CONDITIONS OF UTILIZATION OF EXOGENOUS THYMIDINE FOR LABELLING OF *Bacillus subtilis* DNA

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The conditions were compared under which an increased utilization of exogenous thymidine for DNA synthesis takes place in the prototropic strains of *B. subtilis* and *E.coli*. When thymidylate synthetase is inhibited by 5-fluorodeoxyuridine, the specific radioactivity of DNA from both species is practically the same and comparable with the activity of preparations obtained with thy$^-$ mutants labelled by thymine-[^{3}H]. By contrast, deoxyadenosine increasing the incorporation of thymidine into DNA of *E.coli* is entirely ineffective with *B. subtilis*. When thymidine-[^{3}H] is used as a marker for the preparation of transforming [^{3}H]-DNA only the use of the inhibitory effect of 5-fluorodeoxyuridine can be recommended for a label increase.

The utilization of exogenous thymidine for the incorporation into the DNA of bacterial cells has been devoted considerable attention in view of the importance of labelled DNA preparations in biochemical and biological experiments[1-3]. In most cases the *Escherichia coli* culture served as a model; the results were then applied to other bacterial species, especially to *Bacillus subtilis* whose transforming DNA is becoming still more and more a subject of biochemical investigations. Besides the use of thymineless mutants — the most reliable guarantee of sufficient incorporation of exogenous thymine — several other procedures have been proposed for the increasing of thymidine incorporation into prototrophic bacterial strains: the inhibition of thymidylate synthetase (2.1.1.b) by 5-fluorodeoxyuridine added to the medium[4], the use of the effect of a higher concentration of deoxynucleosides (especially of deoxyadenosine) in the medium[5,6], and the utilization of mutants lacking thymidine phosphorylase[7] (2.4.2.4), which has been considered the main cause of insufficient thymidine uptake from the medium[2,8]. Even thymidylic acid[9] has been recommended for DNA labelling in *E. coli*.

In this study the possibilities of application were compared of some of the procedures mentioned above, which have been tested predominantly with *E.coli*, to DNA labelling in a prototrophic *B. subtilis* strain.

EXPERIMENTAL

Cultures of prototrophic strains *B. subtilis* SMYW and *E.coli* B were grown in a mineral medium[10] with the admixture of 1% of vitamin-free casamino acids (Difco) and of 0·5% of glucose. (^{3}H-Methyl)thymidine (Radiochemical Centre, Amersham, spec. activity 5 Ci/mmol) was added to the medium together with the cold substance adjusting the final concentration to 2 μg/ml (0·2 μCi/ml). The incorporation under different experimental conditions was examined on

membrane filters in terms of the radioactivity of the in trichloroacetic acid precipitable residue of a culture sample. The radioactivity was measured on aluminum planchets in a gas-flow Frieseke-Hoepfner counter. (^{3}H-Methyl)thymine (spec. activity 5 Ci/mmol) was used for the labelling of thymineless mutants. The isolation of DNA was carried out according to the Marmur procedure[11] with the addition of one phenol deproteinization. The culture for the isolation of DNA was allowed to grow in the presence of the radioactive precursor for at least two generations in the logarithmic phase of growth.

RESULTS AND DISCUSSION

The use of 5-fluorodeoxyuridine as thymidylate synthetase inhibitor (Fig. 1) leads with *B. subtilis* to a considerable radioactivity increase (curve *4*) in comparison with the control culture (curve *2*). The difference is even greater when we consider the inhibition of the growth of the culture (compare curves *1* and *3*) caused by the application of the antimetabolite, which, however, does not affect the DNA synthesis. If we try to influence the incorporation of exogenous thymidine by various concentrations of deoxyadenosine added to the culture medium (Fig. 2), then we observe with the same culture (Fig. 2*a*) that the radioactivity incorporated into DNA is not increased. Whereas with 5-fluorodeoxyuridine, used as inhibitor of thymidylic acid synthesis, the values of both cumulative incorporation of labelled thymidine and of the specific radioactivity of isolated DNA (Table I) are in both species comparable, the behavior of the two cultures is entirely different when deoxyadenosine is employed. As shown in Fig. 2*b*, in *E.coli* B – unlike in *B.subtilis* – an increase of thymidine incorporation takes places at all concentrations of deoxyadenosine employed; at the same time,

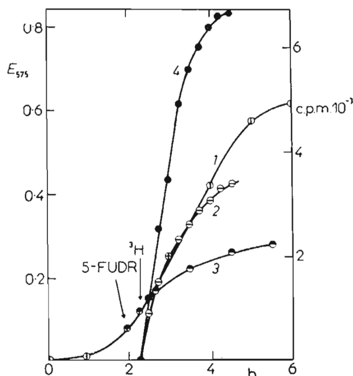

FIG. 1

Incorporation of Exogenous (^{3}H-Methyl)thymidine into *Bacillus subtilis* DNA in Presence of 5-Fluorodeoxyuridine

1,3 Growth curves of culture in the absence (⦶) or presence (◓) of 5-fluorodeoxyuridine (5 μg/ml); *2,4* corresponding radioactivities (⊖, resp. ●) on membrane filter after the extraction of 1 ml samples of culture by 5% trichloroacetic acid.

the highest concentration (500 μg/ml) increases also the initial incorporation rate (curve *4*).

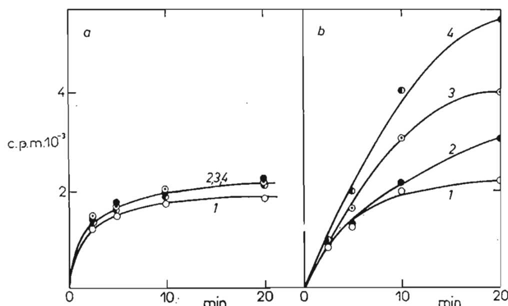

FIG. 2

Incorporation of Exogenous (^{3}H-Methyl)thymidine into *Bacillus subtilis* and *Escherichia coli* DNA in Presence of Deoxyadenosine

a B. subtilis SMYW culture, *b E.coli* B culture; *1,2,3,4* radioactivity on membrane filter after the extraction of 1 ml sample by 5% trichloroacetic acid: (—○—) control culture, cultures influenced by 50 μg (—●—), 150 μg (—⊙—), and 500 μg (—◐—) of deoxyadenosine on 1 ml. Deoxyadenosine was added to the culture when it reached 10^8 cells/1 ml.

TABLE I

Radioactivity of DNA Isolated from *Bacillus subtilis* and *Escherichia coli* Cultures Growing under Conditions Affecting the Incorporation of Exogenous Thymidine

Strain	Thymidine-[^{3}H] μg/ml (μCi/ml)	Inhibitor (μg/ml)	Specific activity of DNA, cpm/μg
B.subtilis SMYW	2 (0·2)	0	5 624
B.subtilis SMYW	2 (0·2)	deAdo (500)	5 932
B.subtilis SMYW	2 (0·2)	5-F-deUrd (5)	11 023
B.subtilis 10 thy$^-$	10 (1·0)[a]	0	18 053
E.coli B	2 (0·2)	0	7 134
E.coli B	2 (0·2)	deAdo (500)	18 762
E.coli B	2 (0·2)	5-F-deUrd (5)	15 052
E.coli thy$^-$	10 (1·0)[a]	0	22 121

[a] Thymine-[^{3}H] was used for the labeling of thy$^-$ strains.

A summary of the values of compared activities of DNA from both strains under conditions which affect the incorporation of radioactive precursors of DNA synthesis (Table I) permits us to evaluate quantitatively the two methods employed and to compare the level of thymidine incorporation with the level of labelled thymine uptake for the dependent cultures of both species. In most cases even the absolute values of specific activity of isolated DNA's are in agreement, except for the incorporation influenced by deoxyadenosine, where the radioactivity of the preparation from *E.coli* approaches the activity of DNA from the thymineless culture and for *B.subtilis* where it remains at the level of the control, unaffected culture. When we consider the fact that *B.subtilis* achieves in the medium used a maximum growth of $5 \cdot 10^8$ cells/ml and *E.coli* approximately 10^9 cells/ml, then the difference given in Fig. 2 could be interpreted in terms of different growth activities of the cultures at the given initial density of 10^8 cells/ml. According to the data given in Table I, however, there is no increase of radioactivity of the DNA preparation from *B.subtilis* even in case that deoxyadenosine has been added to the culture during the early logarithmic phase of growth. Preliminary results of the evaluation of thymidine phosphorylase (2.4.2.4) activity and of the thymidine to thymine[12] conversion in the presence of deoxynucleosides in the medium, indicate, however that the differences between the two species compared must be sought at the level of thymidine kinase (2.7.1.21) or of other enzymes involved in the metabolism of DNA precursors since *E.coli* and *B.subtilis* almost do not differ in these characteristics.

On the basis of the results described above and from the practical viewpoint of obtaining the radioactive preparation of transforming DNA from the prototrophic *B.subtilis* strain, only the incorporation of labelled thymidine with simultaneous inhibition of thymidylic acid synthesis by 5-fluorodeoxyuridine can be recommended. It should be born in mind at the same time that methods tested with only one bacterial species need not be generally applicable.

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