the latter species is not depressed at that stage.

Our experimental results also suggest that

[1] Present address: The Salk Institute for Biological Studies, San Diego, CA 92112.

[2] Present address: Biomedical Science, Stanford Research Institute, Menlo Park, CA 94025.

measurement of in vivo aminoacylation of tRNA by determining the capacity of the tRNA to be reesterified after periodate oxidation and deacylation may be inaccurate because of damage caused to some tRNA's. Reverse-phase column chromatography of periodate-oxidized tRNA revealed that oxidation affected the chromatographic behavior of certain tRNA species. Also, the amino acid acceptor activities of certain species were lowered by periodate oxidation below the values obtained by the amino acid analyzer, implying that the periodate method can give spuriously low values for amounts of intracellular aminoacylation in specific cases. Analysis of amino acids removed from tRNA on an amino acid analyzer proved to a much better method of determining the state of acylation in vivo.

MATERIALS AND METHODS

Bacterial strain and growth conditions. The tryptophan-requiring auxotroph, *B. subtilis* 168 *trpC2*, was grown in tryptone-yeast broth in 2.8-liter Fernbach flasks (7). One liter of the broth was inoculated with approximately 5×10^{10} spores, suspended in sterile water, and heat-shocked for 10 min at 80°C. Growth of the culture was monitored by measuring absorbance at 660 nm (A_{660}) in a Zeiss spectrophotometer. Logarithmically growing cells were harvested after 3 h of growth (A_{660}, 1.15), and stationary-phase cells were harvested at approximately stage III of the sporulation cycle (A_{660}, 5.70).

Preparation of tRNA for determination of intracellular aminoacylation. Cultures were made 10 mM in NaN_3 10 min before harvesting and were quick-chilled in a dry ice-methanol bath at the time of harvesting. Cells were collected by centrifugation and washed with 100 mM sodium acetate (pH 4.5), 0.15 M NaCl, 1 mM ethylenediaminetetraacetic acid, 10 mM NaN_3, and 20% sucrose. Cells were then suspended in 100 mM sodium acetate (pH 4.5), 0.15 M NaCl, 1 mM ethylenediaminetetraacetic acid, 10 mM NaN_3, 20 mM 2-mercaptoethanol, and 1.5 mg of bentonite per ml and disrupted sonically. Deoxyribonuclease (DNase) was added to the sonic extract at 10 μg/ml. The sonic extract was then extracted with an equal volume of water-saturated phenol. After ethanol precipitation, the ribonucleic acid (RNA) from the aqueous phase of the phenol extract was fractionated on a Sephadex G-100 column equilibrated at 9°C with 100 mM sodium acetate (pH 4.5), 0.15 M NaCl, 10 mM $MgCl_2$, and 4 mM 2-mercaptoethanol. tRNA fractions were combined, precipitated with ethanol, and collected by centrifugation or filtration on a Millipore filter (type HA, 0.45 μm).

Determination of levels of in vivo aminoacylation of tRNA by periodate method. Periodate treatment was patterned after the method of Yegian et al. (19). tRNA precipitated from the Sephadex G-100 fraction was dissolved in 100 mM sodium acetate (pH 4.5) and divided into two equal portions. One portion was made 2.5 mM in $NaIO_4$. Periodate-treated and untreated samples were incubated in the dark at 37°C for 30 min. To destroy excess periodate, samples were made 0.1 mM in ethylene glycol and incubated in darkness for an additional 10 min at 37°C. The samples were then dialyzed overnight against 10 mM $MgCl_2$ and 50 mM 2-mercaptoethanol to free them from IO^-_3, a reduction product of periodate that can inhibit acylation of tRNA (11). After dialysis, the oxidized and untreated tRNA were precipitated with ethanol and recovered by filtration on Millipore filters. The tRNA samples were deacylated by incubation in 1.8 M tris(hydroxymethyl)-aminomethane (Tris) (pH 8.0) for 1 h, at 37°C. The nucleic acid was then reprecipitated in ethanol, collected by filtration, and stored in 10 mM $MgCl_2$ and 10 mM 2-mercaptoethanol. Amino acid acceptor activities of periodate-oxidized and untreated tRNA were determined as described previously (18).

Reverse-phase column chromatography. Isoaccepting tRNA species were resolved on reverse-phase columns as described previously (14).

Amino acid analysis. For analysis of amino acids esterified to tRNA by the amino acid analyzer, the tRNA extracted from logarithmic- and stationary-phase cells was deacylated by incubation at 37°C for 90 min in 20 mM ammonium carbonate (pH 10). Amino acids were separated from nucleic acids on a column of Sephadex G-25 equilibrated with 20 mM ammonium carbonate. The amino acids were lyophilized three times and analyzed in a Beckman 121-M amino acid analyzer.

DBAE-cellulose chromatography. Logarithmic or stationary tRNA from the G-100 column was further treated to remove all deoxyribonucleic acid (DNA) fragments because the chromatographic behavior of DNA on DBAE-cellulose is like that of esterified tRNA. After incubation with electrophoretically purified DNase, the tRNA was dialyzed overnight against two 1-liter changes of 10 mM $MgCl_2$ and 10 mM 2-mercaptoethanol. The tRNA was then precipitated with 95% ethanol, recovered by centrifugation, and chromatographed on a column of DBAE-cellulose (10). The buffer used for elution of esterified material was 0.6 M KCl-50 mM morpholine (pH 7.7)-20% (vol/vol) ethanol. The buffer for elution of unacylated tRNA was 0.2 M NaCl-50 mM sodium acetate (pH 4.5).

Isolation of polyribosomes and ribosome-bound tRNA. To inhibit degradation of polyribosomes and tRNA, cultures were made 10 mM in NaN_3 and 1 mM in phenylmethylsulfonylfluoride (PMSF) 10 min before harvesting. At the time of harvesting, cultures were rapidly chilled in a dry ice-methanol bath. Cells were collected by centrifugation and washed with 10% sucrose, 10 mM $MgCl_2$, 10 mM Tris (pH 7.5), 60 mM KCl, 10 mM NaN_3, and 1 mM PMSF. Cells were then resuspended in this washing solution, to which was added 10 μg of DNase and 1 mg of lysozyme per ml, and which was made 20 mM in 2-mercaptoethanol. The cell suspension was incubated for 30 min at 37°C. The lysate was then made 0.5% in sodium deoxycholate, clarified by centrifugation at 10,000 rpm for 10 min and layered onto cushions of 42% sucrose, 10 mM $MgCl_2$, 10 mM Tris (pH 7.5), 60 mM KCl, 10 mM NaN_3, and 1 mM PMSF. Lysates were centrifuged at 40,000 rpm for 3

h in a Beckman type 65 rotor to pellet polyribosomes. To obtain nonribosome-bound tRNA, high-speed supernatants were phenol extracted and fractionated on a Sephadex G-100 column, as described above. To obtain polyribosome-bound tRNA, ribosome pellets were resuspended in cell washing solution containing 20 mM 2-mercaptoethanol and were phenol extracted and fractionated on a Sephadex G-100 column.

RESULTS

The levels of in vivo aminoacylation for tRNA species from logarithmically growing and stationary-phase cells at approximately stage III of sporulation, determined by the periodate method, are shown in Table 1. These levels are given by the ratios of amino acid acceptor activity of periodate-oxidized tRNA to that of untreated tRNA. The more closely the acceptor activity of periodate-treated tRNA approached that of untreated tRNA, the greater was the degree to which tRNA was esterified in vivo and protected from the action of periodate, which renders unacylated tRNA incapable of accepting an amino acid. Experiments in which tRNA, esterified with a ^{14}C-labeled amino acid mixture, was added to preparations of logarithmic and stationary cells during sonic treatment and phenol extraction showed that at least 10% of the tRNA extracted from the cells was deacylated in the course of isolation. The Sephadex chromatography, during which additional deacylation occurred, was desirable in our studies to remove polysaccharides, which were extracted along with tRNA and which interfered with effective periodate oxidation. Sephadex chromatography also removed ribosomal RNA and DNA, which interfered with the RPC-5 and DBAE-cellulose columns. This additional deacylation was responsible for our values of in vivo aminoacylation being about 10% lower than the values given by other investigators who did not use the Sephadex procedure (3, 8, 12, 19). Folk and Berg (4) used another method to stabilize aminoacyl-tRNA in vivo (by adding trichloroacetic acid to the cultures). Since our values for aminoacylation in vivo were not unusually low, there is no reason to doubt that our method was not a satisfactory one for our purposes, especially since we are primarily concerned with relative values comparing cultures from two growth stages.

We also estimated levels of intracellular aminoacylation of tRNA by hydrolyzing amino acids from logarithmic and stationary tRNA and subjecting these amino acids to amino acid analysis. Acceptor activities for tRNA species as determined by this method, which are indicative of the extent of aminoacylation in vivo, are shown in Table 2.

With the exception of proline and glutamic acid, acceptor activities as measured by periodate oxidation were significantly greater than those determined by amino acid analysis. Similar results were reported by Yegian et al. for tRNA of *E. coli* (19). Yegian et al. attributed

TABLE 1. *Levels of aminoacylation of tRNA species in vivo determined by the periodate oxidation method*

Amino acid	Growth stage	Acceptor activity[a]		
		Untreated	After periodate treatment	Percent aminoacylation[b]
Lysine	Logarithmic	95	58	61
	Stationary	79	74	93
Glutamic acid	Logarithmic	95	39	44
	Stationary	87	42	49
Proline	Logarithmic	54	8	14
	Stationary	36	8	23
Leucine	Logarithmic	137	79	58
	Stationary	152	94	62
Tyrosine	Logarithmic	51	20	39
	Stationary	42	32	75
Phenylalanine	Logarithmic	75	34	96
	Stationary	55	35	63
Methionine	Logarithmic	99	75	76
	Stationary	70	65	92
Tryptophan	Logarithmic	20	10	50
	Stationary	13	15	111

[a] Acceptor activity is expressed as picomoles of amino acid accepted per A_{260} unit of tRNA.

[b] The percent aminoacylation was obtained by dividing the amino acid acceptor activity of the periodate-treated tRNA by the activity of untreated RNA.

TABLE 2. *Levels of aminoacylation of tRNA species in vivo determined by amino acid analysis*

Amino acid	pmol of amino acid released/A_{260} unit of tRNA	
	Logarithmic	Stationary
Lysine	19	29
Arginine	20	13
Aspartic acid	33	35
Threonine	20	25
Serine	35	38
Glutamic acid	35	31
Proline	14	13
Glycine	69	56
Valine	24	30
Isoleucine	17	24
Leucine	22	46
Tyrosine	16	10
Phenylalanine	13	16

their results to the esterification of tRNA, with substances other than amino acids protecting the tRNA from periodate oxidation and not removed by alkaline hydrolysis.

To test for the possibility that significant amounts of tRNA of *B. subtilis* may be protected from periodate oxidation by alkali-stable bonds to other substances, we chromatographed alkali-treated logarithmic and stationary tRNA on a column of DBAE-cellulose. The diol groups of terminal adenosine residues of unesterified tRNA formed hydrogen bonds to diol groups attached to the cellulose. Thus, tRNA that has an amino acid or other group on the 3'-terminus does not bind to the DBAE-cellulose and is eluted from the column at high ionic strength and pH, whereas tRNA with a free 3'-terminus is eluted at relatively low ionic strength and pH. Most of the alkali-treated tRNA from logarithmic and stationary cells showed the elution behavior of unacylated material. Of the logarithmic tRNA, 79% was eluted at low ionic strength, whereas 77% of the stationary tRNA was eluted at low ionic strength. The amount of material eluting at high ionic strength was insufficient to account for the difference in amino acid acceptor activity measured by periodate oxidation and by amino acid analysis. Therefore, it is more likely that the difference in acceptor activities as measured by the two methods is accounted for by loss of amino acids before the amino acid analysis because the additional steps necessary to prepare the stripped amino acid for the analyzer include three sequential lyophilizations.

It is possible that there are alkali-labile groups attached to tRNA that are not amino acids and do not show up in the amino acid analyzer analysis, but there does not seem to be any precedence to support such a contention.

Table 3 shows the difference in the relative amount of tRNA aminoacylation for logarithmic and stationary tRNA, as determined by both amino acid analysis and periodate oxidation. With the exceptions of leucine and tyrosine, there is good agreement between the amounts of change in aminoacylation from logarithmic to stationary growth, as determined by the two methods. These results show that in general there is no overall change in the degree of aminoacylation of tRNA in *B. subtilis* cells progressing from the logarithmic to the stationary phase of growth. Confirming evidence was provided by chromatographing undeacylated logarithmic and stationary tRNA on DBAE-cellulose. In these experiments, 79% of the logarithmic tRNA and 75% of the stationary tRNA were eluted from the DBAE-cellulose column at high ionic strength and accordingly consisted of esterified material. However, increases in aminoacylation of stationary-phase lysyl- and tryptophanyl-tRNA were seen and may reflect regulatory changes accompanying the beginning of sporulation. Increases in the amount of aminoacylation of tyrosyl-tRNA and leucyl-tRNA were also seen by one method, but were not detected by both methods. To establish the significance of these small changes, however, was not the point of our investigation. Rather, we were looking for a major decrease in aminoacylation which might have a regulatory role.

TABLE 3. *Relative amount of aminoacylation in vivo of logarithmic tRNA compared with stationary tRNA*[a]

Amino acid	Result from amino acid analysis	Result from periodate oxidation
Lysine	1.5	1.3
Arginine	0.7	
Aspartic acid	1.1	
Threonine	1.3	
Serine	1.1	
Glutamic acid	0.9	1.1
Proline	0.9	1.0
Glycine	0.9	
Valine	1.3	
Isoleucine	1.4	
Leucine	2.1	1.2
Tyrosine	0.6	1.6
Phenylalanine	1.2	1.0
Methionine		0.9
Tryptophan		1.5

[a] Ratios express the amount of aminoacylated tRNA determined for the stationary-phase tRNA sample divided by the amount of aminoacylated tRNA determined in the logarithmic-phase tRNA sample.

Because we were interested in identifying particular isoaccepting tRNA species that might be preferentially aminoacylated at different stages of growth, we co-chromatographed periodate-oxidized and unoxidized tRNA on a reverse-phase-5 column. When reacylated with a labeled amino acid, the periodate-treated tRNA represented those isoaccepting species that were esterified in the cell, protected from oxidation, and capable of reacylation. These chromatography experiments gave the unexpected finding that certain tRNA species are damaged by periodate oxidation.

The cognate tRNA species of phenylalanine, methionine, glutamic acid, leucine, and proline showed normal chromatographic behavior after periodate oxidation since oxidized and unoxidized material gave the same elution profile. As an example, the profile for methionyl-tRNA's is shown in Fig. 1. Other tRNA profiles, however,

were greatly affected by the periodate treatment. For instance, unoxidized tyrosyl-tRNA eluted in the manner described previously by Vold (14). However, both the major and minor isoaccepting species of tyrosyl-tRNA from logarithmically growing and stationary-phase cells were damaged by periodate oxidation to the extent that they no longer eluted at their normal positions in the NaCl gradient (Fig. 2). Susceptibility of certain bases in tyrosyl-tRNA to oxidation by the periodate could be responsible for the nearly complete abolition of the normal elution profile after periodate treatment (6, 9).

The chromatographic mobility of lysyl-tRNA was also altered by periodate treatment (Fig. 3) in that the species designated as 3 on the figure was not present in the periodate-treated sample. Either the lysyl-tRNA, which chromatographed in this position, was not acylated in vivo, or the tRNA eluted at an earlier position after periodate treatment. For a more precise determination of the fate of lysyl-tRNA isoaccepting species after periodate, tRNA from early-exponential-phase cells was aminoacylated with [^{3}H]lysine and chromatographed on a reverse-phase-5 column. Species 1, 2, and 3 were isolated, oxidized with sodium periodate, as described above, and co-chromatographed on the reverse-phase-5 column with unoxidized tRNA aminoacylated with [^{14}C]lysine. The elution profiles indicated that the chromatographic behaviors of the tRNA species designated as 1 and 2 were mainly unaffected by periodate treatment. However, the tRNA species represented as $tRNA_3^{Lys}$ was shifted to the positions in which $tRNA_1^{Lys}$ and $tRNA_2^{Lys}$ normally elute (Fig. 4).

Another interesting effect of periodate treatment occurred with tryptophanyl-tRNA. Although periodate oxidation did not alter the chromatographic behavior of tryptophanyl-tRNA from logarithmically growing cells, an extra species was seen in the elution profile of periodate-oxidized tRNA from stationary-phase cells (Fig. 5). This species, noted in the figure by an asterisk, may have resulted from oxidative activation of a previously inactive isoaccepting species or from oxidative conversion of the specificity of a tRNA species from some other amino acid to tryptophan. Periodate acti-

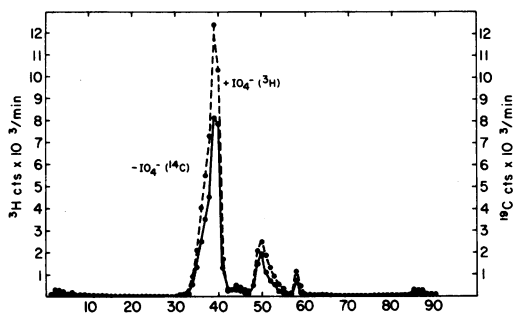

FIG. 1. *Chromatography of methionyl-tRNA's with or without periodate treatment on a reverse-phase-5 column. The tRNA was prepared from stationary-phase cells.*

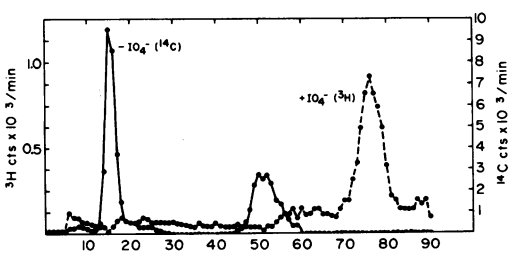

FIG. 2. *Chromatograph of tyrosyl-tRNA's, with or without periodate treatment, on a reverse-phase-5 column. The tRNA was prepared from stationary-phase cells.*

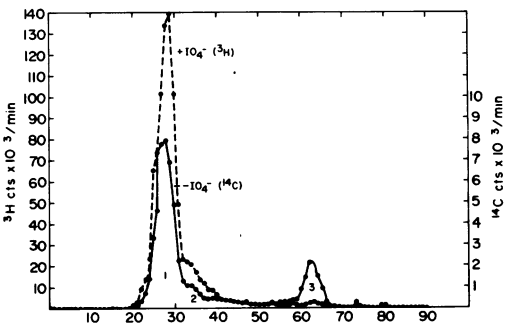

FIG. 3. *Chromatography of lysyl-tRNA's, with or without periodate treatment, on a reverse-phase-5 column. The tRNA was prepared from stationary-phase cells.*

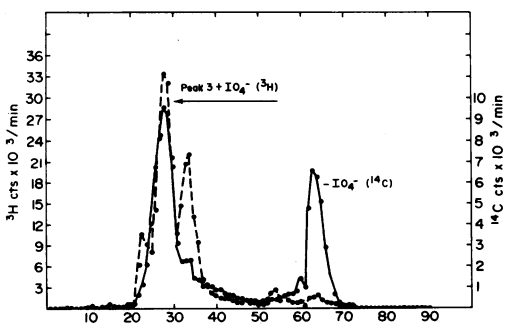

FIG. 4. *Co-chromatography of untreated, total lysyl-tRNA's with a sample of $tRNA_3^{Lys}$ after periodate treatment.*

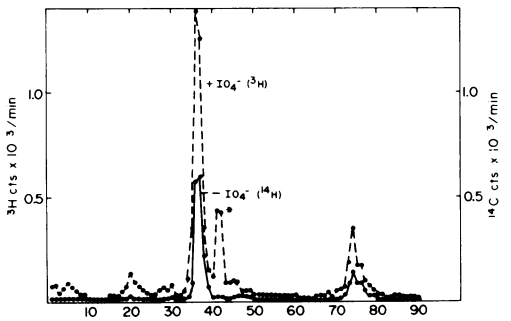

FIG. 5. *Chromatography of tryptophanyl-tRNA's, with or without periodate treatment, on a reverse-phase-5 column. The tRNA was prepared from stationary-phase cells.*

vation of a leucyl-tRNA species has been reported by Yegian and Stent (19). Such an effect of periodate could account for the apparent increase in in vivo aminoacylation of tryptophanyl-tRNA from logarithmic to stationary phase (Table 1).

In addition to identifying isoaccepting species that might have been preferentially aminoacylated, we were interested in identifying species that might be preferentially bound to polyribosomes during protein synthesis. Of particular interest were the isoaccepting species of lysyl-tRNA, since it was shown previously that the pattern of isoaccepting species in this tRNA group changes as cells progress from the logarithmic to the stationary phase (16). Accordingly, we obtained polyribosomes by high-speed centrifugation of lysates of logarithmic and stationary cells and extracted polyribosome-bound tRNA from the resulting pellets and unbound tRNA from the supernatant. Polyribosome-bound tRNA was aminoacylated with [^{3}H]lysine and co-chromatographed on a reverse-phase-5 column with unbound tRNA aminoacylated with [^{14}C]lysine. Table 4 indicates that stationary polyribosomes showed preferential utilization and logarithmic polyribosomes showed a slight preference for one of the isoaccepting species, $tRNA_1^{Lys}$. The relatively constant amounts of $tRNA_2^{Lys}$ in all the preparations attest to the validity of the other differences. The effect is not due to lack of availability or mass action because a majority of the tRNA's remain in the supernatant, and a majority of the tRNA's are aminoacylated. It is also interesting to note that $tRNA_3^{Lys}$ is found on polyribosomes at a level disproportionate to its availability in stationary-phase cells but is utilized in proportion to its concentration in logarithmically growing cells.

TABLE 4. *Differential utilization of lysyl-tRNA's by ribosomes in vivo*[a]

Ribosome	Lysyl-tRNA		
	$tRNA_1^{Lys}$	$tRNA_2^{Lys}$	$tRNA_3^{Lys}$
Logarithmic tRNA			
Nonpolyribosome-bound	38	26	36
Polyribosome-bound	42	24	34
Stationary tRNA			
Nonpolyribosome-bound	61	25	14
Polyribosome-bound	70	25	5

[a] Numbers represent the percentage each isoaccepting species of lysyl-tRNA represents of the total lysyl-tRNA eluted from a reverse-phase-5 column. The amounts of each species were determined by integrating the radioactivity under each chromatographic peak.

DISCUSSION

During cell differentiation, the intracellular condition of tRNA may change in response to the operation of control mechanisms. In this paper, we have questioned whether the level of aminoacylation of tRNA changes between exponential and stationary phase reflect a point of regulatory control. It has been estimated that dormant spores contain tRNA which is 93% deacylated (12) with the exception of the tRNA's for arginine, histidine, isoleucine, and valine, which show 21 to 72% aminoacylation. Also, in *B. megaterium*, about one-third of the spore tRNA's lack the terminal adenosine residue (13) and thus cannot be acylated. In *B. subtilis* 168, we have shown that the loss of the terminal adenosine commences at about stage III in sporulation (15). Since we were concerned with events of possible regulatory significance rather than terminal events, our comparisons of levels of aminoacylation were taken at or before stage III. Also, we observed levels of aminoacylation in a rich medium where pool sizes would not be limiting. Although pool sizes can affect the levels of aminoacylation, we were interested in changes that might have regulatory significance in normal sporulation media in contrast to a mass action effect which might be noted under conditions of amino acid limitations in the medium.

We have shown that, for most of the tRNA species we examined, overall levels of intracellular aminoacylation are not greatly affected as *B. subtilis* progresses from the logarithmic to the stationary phase of growth up to stage III in sporulation. Therefore, it seems that the mech-

anisms of control, which are at work as *B. subtilis* cells begin to prepare for sporulation in a rich medium, are not regulated by the overall level of aminoacylation of tRNA. An important point brought out in these studies is that periodate oxidation has an adverse effect on certain tRNA's. Since periodate oxidation damages some species of tRNA and may affect their capacity for reacylation, the use of periodate may not provide an accurate estimation of the amount of intracellular aminoacylation in vivo. For this reason, the use of amino acid analysis of amino acids removed from the tRNA extracted from cells is desirable to supplement data obtained through the use of periodate.

Control mechanisms operating at the onset of sporulation may affect the use of a particular isoaccepting species of tRNA by polyribosomes. Our data suggest that $tRNA_1^{Lys}$ is preferentially bound to polyribosomes. The lysyl-tRNA bound to polyribosomes is enriched for $tRNA_1^{Lys}$ beyond what would be expected by simple mass action. It has been reported that $tRNA_1^{Lys}$ and $tRNA_3^{Lys}$ show a difference in codon preference in vitro (16). Since the utilization of $tRNA_3^{Lys}$ is greatly diminished in stationary phase, this may reflect a difference in messenger RNA population. Additional experiments with other tRNA groups will be necessary to determine whether particular isoaccepting species for other amino acids might be preferentially bound to polyribosomes at different stages of growth. In addition, significant changes in overall levels of intracellular aminoacylation of tRNA may occur later in the course of sporulation than at the point in time we have studied.

ACKNOWLEDGMENTS

We wish to thank Tony Hugli for completing the amino acid analyzer experiments for us and William Steinberg for reading the manuscript and providing many helpful suggestions. Also, the technical assistance of Nicole Keisel and Arlene Carbone is gratefully acknowledged. J. Ashwell's editorial assistance was invaluable.

This work was supported by Public Health Service grant GM-17421 and Research Career Development award GM-23,736, both from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Clark, V. L., D. E. Peterson, and R. W. Bernlohr.** 1972. Changes in free amino acid production and intracellular amino acid pools of *Bacillus licheniformis* as a function of culture age and growth media. J. Bacteriol. 112:715–725.
2. **Doering, J. L., and K. F. Bott.** 1972. Differntial amino acid requirement for sporulation in *Bacillus subtilis*. J. Bacteriol. 112:345–355.
3. **Ehresmann, G., P. Imbault, and J. H. Weil.** 1974. Determination of the degree of *in vivo* tRNA aminoacylation in yeast cells. Anal. Biochem. 51:548–556.
4. **Folk, W. R., and P. Berg.** 1970. Characterization of altered forms of glycyl transfer ribonucleic acid synthetase and the effects of the alterations on aminoacyl transfer ribonucleic acid synthesis in vivo. J. Bacteriol. 102:204–212.
5. **Kano-Sueoka, T., and N. Sueoka.** 1969. Leucine tRNA and cessation of *Escherichia coli* protein synthesis upon phage T2 infection. Proc. Natl. Acad. Sci. U.S.A. 62:1229–1236.
6. **Kasai, H., Z. Ohaski, F. Hanada, S. Nishimura, N. J. Oppenheimer, P. F. Crain, J. G. Lishi, D. L. Von Minden, and J. A. McCloseky.** 1975. Structure of the modified nucleoside Q isolated from *Escherichia coli* transfer ribonucleic acid 7-(4,5,-dis-dihydroxy-1-cyclopenten-3-ylaminmethyl)-7-deoxyguanosine. Biochemistry 14:4198–4208.
7. **Lazzarini, R. A.** 1966. Differences in lysine-sRNA from spore and vegetative cells of *Bacillus subtilis*. Proc. Natl. Acad. Sci. U.S.A. 56:564–571.
8. **Lewis, J. A., and B. N. Ames.** 1972. Histidine regulation in *Salmonella typhimurium*. XI. The percentage of transfer RNA^{His} charged *in vivo* and its relation to the repression of the histidine operon. J. Mol. Biol. 66:131–142.
9. **Lipsett, N. N., and B. P. Doctor.** 1967. Studies on tyrosine transfer ribonucleic acid, a sulfur-rich species from *Escherichia coli*. J. Biol. Chem. 242:4072–4077.
10. **McCutchan, F. G., P. T. Gilham, and D. Söll.** 1975. An improved method for the purification of tRNA by chromatography on dihydroxyboryl substituted cellulose. Nucleic Acids Res. 2:853–864.
11. **Rizzino, A. A., and M. Freundlich.** 1975. Estimation of *in vivo* aminoacylation by periodate oxidation: tRNA alterations and iodate inhibition. Anal. Biochem. 66:446–449.
12. **Setlow, P.** 1974. Percent charging of transfer ribonucleic acid and levels of ppGpp and pppGpp in dormant and germinated spores of *Bacillus megaterium*. J. Bacteriol. 118:1067–1074.
13. **Setlow, P., G. Primus, and M. P. Deutscher.** 1974. Absence of 3'-terminal residues from transfer ribonucleic acid of dormant spores of *Bacillus megaterium*. J. Bacteriol. 117:126–132.
14. **Vold, B. S.** 1973. Analysis of isoaccepting transfer ribonucleic acid species of *Bacillus subtilis*: chromatographic differences between transfer ribonucleic acids from spores and cells in exponential growth. J. Bacteriol. 113:825–833.
15. **Vold, B.** 1974. Degree of completion of 3'-terminus of transfer ribonucleic acids of *Bacillus subtilis* 168 at various development stages and asporogenous mutants. J. Bacteriol. 117:1361–1362.
16. **Vold, B. S.** 1975. Isoaccepting species of lysyl-transfer ribonucleic acids in *Bacillus subtilis* cells, p. 282–289. *In* H. O. Halvorson, R. Hanson, and L. L. Campbell (ed.), Spores VI. American Society for Microbiology, Washington, D.C.
17. **Vold, B. S., and S. Minatogawa.** 1972. Characterization of changes in transfer ribonucleic acids during sporulation in *Bacillus subtilis*, p. 254–263. *In* H. O. Halvorson, R. Hanson, and L. L. Campbell (ed.), Spores V. American Society for Microbiology, Washington, D.C.
18. **Yegian, C. D., and G. S. Stent.** 1968. Differential aminoacylation of three species of isoleucine transfer RNA from *Escherichia coli*. J. Mol. Biol. 39:59–71.
19. **Yegian, C. D., G. S. Stent, and E. M. Martin.** 1966. Intracellular condition of *Escherichia coli* transfer RNA. Proc. Natl. Acad. Sci. U.S.A. 55:839–846.