ROLE OF ONE-CARBON PRECURSORS IN THE BIOSYNTHESIS OF DEOXYRIBONUCLEIC ACID IN BACTERIOPHAGE-INFECTED AND GROWING CELLS OF ESCHERICHIA COLI

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ABSTRACT

Nester, Eugene W. (Western Reserve University, Cleveland, Ohio) and John Spizizen. Role of one-carbon precursors in the biosynthesis of deoxyribonucleic acid in bacteriophage-infected and growing cells of Escherichia coli. J. Bacteriol. 82:867–874. 1961.—The ability of growing and T2 bacteriophage-infected cells of Escherichia coli to incorporate serine-3-C14, glycine-2-C14, formate-C14, and formaldehyde-C14 into purine and pyrimidine moieties of deoxyribonucleic acid (DNA) was determined. All four one-carbon precursors are effective contributors to the DNA-purines, but only glycine-2-C14 and serine-3-C14 are incorporated into the side chains of the pyrimidines. In addition, formate-C14 becomes incorporated only into position 8 of the purine ring, whereas isotope from serine-3-C14 and glycine-2-C14 is incorporated equally into the 2 and 8 positions. No qualitative differences were observed in the patterns of incorporation of any one-carbon units in growing or bacteriophage-infected cells. However, the 3-carbon of serine serves as a more effective precursor of the 2 and 8 positions of the DNA purine ring when the cells are infected. Formate-C14 and to a slight extent glycine-2-C14 are somewhat better precursors of these positions when the cells are infected under appropriate conditions.

The significance of one-carbon fragments for the biosynthesis of purines and pyrimidines has been delineated in large part from isotope incorporation studies employing a variety of microbial and mammalian systems. These investigations have revealed that the 2 and 8 positions of the purine ring, as well as the methyl side chain moieties of thymine and the bacteriophage pyrimidine hydroxymethylycytosine, have their origin in certain one-carbon precursors. In mammalian systems, studies with intact animals have demonstrated that these one-carbon units are effectively supplied by the 3 carbon of serine, 2 carbon of glycine, as well as formate and formaldehyde (Reichard, 1955). Further, studies on cell-free extracts of mammalian tissues have demonstrated the presence of enzymes capable of carrying out these reactions (Croesbe, 1960; Buchanan, 1960). In growing and T2 bacteriophage-infected cells of Escherichia coli, the 3 carbon of serine has been shown to be an effective precursor of the carbon side chains of both DNA-thymine and hydroxymethylcytosine (Cohen and Weed, 1954). It was reported (Croesbe, 1958) that formate-C14 does not function as a thymine methyl group precursor in exponentially growing cells of E. coli. In contrast to mammalian systems in which one-carbon units from any given precursor are incorporated equally into positions 2 and 8 of the purine ring (Buchanan, 1960), growing cells of the genus Pseudomonas as well as E. coli were shown to be able to incorporate formate-C14 into position 8 of the purine ring, but not appreciably into position 2 (Revel and Magasanik, 1958; Koch, 1955). Depending on the external concentration, glycine was preferentially incorporated into position 8 of the purine ring in the latter species.
Since the rate of deoxyribonucleic acid (DNA) synthesis is markedly increased in T2 bacteriophage-infected cells (Cohen, 1948) with the concomitant synthesis of a unique pyrimidine whose side chain originates from a one-carbon precursor, this system might display different patterns in the incorporation of one-carbon units from various precursors than does the uninfected system.

The present investigation was undertaken to determine in detail the efficiency of several one-carbon precursors in the biosynthesis of the DNA-pyrimidines and purines in exponentially growing cells of E. coli. In addition, studies were concomitantly performed in an attempt to detect any alterations which infection with a virulent bacteriophage would have upon this system.

MATERIALS AND METHODS

Biological systems. E. coli, strain R2 (obtained from A. D. Hershey), a variant of strain B and selected for its resistance to bacteriophage lysis from without, was employed. The bacteriophage T2 was a wild-type stock, and displayed the phenomenon of lysis inhibition. The bacteria were routinely grown on a reciprocal shaker at 37°C in a glucose-salts medium (Spizizen, 1957), supplemented with 0.01% vitamin-free, acid-hydrolyzed casein (Nutritional Biochemicals Co., Cleveland, Ohio). After 18 hr incubation, the cells, at a concentration of approximately $3 \times 10^8$ per ml, were transferred into an identical medium, usually 50 to 100 ml, to yield a final cell concentration of $2 \times 10^8$ per ml. After a doubling of the bacterial mass, determined by the increase in turbidity using a Klett-Summerson Colorimeter (Klett Mfg. Co., New York, N.Y.) with a 660 mμ filter, the culture was infected with bacteriophage at a multiplicity of 5:1. Isotopically labeled compounds were introduced 10 min later, and incubation continued for an additional 2 hr unless otherwise stated. Uninfected cultures were treated in an identical fashion. Bacteriophage-assay procedures were essentially those described by Adams (1950).

Chemical procedures. The culture was added to ice-cold 5% (final concentration) trichloroacetic acid and the acid-insoluble cellular constituents fractionated as follows: After the separation of the DNA from the acid-soluble cellular components, lipids, and ribonucleic acid according to the procedure of Schmidt and Thannhauser (1945), the DNA was extracted from protein with 5% trichloroacetic acid at 90°C as described by Schneider (1945). This solution, containing the moieties of DNA, was heated in a boiling water bath for 3 hr to decompose the trichloroacetic acid (Hitchings, 1941), and then taken to dryness by additional heating. This fraction was hydrolyzed for 30 min with 88% formic acid at 175°C (Wyatt and Cohen, 1952). The formic acid was removed under vacuum, and the purine and pyrimidine bases dissolved in 0.1 N HCl. Aliquots were spotted on Whatman No. 1 filter paper and chromatographed ascending in two dimensions. The initial solvent system (Kirby, 1955) was methanol, coned HCl, water (70:20:10); isopropanol, coned NH₄OH, and water (170:2.5:30) constituted the second solvent system (Hershey, Dixon, and Chase, 1953). This procedure separates unambiguously from one another all five possible bases of the infected system, as well as the two amino acids, glycine and serine.

The ultraviolet-absorbing spots were located with a model (SL Mineralite) (Ultra-Violet Products, Inc., South Pasadena, Calif.) These areas, along with corresponding spots in a blank lane, were cut out and eluted with 0.1 N HCl. The eluates were assayed in a Cary Recording Spectrophotometer (Applied Physics Corp., Pasadena, Calif.) against the corresponding eluates from the blank lanes and the quantity of each base calculated from its extinction coefficient (Bendich, 1957). Aliquots not exceeding 0.01 mg were plated on stainless steel planchets and counted in a Nuclear-Chicago Very-Thin Window Gas Flow Counter (Nuclear-Chicago Corporation, Des Plaines, Ill.). No corrections for self-absorption were necessary.

The purines were degraded by the following procedure. Adenine was eluted from the paper chromatogram and evaporated to dryness in a steam bath. It was converted to hypoxanthine by a procedure similar to that described by Marsh (1951), using 1 ml of 4 N H₂SO₄ and 0.1 g of NaNO₂. The yields were generally 80% of the starting purine. The solution was then neutralized, and the hypoxanthine converted to uric acid with a preparation of xanthine oxidase, prepared from cream by the procedure of Klenow and Embden (1955). Enough enzyme was added to carry the reaction to completion in 30 min. The course of the reaction was followed spectrophotometrically by the increase in optical
density at 290 μm, as described by Kalckar (1947). After completion of the reaction, the solution was boiled for 5 min and filtered to remove enzyme protein. It was then put on a Dowex 50 H⁺ column (Dow Chemical Co., Midland, Mich.) and eluted with H₂O. The resulting effluents were collected in cuvettes and assayed spectrophotometrically at 290 nm for their uric acid content.

The contents of the cuvettes containing uric acid were pooled, and 50.0 mg of recrystallized carrier uric acid added to the pooled samples. The total uric acid was dissolved by the addition of lithium carbonate; then the solution was filtered and enough HCl added to bring the filtrate to pH 7.5. The solution was then gently heated and, after the CO₂ had escaped, more HCl was added to adjust the pH to 4. The uric acid was allowed to crystallize over a 3-day interval at 4 °C. The crystals were washed several times with ice-cold water and then solubilized with 1 ml of 6 N HCl and a minimal amount of potassium chloride. Several drops of sodium thiosulfate were added to reduce any excess chloride. This procedure oxidizes the uric acid to uric acid containing the C-8 moiety of the original purine and alloxan derived from the pyrimidine moiety of the purine.

This urea-alkoxan mixture was put on a Dowex 50 H⁺ column and eluted with H₂O. The fraction containing alloxan does not adsorb to the resin and comes off immediately in the first 30 ml of acid effluent. The next 40 ml of effluent contains no trace of either alloxan or urea. The succeeding 200 ml was collected and shown to contain the entire urea fraction, as determined by the colorimetric procedure of Barker (1944). The alloxan was degraded by a modification of the procedure of Buchanan, Sonne, and Delluva (1947) as follows: The volume was reduced to 2 ml; 10 ml of a saturated solution of barium hydroxide was added and the solution refluxed for 1 hr in a boiling water bath. The precipitate was centrifuged, washed twice in cold water, and the washings added to the original supernatant liquid which contained the C-2 of the purine ring as urea. One ml of 2 N H₂SO₄ was added, and the BaSO₄ removed by filtration through Whatman No. 50 filter paper. The filtrate was then added to a Dowex 50 H⁺ column and the urea eluted with water. The urea fractions were assayed for radioactivity after adding unlabeled carrier urea and dissolving the samples in absolute ethanol. The urea sample was then transferred quantitatively to 10 ml of a solution of toluene containing 12.5% thixin R and 0.3% of 2,5-diphenyloxazole. This mixture was liquified in hot water (50 °C) and then homogenized by vigorous agitation in a commercial-type paint mixer. The contents of the vial were solidified by immersion in ice and assayed in a Packard Automatic Tri-Carb Liquid Scintillation Spectrometer Model 314-DC. The counting efficiency was 40%. The specific activities of the C-2 and C-8 carbon atoms were calculated on the basis of the radioactive assay and the colorimetric assay of urea resulting from degradation of the purines. The same procedure was followed for the degradation and assay of radioactive guanine. In some cases, both adenine and guanine from the same sample of DNA were isolated and degraded. Both purines gave comparable results.

Formate-C¹⁴ and serine-3-C¹⁴ were purchased from Volk Radio-Chemical Co., and glycine-2-C¹⁴ from Traceelab, Inc., Waltham, Mass. All nucleic acid derivatives were purchased from California Corporation for Biochemical Research, and were of special grade. Formaldehyde-C¹⁴ was obtained from Audrey Stevens, Department of Pharmacology, St. Louis University.

Calculations. In all experiments the specific activity of the purines and pyrimidines was calculated on the basis of net synthesis of DNA following the addition of isotope. Since the bases of DNA are not synthesized in equimolar amounts in the bacteriophage-infected cell, one can compare the utilization of the one-carbon precursors for each of the purines and pyrimidines. The following formula was derived for determining the relationship between the amount of thymine or adenine isolated and that present prior to addition of isotope in the infected system. It is based on DNA determinations prior to isotope addition and the fact that the Burton (1956) diphenylamine procedure used to measure the net synthesis of DNA measures only purine-bound deoxyribose, and thymine and adenine each represent approximately 33.3% of the total bases present in the bacteriophage-infected system. In other words, the total μmoles (thymine or adenine) isolated divided by 2 times the increment DNA increase and times 0.667 equal the μmoles present in the infected cell after isotope addition.
TABLE 1. Incorporation of one-carbon units into DNA*

<table>
<thead>
<tr>
<th>Product analyzed</th>
<th>Specific activity (counts per min/μmole)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Formate-C(^{14})</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
</tr>
<tr>
<td>Thymine</td>
<td>160</td>
</tr>
<tr>
<td>Hydroxymethylcytosine</td>
<td>130</td>
</tr>
<tr>
<td>Adenine</td>
<td>26,000</td>
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</tbody>
</table>

* Bacteria (100 ml) were grown on a rotary shaker after overnight cultivation and treatment as described in the text. Isotope additions were the following: formate-C\(^{14}\), 10 μmoles, 56,000 count/min; formaldehyde-C\(^{14}\), 10 μmoles, 25,000 count/min; dL-serine-3-C\(^{14}\), 100 μmoles, 180,000 count/min; glycine-2-C\(^{14}\), 100 μmoles, 160,000 count/min. Incubation was continued 120 min after isotope addition. The flasks with the formate and formaldehyde were fitted with a sodium hydroxide trap to collect CO\(_2\). The values for guanine are comparable to those reported for adenine.

Since the base ratio remains constant in the uninfected system, the μmoles initially present equal the total μmoles (thymine or adenine) isolated divided by 2 times the increment DNA increase.

RESULTS

The initial experiments were designed to determine the efficiency of the incorporation of one-carbon units derived from a variety of precursors into DNA-purines and pyrimidines in growing and bacteriophage-infected cells. Table 1 summarizes the data on the incorporation of four one-carbon precursors: formate-C\(^{14}\), formaldehyde-C\(^{14}\), glycine-2-C\(^{14}\), and serine-3-C\(^{14}\). The ability of formaldehyde-C\(^{14}\) to be incorporated into DNA was determined in the infected system only. These results indicate that in growing and bacteriophage-infected bacteria neither formate nor formaldehyde are incorporated to any significant degree into either of the two pyrimidines in the DNA (thymine and hydroxymethylcytosine). In contrast, however, both of these compounds are precursors of the purines, and therefore are entering a one-carbon pool in the cell. The reason for their inability to become incorporated into the pyrimidines is not a result of their inability to integrate into the metabolism of the organism.

The data further illustrate that in contrast to formate and formaldehyde, both glycine and serine are capable of acting as one-carbon donors for the biosynthesis of the two pyrimidines. Friedkin and Kornberg (1957) demonstrated that serine could substitute for hydroxymethyl tetrahydrofolic acid in the synthesis of thymidylate acid in cell-free extracts of E. coli. Thus, serine would be expected to give rise to hydroxymethyl tetrahydrofolic acid in the course of its intermediary metabolism. Likewise, Sagers and Gunsalus (1958) reported the presence of an enzyme in two species of Clostridium which converts the 2 carbon of glycine to hydroxymethyl tetrahydrofolic acid. The biosynthetic capabilities of the various one-carbon precursors as determined from the present study are illustrated in Fig. 1. If the data obtained from other microorganisms can be applied to the present system, it appears that precursors, which give rise endogenously to one-carbon units at the hydroxymethyl form (reduced) of tetrahydrofolic acid.
acid, incorporate these units as the side chain moieties of the pyrimidines as well as into the purine ring. To account for the inertness of formaldehyde in pyrimidine but not purine biosynthesis on this basis, an obligatory oxidation to formate must occur prior to its further metabolism.

Although there are no appreciable qualitative differences in the utilization of the one-carbon units of formate, glycine, and serine for purine and pyrimidine biosynthesis (Table 1) the infected cell does display significant quantitative differences in its utilization of formate-C^14, and to an even more marked degree, serine-3-C^14, when the growing and bacteriophage-infected cells are compared. Thus, in the infected system, the proportion of one-carbon units in DNA-purines and pyrimidines derived from serine-3-C^14 is two to three times higher than in the uninfected system. In contrast, there is no increased utilization of the one-carbon moieties of glycine-2-C^14 beyond the general increased utilization of all DNA precursors. This reflects the increased rate of DNA synthesis in the bacteriophage-infected cell.

To determine how the quantitative differences observed between the uninfected and infected cell in the synthesis of the entire purine molecule are reflected in the synthesis of the individual 2 and 8 positions of the purine ring, a precise determination of the extent of incorporation into these two positions was made, employing formate-C^14, glycine-2-C^14, and serine-3-C^14 as the one-carbon precursors. The DNA-purines were isolated and degraded so as to yield the 2 and 8 positions of the molecule in separate fractions. The ratio of the specific activity of carbon 2 to carbon 8 serves as a measure of the comparative utilization of the one-carbon precursor in the biosynthesis of these two positions. To fully magnify any differences between the infected and uninfected system, the cultures were incubated for only 60 min after infection, the duration of greatest divergence in the rates of DNA synthesis in the two systems. The results on the utilization of formate-C^14, glycine-2-C^14, and serine-3-C^14 for the 2 and 8 positions of the purine molecule are shown in Table 2. For any single precursor the ratio of the specific activities of the C-2:C-8 positions of the purine ring is the same whether or not the cell has been infected. Therefore, bacteriophage infection does not alter the source of the one-carbon units used in the biosynthesis of the 2 and 8 positions relative to each other. Under the experimental conditions of this study, glycine-2-C^14 and serine-3-C^14 enter one-carbon precursor pools which contribute equally to the biosynthesis of the 2 and 8 positions. Formate displays a considerably different behavior. Although it enters a one-carbon pool which labels the 8 position, this same pool is not tapped as a source of one-carbon units for the 2 position. It follows, therefore, that the one-carbon pool derived from glycine and serine is not in equilibrium with the one derived from formate.

Although bacteriophage infection does not appear to alter the precursor pools of these two positions qualitatively, there are significant quantitative differences. When the increased rate of DNA synthesis is taken into consideration, the infected cell employs serine as a one-carbon precursor for the 2 and 8 positions of the purines approximately six times more effectively than does the uninfected cell. Formate also supplies a con-

<table>
<thead>
<tr>
<th>Condition of culture</th>
<th>Supplement*</th>
<th>Amount of supplement (µmole/ml)</th>
<th>Specific activity (count/min/µmole) C-2</th>
<th>Specific activity (count/min/µmole) C-8</th>
<th>Ratio of specific activities C-2:C-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected</td>
<td>L-Serine-3-C^14</td>
<td>0.25</td>
<td>59.4</td>
<td>55.0</td>
<td>1.07</td>
</tr>
<tr>
<td>Uninfected</td>
<td>L-Serine-3-C^14</td>
<td>0.25</td>
<td>9.4</td>
<td>9.1</td>
<td>1.03</td>
</tr>
<tr>
<td>Infected</td>
<td>Glycine-2-C^14</td>
<td>1.0</td>
<td>5.6</td>
<td>5.1</td>
<td>1.10</td>
</tr>
<tr>
<td>Uninfected</td>
<td>Glycine-2-C^14</td>
<td>1.0</td>
<td>3.3</td>
<td>3.0</td>
<td>1.10</td>
</tr>
<tr>
<td>Infected</td>
<td>Formate-C^14</td>
<td>1.0</td>
<td>24.6</td>
<td>292.0</td>
<td>0.082</td>
</tr>
<tr>
<td>Uninfected</td>
<td>Formate-C^14</td>
<td>1.0</td>
<td>8.3</td>
<td>90.4</td>
<td>0.092</td>
</tr>
</tbody>
</table>

* Glycine-2-C^14 (specific activity 2 × 10⁴ count/min/µmole), serine-3-C^14 (specific activity 1 × 10⁴ count/min/µmole), and formate-C^14 (specific activity 1 × 10⁴ count/min/µmole) were all added 10 min after phage infection. Incubation was continued for an additional 60 min.
siderably greater fraction of the one-carbon units entering the 8 position when the cell is infected. This marked effect is not seen with glycine-2-C¹⁴. These results generally confirm the data presented in Table 1. The magnification observed in the utilization of all three precursors when the cells are infected is very likely a result of the shortened time of incubation, 60 min compared to the 120 min in the previous experiment.

**DISCUSSION**

The studies reported in the present paper on the role of one-carbon metabolites in the biosynthesis of DNA-purines and pyrimidines in growing and bacteriophage-infected cells of *E. coli* resulted in several unexpected observations. Perhaps the most surprising is the finding that neither formate nor formaldehyde are incorporated into the DNA-pyrimidines though they serve as effective precursors of the purines. This pattern is not changed when the cells are infected with bacteriophage T2. This inertness of formate and formaldehyde in pyrimidine biosynthesis is in marked contrast to observations in mammalian systems and even in cell-free bacterial extracts. In mammalian cell suspensions, as well as in the intact animal, formate is incorporated into thymine (Elwyn and Sprinison, 1954; Herrmann, Fairly, and Byerrum, 1955; Kit, 1957). Similarly, in extracts prepared from T6 bacteriophage-infected cells, formaldehyde is incorporated into both thymine and hydroxymethylcytosine (Flaks and Cohen, 1957). The lack of incorporation of formaldehyde and formate in the whole cell has its cause most likely at a different site than the thymidylate or hydroxymethyldeoxythymidylate synthetase enzyme level. One interpretation of these data is that there are a number of one-carbon precursor pools in an intact actively growing and metabolizing bacterial cell. The observation that a single one-carbon precursor may serve as a source of one-carbon units for the number 8 carbon of the purine, but not of the methyl group of thymine, further indicates that the one-carbon precursor pools are not necessarily in equilibrium. As can be seen in Fig. 1, the enzymatic reaction which may account for the differential effect of formate-C¹⁴ on purine and pyrimidine biosynthesis is the reaction between formyl and hydroxymethyltetrahydrofolic acid. In mammalian systems this reaction is reversible with an equilibrium constant close to 1 ( Hatefi et al., 1957). In the growing bacterial cell, however, this reaction does not appear to operate reversibly, the equilibrium being strongly in favor of the oxidized (formyl) form.

The further observation that formate is incorporated only into position 8 of the purine ring, and not into position 2 presumably supplied from the same one-carbon pool, leads to a different interpretation: the exclusiveness of one-carbon pools for biosynthesis.

Since both of these positions have as their immediate precursor formyl tetrahydrofolic acid (Warren and Buchanan, 1957; Warren, Flaks, and Buchanan, 1957), the explanation for the incongruous behavior of formate probably has its explanation on a level of organization higher than the enzymes involved in the biosynthesis of these two positions. This observation, previously noted in *E. coli* by Koch (1955) and in *Pseudomonas* by Revel and Magasanik (1958), again can be best interpreted on the basis that in bacteria there are a number of one-carbon precursor pools not in equilibrium with each other. Therefore, the one-carbon moieties of serine and glycine enter a precursor pool which contributes equally to the synthesis of the 2 and 8 positions. Formate enters a different pool which is sampled only for position 8. Since there is no obvious chemical distinction between the carbon atoms in the 2 and 8 position, or in the immediate precursor of these two positions, it appears that there are chemically identical precursors which are incorporated into only one of two identical positions. The fact that glycine-2-C¹⁴ and serine-3-C¹⁴ do give rise to equal labeling in the two positions precludes the possibility that there was equal incorporation of the formate into the 2 and 8 positions and subsequent exchange of carbon 2 with an unlabeled one-carbon unit. Whether this apparent compartmentalization of one-carbon precursors and biosynthetic products of one-carbon units represents a structural feature or an undefined subtle chemical barrier is not clear from the present data. Previous work by Koch (1955) indicates that certain one-carbon precursors display different incorporation patterns dependent on their concentration in the external medium. Such patterns are not limited to formate. He observed that in growing cells of *E. coli* glycine-2-C¹⁴ at a concentration of 0.1 µmole per ml labeled position 8 exclusively, whereas at 10 µmoles per ml the isotope was incorporated...
equally into positions 2 and 8. In the present investigation, only formate was observed to display an anomalous behavior as a one-carbon precursor. It is not incorporated into the 2 position of the DNA-purines, DNA-pyrimidines, thymine, or hydroxymethylcytosine. At first sight there is no obvious relationship between the inability to become incorporated as a reduced methyl group, and into a particular position as an oxidized carbon moiety. However, this observation of the apparent compartmentalization of precursors and their end products could explain the inability of formate to label the pyrimidines.

The observation that serine is a much more effective precursor of one-carbon units in the bacteriophage-infected cell may be a reflection of the increased synthesis of an enzyme(s) resulting from bacteriophage infection. Previous studies have shown increased synthesis of a variety of enzymes upon bacteriophage infection (Flaks and Cohen, 1957; Bessman, 1959), as well as the synthesis of some which cannot be demonstrated in the uninfected cell (Flaks and Cohen, 1959; Kornberg et al., 1959; Somerville et al., 1959). The increase in the utilization of serine as a one-carbon donor, in comparison to glycine, upon infection indicates that any modification in one-carbon metabolism may be closer to the specific metabolism of serine than to the common pathway of one-carbon metabolism of serine and glycine.

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LITERATURE CITED


Kirby, K. S. 1955. Some new solvent systems for

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