Research Note
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ROLE OF AMINOTRANSFERASE IN THE SYNTHESIS OF 5-METHYLCYTOSINE IN PHAGE XP12 INFECTED CELLS.[1]

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Phage Xp12 with a unique pyrimidine constituent was previously isolated from *Xanthomonas oryzae*, and it was found that the pyrimidine, 5-methylcytosine, completely replaced cytosine in the phage deoxyribonucleic acid (Kuo *et al.* 1968). Presence of the unusual pyrimidine in the viral DNA and its absence in the nucleic acid of host organism raises the questions as to what is the mechanism of the pyrimidine biosynthesis and the metabolic alterations within the virus infected cell to make the synthesis possible.

Flaks and Cohen (1959) suggested that 5-methylcytosine might arise through a reaction similar to that involved in the formation of thymidylate but with a cytosine derivative as substrate or conceivably via an amination reaction involving a thymine derivative. In order to see if the suggested scheme is indeed operative in the Xp12 infected *Xanthomonas oryzae* syetem, aminotransferase from the phage infected cells were compared with that from non-infected bacteria.

X. oryzae, strain 507, was grown with vigorous aeration to 1×10^9 cells/ml on the semisynthetic medium containing 5g of bactopeptone, 15 g of sucrose, 10 ml of 1 M tris-buffer at pH 7.0, 0.1 g of $Ca(NO_3)_2$, 0.5 mg of $FeCl_2$, 1 mg of $MgSO_4$ and 0.5 g of NH_4Cl in a liter of distilled water. Bacterial cells were infected with phage Xp12 for various periods time at a phage to bacteria ratio of 5:1. The infected cells were chilled and harvested by centrifugation, washed once before resuspended in 1/40 volume of cold 0.01 M phosphate buffer at pH 7.0. After breaking the cells with Brownwill biosonik, the extract was clarified by centrifugation at 100,000 G for 30 minutes. The supernatant was dialyzed against the same buffer for 7 hours at 4°C to serve as crude enzyme preparation. The enzyme was then partially purified by ammonium sulfate precipitation between 30% to 60% saturation. The precipitate was resuspended and dialyzed in the same phosphate buffer for 7 hours at 4°C. Uninfected

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bacterial cells were treated and the enzyme preparation obtained in the same manner to serve as control.

Aminotransferase activities were measured by the absorption maxima of respective aromatic—keto acids formed (Lin *et al.* 1958, Gamborg *et al.* 1962). Standard reaction mixture contains 5 μ moles of L-phenylalanine or L-tryptophane, 8 μ moles of α-ketoglutarate, 0.0025 μ moles of pyridoxal phosphate, enzyme preparation and 5 μ moles of sodium phosphate buffer at pH 7.0 in a total volume of 2.0 ml. The reaction was allowed to proceed at 28°C. Samples of 0.4 ml were withdrawn from the reaction mixture at 10-minutes intervals starting with zero time (when α-ketoglutarate was added). The samples were immediately added to a test tube containing 2.5 ml of 0.5 N NaOH to stop further reactions. The activities of aminotransferases were measured with Gilford 2400 S spectrophotometer at 320 mμ and 328 mμ respectively for L-phenylalanine and L-tryptophane transaminations.

Assay mixture for tyrosine transaminase contains 6 μmoles of L-tyrosine instead of L-phenylalanine. A sampling schedule similar to the previous one was employed except in this case, 1/2 of each samples (0.2 ml was added to 2.3 ml of 0.5 M sodium arsenate at pH 7.0 whereas the remaining half to 2.3 ml of 0.5 M sodium arsenate with 0.5 M borate at the same pH. Transamination was indicated by the *p*-hydroxyphenylpyruvic acid formed. *p*-hydroxyphenylpyruvic acid concentration was determined by the absorption difference of the two solutions at 310 mμ.

The activities of aminotransferases from infected bacteria using phenylalanine, tryptophane and tyrosine respectively as substrate, α-ketoglutarate as amino acceptor in each case, are shown in Fig. 1. The curves are linear up to 30 minutes of incubation. The results for aminotransferases from uninfected cells, assay with the same system, were almost identical to those of infected cells.

To examine the possible amination of thymine derivatives by these enzymes, thymine, thymidine, thymidine monophosphate and thymidine triphosphate were respectively employed as amino acceptor in the standard reaction mixture. Since the lactam form of thymine predominates at pH 7.0, all enzyme assays were carried out in buffer with that pH. As indicated in Table 1, thymine and its derivatives failed to serve as amino acceptors from aromatic amino acids in the systems.

The failure of transamination in above system might be due to the unproper amino donors from aromatic amino acids for 5-methylcytosine:thymine transaminase, therefore, 5-methylcytosine was employed as amino donor, and its amino group was expected to be transferred to thymine-H^3 to become 5-methylcytosine-H^3. In standard reaction mixture, 5-methylcytosine was used

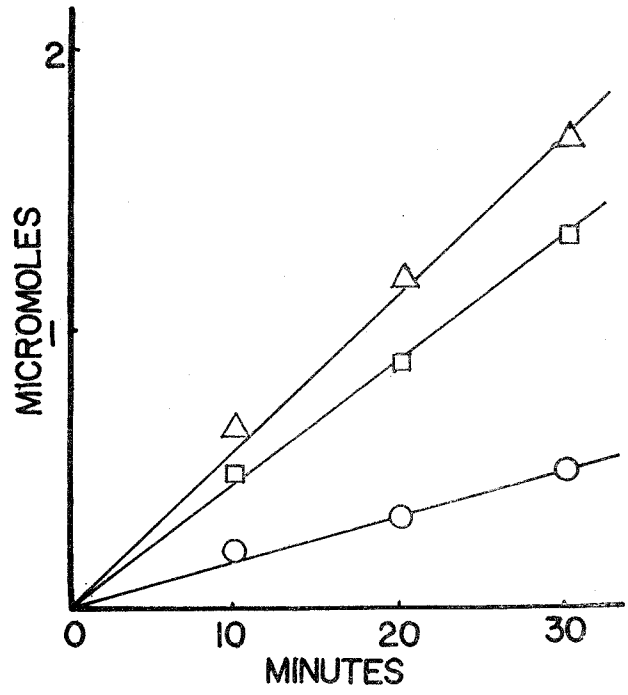

Fig. 1. The time course study of transamination for L-phenylalanine, L-tryptophane and L-tyrosine.
Enzyme was prepared from bacterial cells infected with Xp12 for 40 minutes. The reaction was carried out under standard conditions, and the activity was defined as the amount of the aromatic keto acid formed in μmoles.
△-△-△- L-phenylalanine α-ketoglutarate transamination
□ □ □- L-tryptophane-α-ketoglutarate transamination
○-○-○- L-tyrosine-α-ketoglutarate transamination

Table 1. *Thymine derivatives as NH_2-acceptors for transaminases isolated from phage infected X. oryzae with partial purification. The enzyme activity was expressed as μ moles aromatic keto acid formed in 30 min.*

donor / acceptor	phenylalanine	tryptophan	tyrosine
buffer	0	0	0
α-ketoglutarate	1.86	0.25	1.19
thymine	0	0	0
thymidine	0	0	0
thymidine monophosphate	0	0	0
thymidine triphosphate	0	0	0

as donor, and 1 μc of thymine-H^3 was added as acceptor in a total volume of 120 μl. 20 μl of the reaction mixture was withdrawn at zero time, 10 minutes,

30 minutes and 60 minutes after incubation. The samples were heated and applied on Whatman No. 1 filter paper, and ran chromatography with authentic 5-methylcytosine and thymine as indicators. Paper at the 5-methylcytosine and thymine positions after development were cut, placed in scintillation fluid, and counted with Packard Liquid Scintillation Spectrometer. Neither the formation of 5-methylcytosine-H^3 nor the disappearance of thymine-H^3 was detected. The same enzyme prparation was used for checking the transamination of amino group from L-glutamic acid to α-ketoglutarate, 50% of L-glutamic acid-C^{14} was found to convert to α-ketoglutarate-C^{14} in 10 minutes.

Possible induction of aminotransferase in phage infected cells was also studied. Table 2 shows that transamination does occur from phenylalanine to α-ketoglutarate in the bacterial cells but no further induction due to the phage infection. Transamination from phenylalanine to thymine does not take place in the bacteria and no induction was detected after phage infection.

Table 2. *Transaminase activity in cells infected and uninfected with phage. Crude emzyme preparations were used in this experiment. The enzyme activity was expressed as μ moles of phenyl pyruvate formed in 30 min.*

treatment / acceptors	infected cells (time after phage infection)				Uninfected cells
	0	20′	40′	60′	0
Buffer	0	0	0	0	0
α-ketoglutarate	0.37	0.41	0.37	0.38	0.42
thymine	0	0	0	0	0

From the above data it is concluded that the pathway, i.e., amination of thymine and its derivatives to 5-methylcytosine and its derivatives by aminotransferases do not operate in Xp12 infected cells.

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