Triazine Nucleic Acid Interrelationship in *Escherichia coli* 15 arg⁻ *t*⁻ *u*⁻

Since it was demonstrated in 1958 that triazines inhibit the Hill reaction, various attempts have been undertaken to obtain more chemical information for their outstanding herbicidal properties (see for reviews[1,2]). In one of these studies, the structure of the triazines gave rise to the speculation that this heterocycle could be incorporated into the DNA or RNA by substituting the pyrimidine bases thymine and uracil. This hypothesis was investigated by ERCEGOVICH et al.[3] and TEMPERLI et al.[4] with ring-^{14}C labelled cyanuric acid and Prometryne. They observed no incorporation of cyanuric acid or Prometryne into the nucleic acids of *Escherichia coli* 15 arg⁻ *t*⁻ *u*⁻ after they had been grown in a complete nutrient medium. However, when these bacteria were grown in nutrient media deficient in either thymine or uracil, very low amounts of radioactivity derived from the added cyanuric acid or Prometryne were found in the DNA or RNA fraction.

Because the information presented was rather limited, it was difficult to judge the efficiency of the described test system and the significance of the results. We therefore repeated these investigations including as a competent positive control 2-^{14}C-5-bromouracil which is known to be incorporated into the DNA fraction of microorganisms. As a consequence of the importance of the problem under discussion, one representative of the chlorotriazines (2-ethylamino-4-isopropylamino-6-chloro-*s*-triazine) and one of the methylthiotriazines (2,4-*bis*-(isopropylamino)-6-methylmercapto-*s*-triazine) were investigated[5].

The triple auxotrophe *E. coli* 15 arg⁻ *t*⁻ *u*⁻ which requires arginine, thymine and uracil was used in these studies[6]. Log phase inocula for these experiments were obtained according to the method described by TEMPERLI et al.[4], except that a synthetic medium[7] substituted the minimal medium. The strain used grew more vigorously in this synthetic medium. The washed cells were transferred to 300 ml Erlenmeyer flasks containing 80 ml fresh synthetic medium and the supplements as listed in the Table. In each experiment 25 flasks were incubated for approximately 100 min at 37 °C while being aerated. Turbidity readings in control experiments in complete medium were in the range of 80–100 Klett units at the start and 200–230 Klett units after the incubation period.

The cells were harvested by centrifugation and washed repeatedly with 95% ethanol to remove adherent radioactivity. Approximately 2.5 g of lyophilised material were obtained from 2 l of culture broth. The nucleic acids were isolated by the procedure described by RUDNER et al.[8], except that the step in which the RNA is removed by ribonuclease was omitted. The final separation of DNA and RNA was performed according to MARMUR[9]. Both the RNA and the DNA contained only small amounts of protein not exceeding 1%, but were contaminated with polysaccharides. The mean values for the N/P ratios of the nucleic acids were found to be 1.6–1.8.

The radioactivity incorporated into the nucleic acids of *E. coli* was determined by liquid scintillation counting after combustion in an oxygen atmosphere[10]. The values presented are expressed as dpm/μg atom phosphorus. The results of the various experiments are summarized in the Table.

The results with 2-^{14}C-5-bromouracil clearly demonstrate the capability of *E. coli* 15 arg⁻ *t*⁻ *u*⁻ to incorporate base analogs of the pyrimidine into their nucleic acids. However, in experiments with the ring-^{14}C labelled triazines (Atrazine and Prometryne) no significant amount of radioactivity was detected in neither DNA nor RNA respectively. Therefore, the incorporation of Prometryne into DNA or RNA of these microorganisms as reported by ERCEGOVICH et al.[3] and TEMPERLI et al.[4] could not be confirmed.

Zusammenfassung. Mit Hilfe von 5-Bromuracil wurde die prinzipielle Fähigkeit von *Escherichia coli* 15 arg⁻ *t*⁻ *u*⁻, Basenanaloge des Pyrimidins für die Synthese ihrer Nukleinsäuren zu verwenden, gezeigt. Ein Einbau der Triazinverbindungen Atrazin und Prometryn in die Nukleinsäuren von *E. coli* 15 arg⁻ *t*⁻ *u*⁻ findet nicht statt, unabhängig, ob die Mikroorganismen in komplettem oder Basen-defizitärem Nährmedium wuchsen.

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Radioactive content of nucleic acids of *E. coli* 15 arg⁻ *t*⁻ *u*⁻ grown in the presence of ^{14}C-labelled 5-bromouracil, Atrazine and Prometryne. Negative control experiments (addition of 2-^{14}C-5-bromouracil after cultivation of the microorganisms and directly before starting the procedure) showed that no unspecific absorption of radioactivity had occurred (incorporation: DNA 78 and RNA 9 dpm/μg atom *P*).

Supplements to medium						Incorporation [dpm/μg atom *P*]	
Arginin	Thymine	Uracil	2-^{14}C-5-Bromouracil[a]	^{14}C-Atrazine[b]	^{14}C-Prometryne[c]	DNA	RNA
+	—	+	+			64,440	2,180
+	+	+		+		11	3
+	—	+		+		4	6
+	+	—		+		12	4
+	+	+			+	6	3
+	—	+			+	1	4
+	+	—			+	17	6

[a] 227 μC (spec. act.: 16.9 μC/mg) dissolved in 2.5 ml ethanol-water (9:1 v/v) were added to 2 l of medium. [b] 285 μC (spec. act.: 6.0 μC/mg) of uniformly ring labelled Atrazine dissolved in 6.25 ml ethanol were added to 2 l of medium. [c] 275 μC (spec. act.: 4.0 μC/mg) of uniformly ring labelled Prometryne dissolved in 2.5 ml ethanol were added to 2 l of medium.

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[5] 2-ethylamino-4-isopropylamino-6-chloro-s-triazine (Atrazine), active ingredient of Primatol A® and Gesaprim®, 2,4-*bis*-(isopropylamino)-6-methylmercapto-s-triazine (Prometryne), active ingredient of Gesagard®. Both herbicide formulations are manufactured by J. R. Geigy S.A., Basel (Switzerland).
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