DNA SYNTHESIS DURING NIACIN STARVATION IN NIACIN REQUIRING ESCHERICHIA COLI

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Recently, joining enzymes which repair single-stranded breaks in the DNA duplex covalently have been found in T4 phage-infected (Weiss and Richardson, 1967) and non-infected E. coli (Gellert, 1967). The purified DNA joining enzyme requires ATP as a cofactor in T4-infected (Weiss and Richardson, 1967) and NAD in non-infected E. coli (Zimmerman et al., 1967)

The biological importance of the DNA joining enzyme was suggested by its requirement for multiplication of T4 phage (Fareed and Richardson, 1967) and by the demonstration of its joint action with DNA polymerase in the synthesis of infective ØX174 DNA in vitro (Goulian and Kornberg, 1967; Goulian et al., 1967). However, it is uncertain how this enzyme is involved in duplication of chromosomes in vivo.

We isolated an E. coli mutant requiring niacin, a precursor of NAD, and examined its physiology in respect to DNA synthesizing activity during niacin starvation. DNA's of small size were found using an alkaline sucrose gradient. These were found to accumulate during starvation and might have been produced due to decreased activity of the DNA joining enzyme. It was shown that these smaller DNA's were integrated stepwise into larger DNA molecules on addition of niacin.

MATERIALS AND METHODS

A niacin requiring mutant of E. coli was isolated from E. coli W3110 thy⁻ by treatment with N-methyl-N'-nitro-N-nitrosoguanidine (Apirion and Schlessinger, 1967). CG medium which contains per liter: casamino acid (Difco vitamin free), 10 g; glucose, 2 g; NaCl, 3 g; KH_2PO_4, 1.3 g; $MgSO_4 \cdot 7H_2O$, 250 mg; $CaCl_2$, 11 mg; thymine, 2 mg and Tris, pH 7.4, 6 g was used as niacin-free medium and niacin was added to a concentration of o.1 μg/ml, when needed. The concentration of thymine was reduced to one fifth when labeling DNA. Nutrient agar supplemented with 3 g of yeast extract and 15 g of agar per liter of CG medium was used for viable counts.

Cells of E. coli niacin⁻ were grown at 37° to the early logarithmic phase, harvested, washed once with saline, and transfered to niacin-free medium. Synthesis of RNA and DNA was followed by incorporation of ^{14}C-adenine into the RNA and DNA fractions (Schmidt and Thannhauser, 1945) and that of protein by incorporation of ^{14}C-amino acid mixture into the hot TCA insoluble fraction. For sedimentation analysis of DNA in alkaline sucrose, spheroplasts were produced at 0° by suspending about 5 x 10^8 cells in 0.2 ml 0.033 M Tris (pH 7.4) containing 20 % sucrose, 200 μg/ml lysozyme and 5 mM EDTA. Ten μl of the spheroplast solution were placed on a 5 to 20 % sucrose gradient containing 1 mM EDTA, 0.8 M NaCl and 0.1 N NaOH according to the procedure of Rupp and Howard-Flanders (1968). The tubes were centrifuged in a Hitachi RPS40 rotor for 120 min at 30,000 rev./min. After centrifugation, fractions were collected, treated with cold TCA and washed repeatedly on Whatman GF/C discs with TCA. They were counted in a Tri-Carb liquid scintillation spectrometer. Phage particles of T4, labeled with ^{14}C-thymine, were layered onto an alkaline sucrose gradient (Rupp and Howard-Flanders, 1968). N-methyl-N'-nitro-N-nitrosoguanidine was purchased from Aldrich Chemical Co., and ^{3}H-thymidine

(5 C/mM), ^{14}C-thymine (24 mC/mM), ^{14}C-adenine (22 mC/mM) and ^{14}C-amino acid (0.7 mC/mg) were from Daiichi Kagaku.

RESULTS

When a niacin requiring mutant of E. coli W3110 thy$^-$ was transfered to niacin-free medium, the turbidity at 650 mμ gradually increased, but the number of viable cells reached a plateau in one hour and remained so for a further three hours (Fig. 1). The synthesis of DNA,

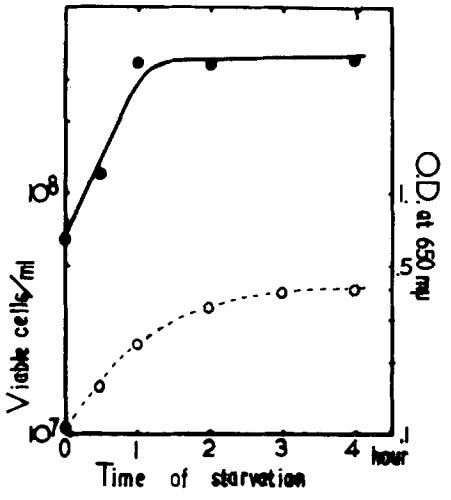

Fig. 1 Turbidity at 650 mμ and viable count of cell during the niacin starvation. ●——● , viable cells; o---o , turbidity

RNA and protein during this time is shown in Fig. 2. DNA and RNA syntheses decreased 30 min after the transfer while protein continued to be synthesized at steady rate for three hours. Therefore, the niacin within the cells seems to become exhausted in 30 min to one hour after the shift but the time needed for niacin-exhaustion varies in different experiments.

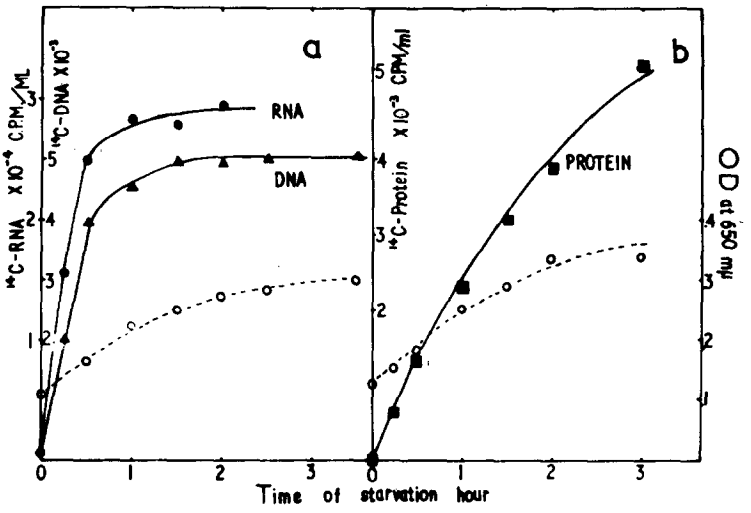

Fig. 2 Synthesis of DNA, RNA and protein after transfer to niacin-free medium. At time 0 14C-adenine (a) and 14C-amino acid (b) were added.
(a) ●——● , ^{14}C-adenine in RNA fraction; ▲——▲ , ^{14}C-adenine in DNA fraction; o---o , turbidity at 650 mμ
(b) ■——■ , ^{14}C-amino acid; o---o , turbidity at 650 mμ

The DNA's synthesized during the period of niacin-starvation were sedimented by alkaline sucrose gradient centrifugation. Their profiles are shown in Fig. 3 where rapidly sedimenting DNA's, like normal DNA's, were observed together with new DNA's with a slower rate of sedimentation. When the time of harvest was varied the latter DNA's could be found alone (Fig. 4). These two types of profile were reproducible and depended on the conditions used. These DNA's sedimented faster than T4 DNA which was used as an internal reference.

To see whether these DNA's were intermediates of normal DNA's or were simply artifacts, the change in the DNA's within the cell was studied by chasing with ^{1}H-thymidine added to the growth medium in which niacin was added to abolish the deficiency of NAD (Fig. 4). These accumulated DNA's with a reduced rate of sedimentation were

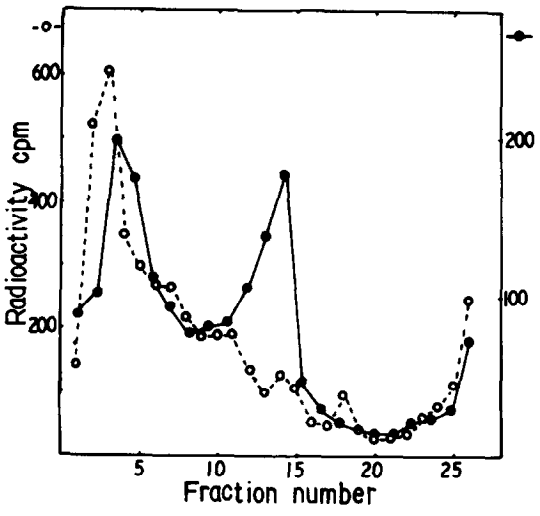

Fig. 3 Sedimentation in alkaline sucrose of DNA accumulated during niacin starvation. Cells were labeled with 3H-thymidine for 30 min after 75 min starvation of niacin ●——● , and before starvation, o---o . Gradient from right to left.

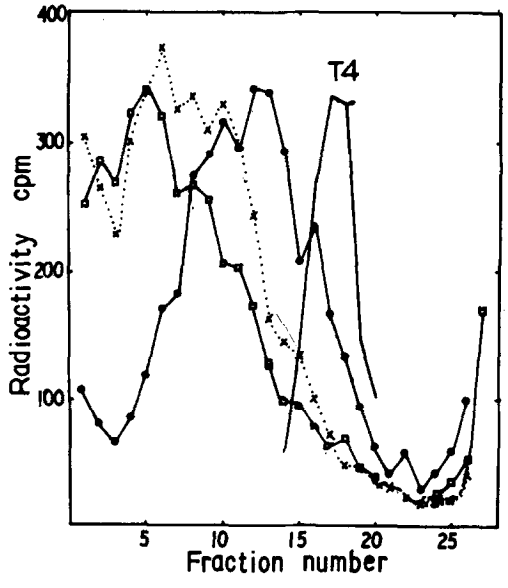

Fig. 4 Effect on sedimentation of DNA of addition of niacin after starvation. Cells were labeled with 3H-thymidine for 30 min after 75 min starvation of niacin, ●——● , then niacin was added and cells were chased with 1H-thymidine for 15 min, x···x , and for 30 min, □——□ , respectively.

integrated into faster sedimenting DNA's 15 min after addition of niacin to the medium. Further, 30 min after addition of niacin the profile of these faster sedimenting DNA's was converted to that of the normal type of DNA. The radioactivities of these DNA's were conserved almost completely throughout the chase-experiment. This suggests that these DNA's are intermediates and that the smaller DNA's accumulating in NAD deficiency are converted stepwise to the larger ones and eventually to normal DNA's. Since the de novo synthesis of DNA was not restored within 15 min after the addition of niacin (Fig. 5), the conversion from the smaller DNA's to the larger ones is considered to be due to the action of NAD which was formed on addition of niacin.

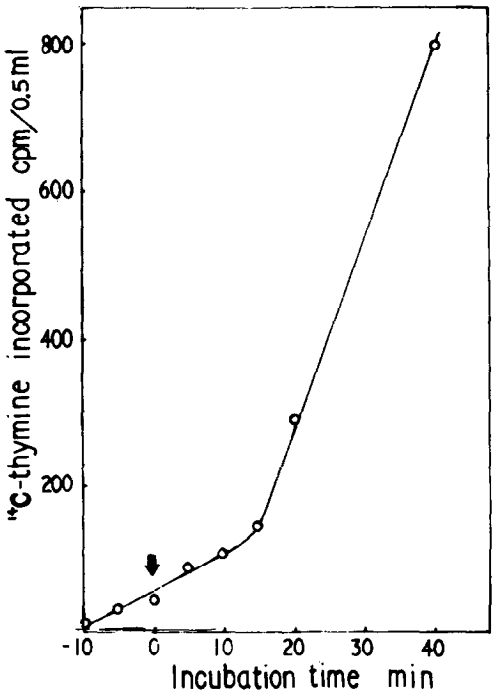

Fig. 5 Incorporation of ^{14}C-thymine into cold TCA-insoluble fraction after addition of niacin. Cells were starved of niacin for 120 min, labeled with 14C-thymine and at time 0 niacin was added.

DISCCUSION

The metabolic role of NAD is mainly concerned with respiratory chain, where NAD is maintained in equilibrium with NADH. Therefore, a limited but steady supply of energy must remain during niacin starvation as suggested by the observed continuation of protein synthesis (Fig. 2). On the other hand, since NAD, a cofactor of the DNA joining enzyme, is degraded to NMN and AMP (Zimmerman et al., 1967), niacin starvation is considered to decrease irreversibly the supply of NAD for the reaction of the joining enzyme.

The DNA's synthesized during niacin starvation are smaller than normal DNA of E. coli but larger than that of T4 phage DNA, since DNA's in alkaline sucrose are in the form of random coils (Studier, 1965). Okazaki et al. (1968) presented a model to show the discontinuous synthesis of DNA from 1,000 to 2,000 nucleotides to larger melecules. Our results are essentially consistent with theirs, although the size of our intermediates is quite different.

The accumulation of these DNA's can be considered to be due to the diminished action of the DNA joining enzyme which requires NAD as a cofactor, since these DNA's were formed during niacin deficiency. However, it is nor known whether NAD is essential to their formation.

It is noted that these intermediate DNA's are not converted directly to normal DNA's, but are converted via moderately larger DNA's. Since this conversion occures after the addition of niacin, and hence, after the formation of NAD, the DNA joining enzyme must be responsible for the conversion.

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