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^{18}O Studies on the Oxidative and Nonoxidative Pentose Phosphate Pathways in Wild-Type and Mutant *Escherichia coli* Cells†

Richard Johnson,‡ Alvin I. Krasna,* and David Rittenberg§

ABSTRACT: The experiments described were carried out in order to determine the role of the oxidative and the nonoxidative pathways for the synthesis of pentose phosphate in *Escherichia coli*. The experimental approach involved growth of the organism on [1-^{18}O]glucose or [2-^{18}O]fructose and isolation of the ribonucleosides. Mass spectrometry of the nucleosides gave the ^{18}O content of each of the oxygen atoms in the ribose moiety. Incubations were carried out with wild-type *E. coli* K10 which utilizes both pathways, a mutant lacking transketolase (*tkt*$^-$) which utilizes the oxidative pathway exclusively, and a mutant lacking glucose-6-phosphate dehydrogenase (*zwf*$^-$) which utilizes the nonoxidative pathway exclusively. Growth of the *tkt*$^-$ mutant on [1-^{18}O]-glucose yielded unlabeled pentose phosphate showing that there was no equilibration between the C-1 oxygen and C-6 oxygen of glucose *via* the trioses. Growth of the *zwf*$^-$ mutant on [1-^{18}O]glucose yielded pentose phosphate containing 48% of the ^{18}O in the 5′-oxygen atom, suggesting that the nonoxidative pathway in this organism consists mainly of a single transketolase reaction. From growth of this mutant on [2-^{18}O]fructose it was concluded that 35% of the label of the intermediate [2-^{18}O]dihydroxyacetone phosphate exchanged with water during the reaction. The results of growth of wild-type *E. coli* K10 on [1-^{18}O]glucose and [2-^{18}O]fructose were analyzed with respect to two models; one which proposes that both pathways operate simultaneously for the synthesis of pentose phosphate, and a second model which proposes that only the oxidative pathway is utilized for the synthesis of pentose phosphate. The contributions of both pathways were also studied in *E. coli* K12 W6, which is relaxed in RNA synthesis, both during growth as well as during starvation for methionine when the cells synthesize RNA but not protein or DNA.

The synthesis of pentose phosphate from glucose supplies the ribose and deoxyribose necessary for RNA and DNA synthesis as well as the NADPH required for various biosynthetic processes. Ribose phosphate can be formed from glucose by two routes, the oxidative pathway and the nonoxidative pathway. In the oxidative pathway, glucose 6-phosphate is oxidized by $NADP^+$ to gluconate 6-phosphate and the latter is oxidized by $NADP^+$ to CO_2 and ribulose 5-phosphate. In this conversion 1 mol of hexose is converted to 1 mol of pentose phosphate, 1 mol of CO_2, and 2 mol of NADPH. This pathway is essentially irreversible.

The nonoxidative pathway branches from the glycolytic pathway at fructose 6-phosphate and glyceraldehyde 3-phosphate. The action of transketolase on these two substrates yields xylulose 5-phosphate and erythrose 4-phosphate. If erythrose 4-phosphate reacts with another molecule of fructose 6-phosphate in a reaction catalyzed by transaldolase, sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate are produced which, if acted upon by transketolase, give rise to xylulose 5-phosphate and ribose 5-phosphate. In this series of reactions, 2.5 mol of hexose phosphate gives rise to 3 mol of pentose phosphate. These reactions are shown in Figure 3.

Though there are other pathways which could give rise to pentose phosphate, it has been shown (Caprioli and Rittenberg, 1969) that, quantitatively, they do not play an important role in ribose phosphate synthesis in *E. coli*. Since both the oxidative and nonoxidative pathways can operate simultaneously, there has been much interest in evaluating the contribution of each pathway to ribose phosphate synthesis. A major function of the oxidative pathway, in addition to supplying pentose phosphate, is the production of NADPH.

The contribution of the oxidative pathway to pentose phosphate synthesis has been measured by the radiorespirometric method (Wang and Krackov, 1962) in which the ratio of the radioactivity of $^{14}CO_2$ produced by cells grown on [1-^{14}C]glucose to that of cells grown on [6-^{14}C]glucose is mea-

† From the Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York, New York 10032. *Received June 5, 1972.* This work was supported by a grant from the National Science Foundation (NSF GB-8559).

‡ Predoctoral trainee of the National Institutes of Health (Training Grant No. 5T01 GM-00255). This work is from a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the Faculty of Pure Science, Columbia University. Present address: Department of Medicine, Albert Einstein College of Medicine of Yeshiva University, Bronx, N. Y. 10461.

§ Deceased Jan 24, 1970. This investigation was initiated by the late Professor David Rittenberg and was begun in his laboratory. His insight and enthusiasm laid the scientific approach for the work reported here and served as an inspiration to all his students and colleagues.

sured. This method cannot detect the extent of contribution of the nonoxidative pathway. The conversion of ^{14}C-labeled substrates to the pentose phosphate moieties of DNA and RNA has been used to estimate the relative contribution of both pathways (Bagatell *et al.*, 1959; Sable and Cassisi, 1962; Wood *et al.*, 1963). It was shown (Szynkiewicz *et al.*, 1961; Model and Rittenberg, 1967) that the activities of the two pathways varied considerably with the conditions of growth of the organism.

The ^{14}C data from *E. coli* and other organisms were interpreted by some laboratories (Horecker and Mehler, 1955; Horecker, 1965; Sable, 1966; Caprioli and Rittenberg, 1969) to suggest that the oxidative and nonoxidative pathways operate simultaneously to synthesize pentose phosphate for nucleic acid biosynthesis and estimates were made of the contribution of each pathway. A different view of the ^{14}C data was presented by Wood and Katz (Wood *et al.*, 1963; Katz and Rognstad, 1967). They calculated the rates of the different pathways taking into account the reversibility of the individual reactions which could exchange the ^{14}C into different positions of pentose phosphate. They concluded that the formation of pentose phosphate by the oxidative pathway greatly exceeds its utilization for synthesis and that this pathway is the only one utilized for net synthesis of pentose phosphate with no contribution from the nonoxidative pathway. They suggested that the bulk of pentose phosphate is recycled to fructose phosphate in the nonoxidative pathway with the net flow being from pentose phosphate to fructose phosphate.

Another approach to the evaluation of the relative contributions of both pathways involves the use of ^{18}O-labeled hexoses. When either [1-^{18}O]glucose or [6-^{18}O]glucose passes through the glycolytic pathway 2-[3-^{18}O]phosphoglyceric acid is formed which loses the ^{18}O on dehydration to phosphoenolpyruvate, and oxidation of pyruvic acid yields unlabeled CO_2. When [1-^{18}O]glucose is converted to pentose phosphate by the oxidative pathway, the CO_2 evolved contains ^{18}O, while with [6-^{18}O]glucose the CO_2 is unlabeled. Rittenberg and Ponticorvo (1962) measured the ^{18}O content of the CO_2 produced when *E. coli* W was grown on [1-^{18}O]glucose and [6-^{18}O]glucose. Only the [1-^{18}O]glucose gave rise to labeled CO_2, and it was estimated that between 10 and 30% of the glucose was metabolized by the oxidative pathway. Model and Rittenberg (1967) measured the yields of ^{18}O-labeled CO_2 produced from different ^{18}O-labeled sugars in *E. coli* and estimated that 24% of the glucose was metabolized by the oxidative pathway. In both of these studies, it was shown that there was very little equilibration between [1-^{18}O]glucose and [6-^{18}O]glucose *via* the trioses which could take place theoretically by the action of aldolase or transketolase.

The contribution of the nonoxidative pathway cannot be determined from the ^{18}O content of the evolved CO_2. When *E. coli* was grown on [1-^{18}O]glucose, Nicholson *et al.* (1965) showed that the ribose portion of the RNA contained ^{18}O. In order to study the relative contributions of the oxidative and nonoxidative pathways to ribose phosphate and deoxyribose phosphate synthesis, Caprioli and Rittenberg (1968a,b, 1969) isolated the RNA and DNA of *E. coli* B grown on [1-^{18}O]-, [2-^{18}O]-, and [6-^{18}O]glucose and [2-^{18}O]fructose as sole carbon sources. They degraded the nucleic acids to the nucleosides which were analyzed by mass spectrometry to determine the distribution of ^{18}O in each of the oxygen atoms of the pentose. They concluded that both pathways operated simultaneously and that the nonoxidative pathway consisted of a single reaction catalyzed by transketolase which accounted for 70% of the pentose phosphate synthesized during log phase growth while the oxidative pathway accounted for only 30% of pentose phosphate synthesis.

In the experiments of Caprioli and Rittenberg, both pathways were operating simultaneously in the growing organism and certain assumptions had to be made regarding the rate of nonenzymatic exchange of [2-^{18}O]dihydroxyacetone phosphate with water, the mechanism of action of the aldolase of *E. coli*, and the equivalence of glucose and fructose as carbon sources. In this article, the method of Caprioli and Rittenberg was used to study the oxidative and nonoxidative pathways individually. This was accomplished by the use of mutants of *E. coli* which lack enzymes for each pathway. One mutant lacked transketolase and synthesized ribose phosphate solely by the oxidative pathway. A second mutant lacked glucose-6-phosphate dehydrogenase and made ribose phosphate only by the nonoxidative pathway. The use of these mutants provided values for each pathway when operating separately and could be used to calculate rates of exchange and randomization reactions. The parent strain, which has both pathways, was then studied in order to estimate the relative contributions of each pathway when operating simultaneously.

Experiments were also carried out with a mutant of *E. coli* which had relaxed control of RNA synthesis and required methionine for growth. The contributions of the oxidative and nonoxidative pathways for ribose phosphate synthesis were studied during normal growth on methionine and during starvation for methionine when the organism continued to synthesize RNA in the absence of growth.

Experimental Section

$H_2{}^{18}O$ (>90 atom % excess ^{18}O) was obtained from the Weizmann Institute of Science. All other chemicals were obtained either from the Sigma Chemical Co. or from the J. T. Baker Chemical Co.

Preparation of Labeled Substrates. [1-^{18}O]Glucose was prepared by exchanging the anomeric oxygen in D-glucose with $H_2{}^{18}O$ (Rittenberg *et al.*, 1961). [2-^{18}O]Fructose was prepared in a similar manner. The ^{18}O content of the sugars was determined by pyrolysis to CO_2 (Rittenberg and Ponticorvo, 1956) and analysis of the $C^{18}O_2$ in the mass spectrometer.

E. coli Strains. E. coli K12 W6 was obtained from Dr. P. R. Srinivasan of this department. This mutant (*bio*$^-$, *met*$^-$, *rel*$^-$) required biotin and methionine for growth and was relaxed for RNA synthesis (Borek *et al.*, 1955). *E. coli* K10 DF1000, DF2001 (Fraenkel, 1968a), and BJ502 (Josephson and Fraenkel, 1969) were kindly supplied by Dr. D. G. Fraenkel of the Department of Microbiology and Molecular Genetics, Harvard Medical School. DF1000 is the wild-type strain of *E. coli* K10 from which the two mutants were isolated and is an Hfr C of *E. coli* K12. It is wild type in sugar metabolism, prototrophic (Fraenkel and Levisohn, 1967), and RC relaxed. Strain DF2001 lacks glucose-6-phosphate dehydrogenase (*zwf*$^-$) and strain BJ502 lacks transketolase (*tkt*$^-$).

Growth of Organisms. The growth medium consisted of M9 salts medium (Clowes and Hayes, 1968) and either glucose or fructose as the carbon source. The sugar was added at the same time as the inoculum to give a final sugar concentration of 0.1% (w/v). For growth of *E. coli* K12 W6, the medium was supplemented with 0.02 g/l. of L-methionine and 0.012 mg/l. of biotin. For growth of *E. coli* K10 BJ502 (*tkt*$^-$), which cannot synthesize the aromatic amino acids, the medium was supplemented with 25 mg/l. of shikimic acid.

A stock culture was grown twice on normal hexose and

TABLE I: ^{18}O in Ribose Moiety from Cells Grown on [1-^{18}O]Glucose and [2-^{18}O]Fructose.

Strain	Substrate	% ^{18}O in Labeled O Atom[a]	^{18}O in Ribose Oxygen Atoms (% Excess)[b]			
			2',3',4',5'	2',3',4'	2'	4'
E. coli K10 BJ502 (*tkt*$^-$)	[1-^{18}O]Glucose	10.1	0	0	0	0
E. coli K10 DF2001 (*zwf*$^-$)	[1-^{18}O]Glucose	10.1	4.8	0	0	0.2
	[2-^{18}O]Fructose	52.4	21.3	20.4	4.7	16.5
E. coli K10 DF1000 (wild)	[1-^{18}O]Glucose	39.5	10.3	0	0	0.8
	[2-^{18}O]Fructose	39.5	13.5	14.0	3.8	9.5
E. coli K12 W6 (wild, relaxed)	[1-^{18}O]Glucose	67.4	17.6	1.7	0.4	0.6
during log phase growth	[2-^{18}O]Fructose	52.6	21.1	20.7	5.6	15.1
E. coli K12 W6	[1-^{18}O]Glucose	65.7	8.4	0	0	0
during starvation for methionine	[2-^{18}O]Fructose	57.8	7.3	7.3	1.8	5.5

[a] Average ^{18}O content of substrate corrected for exchange as described in text. [b] Average of values obtained from adenosine and guanosine analyzed in triplicate. The values were obtained from a single incubation of each strain with the appropriate hexose and are accurate to ±10%.

finally once on labeled hexose. The latter culture was used for the inoculation (1%) of large cultures containing the labeled hexose. Growth began immediately and was continued for 5 hr at 37° to a Klett density of 100 (600 nm), which corresponded to 10^9 cells/ml (midexponential phase). The cells were harvested by centrifugation, washed with 0.8% saline, and stored at −20°.

In the starvation experiments, *E. coli* K12 W6 was first grown on unlabeled hexose in the presence of methionine and biotin. The cells were harvested, washed, and then resuspended in minimal medium lacking methionine which contained ^{18}O-labeled hexose. The cells were shaken at 37° for 2 hr, during which time the Klett density did not change but the RNA content increased as measured by the orcinol procedure.

Isolation and Analysis of Nucleosides. DNA and RNA were isolated from the harvested cells and degraded to the nucleosides which were separated and analyzed for ^{18}O distribution by fragmentation mass spectrometry (Caprioli and Rittenberg, 1969). Only adenosine and guanosine were routinely analyzed since it has been shown (Caprioli and Rittenberg, 1969) that the ribose and deoxyribose moieties have the same labeling pattern.

Results

The oxygen atoms at C-1 of glucose and at C-2 of fructose exchange slowly with water, and decrease the ^{18}O content of the sugar added as carbon source. The rate constant for the exchange of oxygen at C-1 of glucose at pH 7.0 is 0.0225 hr^{-1} (Rittenberg and Graff, 1958), and the rate of exchange of oxygen at C-2 of fructose is 0.01409 hr^{-1} (Model *et al.*, 1968). These exchange rates were used to calculate the average ^{18}O content of the added sugar when the culture contained 50% of the number of cells present at the completion of the incubation. In general, about 10% of the initial ^{18}O content was lost during incubation, and these corrected values are given in the tables and are used in all calculations. For example, in the first two entries in Table I, the glucose contained 11.8 atom % excess ^{18}O and the growth time was 7.5 hr. The average concentration of ^{18}O in the glucose was calculated to be 10.1 atom % excess.

Nucleosides from E. coli K10 BJ502 (tkt$^-$*).* In this organism which lacks transketolase, all of the ribose phosphate for nucleic acid synthesis must be made by oxidative pathway. When grown on [1-^{18}O]glucose, ^{18}O would be lost as CO_2 and the pentose phosphate should be unlabeled. The results (Table I) show that the nucleosides contained no excess ^{18}O. To confirm this finding, uridine was also isolated and analyzed for total ^{18}O. It contained only 0.12 atom % excess ^{18}O.

Nucleosides from E. coli K10 DF2001 (zwf$^-$*).* This organism, which lacks glucose-6-phosphate dehydrogenase, must make all of its ribose phosphate by the nonoxidative pathway. When grown on [1-^{18}O]glucose, all the ^{18}O was found in the 5' position of the nucleosides (Table I). Combustion of the uridine to CO_2 showed that it contained 4.6 atom % excess ^{18}O. When grown on [2-^{18}O]fructose, the nucleosides contained ^{18}O only in the 2' and 4' positions (Table I) with about 80% of the label in the 4' position.

Nucleosides from E. coli K10 DF1000 (Wild Type). In this wild strain, from which the two mutants were derived, both pathways could contribute to ribose phosphate synthesis, and the ^{18}O content of the nucleosides could be used to evaluate the relative contributions of both pathways. Growth of the wild-type cells on [1-^{18}O]glucose gave nucleosides containing ^{18}O predominantly in the 5' position (Table I). Analysis of the uridine by combustion indicated that it contained 11.2 atom % excess ^{18}O. Since nucleosides made by the oxidative pathway from [1-^{18}O]glucose contain no ^{18}O, the amount of ^{18}O in the 5' position should be an indication of the contribution of the nonoxidative pathway or the extent of isotopic equilibration of the pentose phosphates *via* the transketolase reaction. The wild strain was also grown on [2-^{18}O]fructose (Table I). The ^{18}O was found only in the 2' and 4' positions with about 70% in the 4' position.

Nucleosides from E. coli K12 W6. When grown in log phase in the presence of methionine and biotin on [1-^{18}O]glucose or [2-^{18}O]fructose, the labeling pattern in the nucleosides (Table I) was similar, though not identical, with that found with the *E. coli* K10, wild type.

In the absence of methionine, RNA synthesis continues although DNA and protein synthesis stop. Cells grown on normal glucose were harvested and then resuspended in minimal medium without methionine which contained [1-^{18}O]-glucose. The cells were shaken at 37° for 2 hr during which time the RNA concentration increased by 90%. Thus, 47.4% of the RNA present in the cells was made during the period

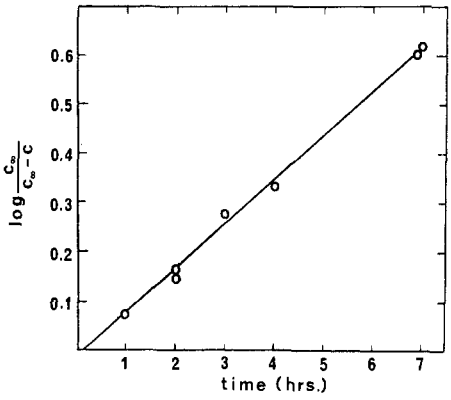

FIGURE 1: Exchange rate of ribose 5-phosphate with $H_2{}^{18}O$. The disodium salt of ribose 5-phosphate was dissolved in $H_2{}^{18}O$ (40% ^{18}O) to a concentration of 10% (w/v) and incubated at 25°. Samples were taken at the indicated time intervals, precipitated with ethanol, dried over P_2O_5 *in vacuo*, and analyzed for total ^{18}O by combustion. C is the atom % excess ^{18}O at the indicated time and C_∞ is the ^{18}O content at equilibrium (t_∞ = 48 hr).

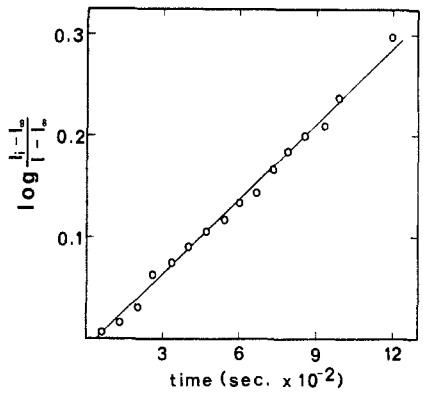

FIGURE 2: Exchange rate of ribulose 5-phosphate with $D_2{}^{18}O$. The exchange was studied by measuring the decrease in intensity of the carbonyl stretching frequency at 5.795 μ (C=^{16}O) as ^{18}O replaced ^{16}O at position 2 of the sugar. The carbonyl frequency in ^{18}O at equilibrium was at 5.903 μ. The peaks at all other wavelengths were identical. The infrared spectrum was measured on a 10% (w/v) solution of ribulose 5-phosphate in $D_2{}^{18}O$ in a calcium fluoride cell. I is the intensity of the peak at 5.795 μ at the indicated time, I_i the intensity at the beginning of the reaction, and I_∞ the intensity at equilibrium (24 hr).

of starvation. When the ribonucleosides were isolated and the amount of label measured, it was found to be present predominantly in the 5′ position (Table I). The values listed have not been corrected for the unlabeled RNA present before incubation with the labeled glucose. In this experiment deoxyadenosine was also analyzed. It was found to contain no excess ^{18}O, as expected, since DNA synthesis did not occur during the starvation period.

E. coli K12 W6 was also incubated in the absence of methionine with [2-^{18}O]fructose as the carbon source during which time the RNA concentration increased 46%, *i.e.*, 31.5% of the RNA present in the cell was made during the period of starvation. The ribonucleosides were isolated and found to contain label only in the 2′ and 4′ positions (Table I). These values have not been corrected for the unlabeled RNA present.

Exchange of Ribose 5-Phosphate and Ribulose 5-Phosphate with $H_2{}^{18}O$. In the interpretation of the ^{18}O data (see below), one must consider the loss of ^{18}O from intermediates by nonenzymatic exchange with water. Accordingly, the loss of ^{18}O from pentoses by exchange at O-1 of ribose 5-phosphate and O-2 of ribulose 5-phosphate was measured.

Ribose 5-phosphate was dissolved in $H_2{}^{18}O$ and samples of the sugar were removed at appropriate time intervals and analyzed for ^{18}O. The data for the incorporation of ^{18}O are given in Figure 1, from which a half-life of 200 ± 20 min was calculated.

The ribulose 5-phosphate exchange with $D_2{}^{18}O$ was studied by measuring the decrease in intensity of the carbonyl stretching frequency at 5.795 μ in the infrared spectrum. Figure 2 shows the decrease in the C=^{16}O peak, from which a half-life of 20 min was calculated.

Discussion

Before comparing the results with the different strains of *E. coli* grown on [1-^{18}O]glucose and [2-^{18}O]fructose, the values obtained must be normalized to account for the fact that each experiment was done with a different concentration of ^{18}O in the hexose used as carbon source. These calculations are described in footnote *a* of Table II. Table II lists the corrected, normalized ^{18}O content in each of the pentose atoms of the nucleosides in a form in which they can be directly compared.

In the discussion which follows, the ^{18}O data will be analyzed in terms of two models proposed for the role of the oxidative and nonoxidative pathways for pentose phosphate synthesis. Model I proposes that both pathways operate simultaneously for the synthesis of pentose phosphate for DNA and RNA. This was first proposed by Horecker (Horecker and Mehler, 1955; Horecker, 1965; Sable, 1966; Caprioli and Rittenberg, 1969). Model II proposed by Wood and Katz (Wood *et al.*, 1963; Katz and Rognstad, 1967) suggests that the pentose phosphate is synthesized solely by the oxidative pathway while the nonoxidative pathway serves to recycle pentose phosphate to fructose phosphate. It suggests that the reversibility of the individual reactions of the nonoxidative pathway could explain the distribution of isotope in pentose phosphate found on incubation of cells with labeled hexose. Of course, one can also consider a third model which combines features of the first two; *i.e.*, in addition to synthesis of pentose phosphate *via* the oxidative pathway, some pentose phosphate could be synthesized *via* the nonoxidative pathway with isotopic equilibration by reversible reactions in this pathway.

E. coli K10 BJ502 (tkt$^-$). Glucose metabolized exclusively *via* the oxidative pathway would be converted to ribulose 5-phosphate through the loss of C-1 as carbon dioxide. Positions C-2 through C-6 of glucose would become positions C-1 through C-5 of pentose phosphate and [1-^{18}O]glucose would not give rise to labeled pentose phosphate. Since strain BJ502 lacks transketolase, it can only make ribose phosphate by the oxidative pathway and the nucleosides should not contain ^{18}O when the cells are grown on [1-^{18}O]glucose. This is the case as shown by the normalized data in Table II.

These results rule out any equilibration of the C-1 oxygen and C-6 oxygen atoms of glucose *via* the triose phosphates. If [1-^{18}O]glucose were metabolized *via* the glycolytic pathway to [3-^{18}O]dihydroxyacetone phosphate (from C-1, -2, and -3) and glyceraldehyde phosphate (from C-4, -5, and -6), isomerization of these triose phosphates would give [3-^{18}O]glyceraldehyde phosphate. Condensation of this labeled glyceraldehyde phosphate with dihydroxyacetone phosphate and reversal of glycolysis would give rise to [6-^{18}O]glucose. The latter hexose, *via* the oxidative pathway, would give ribose

TABLE II: Corrected, Normalized ^{18}O Content in Oxygen Atoms of Ribose Moiety.[a]

Carbon Source	Position of O Atom	Atom % Excess ^{18}O				
		E. coli K10 BJ502 (*tkt*⁻)	*E. coli* K10 DF2001 (*zwf*⁻)	*E. coli* K10 DF1000 (Wild)	*E. coli* K12 W6 (Wild, Relaxed) during Growth	*E. coli* K12 W6 (Wild, Relaxed) during Starvation
[1-^{18}O]Glucose	2′	0	0	0	0.6	0
	3′	0	0	0	1.1	0
	4′	0	2.0	2.0	0.9	0
	5′	0	48.0	26.0	23.6	26.7
[2-^{18}O]Fructose	2′		9.0	9.7	10.6	10.1
	3′		0	1.8	0	0
	4′		31.5	24.0 (18.3)[b]	28.7 (21.8)[b]	30.6 (23.2)[b]
	5′		0	0	0.7	0

[a] The values listed in this table are normalized to 100% ^{18}O in the hexose to account for the fact that each experiment in Table I was done with a different concentration of ^{18}O in the hexose used as carbon source. The normalized values were obtained by dividing the ^{18}O content in each position of the nucleosides (listed in Table I) by the ^{18}O content of the glucose or fructose used as carbon source and multiplying by 100. These calculated values are those that would have been found if the ^{18}O content of the hexose in each experiment had been 100% in the position indicated. In the case of *E. coli* K12 W6, starved for methionine, the ^{18}O content in the nucleosides is corrected for the unlabeled RNA present before the incubation was started. This was done by dividing the ^{18}O values for this experiment (listed in Table I) by the fraction of RNA synthesized during starvation in the presence of [^{18}O]hexose (47.4% for [1-^{18}O]glucose and 31.5% for [2-^{18}O]fructose). These two corrections have been applied to the data in Table I and the corrected, normalized values are listed in this table. [b] These values are the ^{18}O content in the 4′-oxygen atom calculated for growth on [2-^{18}O]glucose. They were calculated as described in the text.

phosphate labeled with ^{18}O in the 5′ position. The finding that the nucleosides in this experiment contained no ^{18}O shows that the redistribution of ^{18}O from C-1 to C-6 of glucose does not take place to any significant extent. The same conclusion was reached by Rittenberg and Ponticorvo (1962) and Model and Rittenberg (1967) on the basis of the $C^{18}O_2$ production from [1-^{18}O]glucose and [6-^{18}O]glucose in wild-type *E. coli* cells. Sable and coworkers (Sable and Cassisi, 1962; Szynkiewicz *et al.*, 1961) arrived at the same conclusion from a study of the distribution of ^{14}C in glucose isolated from the glycogen of wild-type *E. coli* which was grown on [2-^{14}C]glucose. In this organism, where there is no nonoxidative pathway, one cannot make any conclusions regarding model I or model II.

Josephson and Fraenkel (1969) reported that mutant BJ502 was leaky and could metabolize glucose to a small extent *via* the nonoxidative pathway. We found no growth at all on minimal media (without added shikimic acid) even after 48 hr and our ^{18}O data indicate no significant synthesis of ribose phosphate *via* the nonoxidative pathway in this mutant. However, since [1-^{18}O]glucose metabolized by this pathway loses half of its ^{18}O (see below), it is conceivable that if this pathway was used to a very small extent it might not be detectable in these experiments.

E. coli K10 DF2001 (*zwf*⁻). This organism permits us to evaluate the ^{18}O distribution when the nonoxidative pathway is used exclusively. When grown on [1-^{18}O]glucose, the nucleosides contained ^{18}O predominantly in the 5′-oxygen atom and had a normalized ^{18}O content of 48 atom % excess (Table II). From this value we can deduce the reactions in the nonoxidative pathway (see Figure 3).

The cleavage of fructose 1,6-diphosphate by aldolase produces unlabeled glyceraldehyde 3-phosphate and [3-^{18}O]-dihydroxyacetone phosphate. These two triose phosphates are in isotopic equilibrium due to the reversible action of triosephosphate isomerase (Figure 3). The [3-^{18}O]glyceraldehyde 3-phosphate would contain one-half the initial ^{18}O concentration of the hexose. Transfer of an active glycolaldehyde group from a 2-keto sugar like fructose 6-phosphate, by the enzyme transketolase (reaction A, Figure 3), would give rise to a pentose phosphate containing 50 atom % ^{18}O in the 5′ position. The ^{18}O in the 1′ position is lost in the glycosidic bond formation. This predicted value of 50% is very close to the 48% actually found (Table II).

The nonoxidative pathway is generally considered to consist additionally of a transaldolase reaction (reaction B, Figure 3) and a second transketolase reaction (reaction C). This series of reactions (A, B, and C) produces 3 pentose phosphate molecules from 2.5 hexose molecules. It is clear that the finding of 48 atom % excess ^{18}O in the 5′ position of the ribose phosphate rules out this series of reactions as being involved in ribose phosphate synthesis in the *zwf*⁻ mutant. Of the two pentose phosphates produced by reactions B and C, one (Xu5P) would contain 50 atom % excess in the 5′ position and the other (R5P) would be unlabeled. These two pentose phosphates, together with the labeled pentose phosphate produced in reaction A, would give ribose with an average ^{18}O content of 33 atom % excess in the 5′ position. This value is much lower than actually found and indicates that, in the *zwf*⁻ mutant, the nonoxidative pathway consists mainly of a single transketolase reaction (reaction A). Reactions B and C could operate to the extent of 5–10% of reaction A and still be consistent with our finding of 48 atom % excess ^{18}O in the 5′ position. If the nonoxidative pathway consists mainly of reaction A, erythrose 4-phosphate would be expected to accumulate and would be used for aromatic biosynthesis. As will be pointed out below, there is reason to believe that fructose 6-phosphate does not function as the major trans-

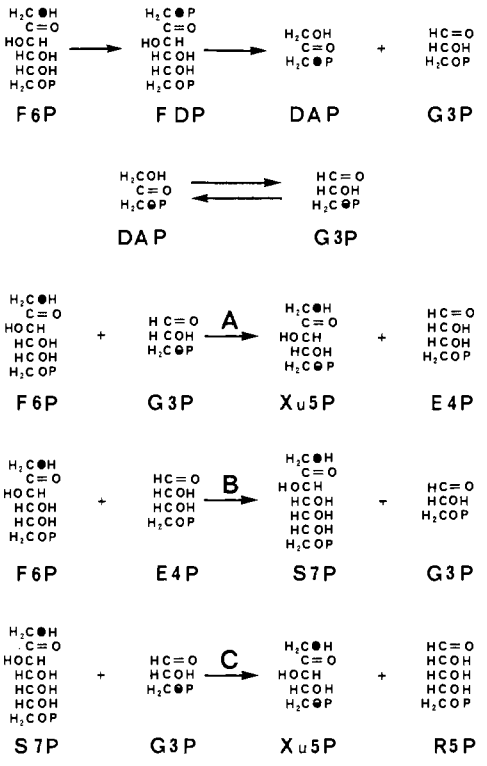

FIGURE 3: Reactions of the nonoxidative pathway. FDP is fructose 1,6-diphosphate, F6P is fructose 6-phosphate, DAP is dihydroxyacetone phosphate, G3P is glyceraldehyde 3-phosphate, Xu5P is xylulose 5-phosphate, E4P is erythrose 4-phosphate, S7P is sedoheptulose 7-phosphate, and R5P is ribose 5-phosphate. In this figure the labeling pattern with [1-^{18}O]glucose as the carbon source is illustrated. Filled circles indicate 100 atom % excess ^{18}O and half-filled circles indicate 50 atom % excess ^{18}O.

ketolase donor and that other donors may function which would not produce erythrose 4-phosphate.

Recently, Kupor and Fraenkel (1972) reported that in this *zwf*$^-$ mutant the specific activity of the ribose produced from [1-^{14}C]glucose was 1.32 times that of the glucose. This specific activity cannot be explained if the nonoxidative pathway consisted of reactions A, B, and C. However, if the pathway consists only of reaction A, this value is quite reasonable.

To confirm the results obtained with [1-^{18}O]glucose, a similar experiment was conducted with [2-^{18}O]fructose as the sole carbon source. The expected pattern of labeling can be deduced from the reactions illustrated in Figure 3, in which the initial label would be at the 2 position of fructose 6-phosphate.

With [2-^{18}O]fructose as the sole carbon source, the nucleosides contained ^{18}O only in the 2′ (9.0%) and 4′ (31.5%) positions. If the nonoxidative pathway consisted of reactions A, B, and C, one would have expected to find 33 atom % excess ^{18}O in the 4′ position which is very close to the 31.5% actually found. However, the results just described with [1-^{18}O]glucose in this mutant rule out this possibility and suggest that the nonoxidative pathway consists mainly of a single transketolase reaction (reaction A). In this case it would be expected that if the transketolase reaction uses fructose 6-phosphate as the active glycolaldehyde donor, the resulting pentose phosphate should contain 100 atom % excess ^{18}O in the 2′ position and 50 atom % excess ^{18}O in the 4′ position (actually 48%, the same amount as in the 5′ position when the cells are grown on [1-^{18}O]glucose). The lower value found (31.5%) probably reflects the loss of ^{18}O from [2-^{18}O]dihydroxyacetone phosphate by exchange with water. The half-life for the exchange of [2-^{18}O]dihydroxyacetone phosphate with water is less than 1 min (Model *et al.*, 1968) and one would expect loss of ^{18}O from this position. The fact that 65% of the label is still retained in the pentose phosphate suggests that under conditions of log phase growth, 35% of the ^{18}O is lost by exchange. Caprioli and Rittenberg (1969) estimated this exchange in *E. coli* B to be 40%.

The loss of ^{18}O in these reactions is probably not enzymatically catalyzed. The mechanism of the triosephosphate isomerase reaction has been shown (Rieder and Rose, 1959) to involve an enzyme-bound enolate anion which would not lead to exchange of ^{18}O with water. The data also suggest, as has been proposed by Caprioli and Rittenberg (1969), that the aldolase of *E. coli*, which involves a metal chelate intermediate and not a Schiff base, does not lead to loss of the ^{18}O label from [2-^{18}O]fructose 6-phosphate. Muscle aldolase, on the other hand, which proceeds *via* a Schiff base (Horecker *et al.*, 1963), causes the loss of ^{18}O from this substrate (Model *et al.*, 1968).

From the reactions outlined in Figure 3, with [2-^{18}O]fructose 6-phosphate as the sole glycolaldehyde donor, one would have predicted 100% ^{18}O in the 2′-oxygen atom. Actually, only 9% ^{18}O is found. The loss of ^{18}O at the 2′ position cannot be due to exchange with H_2O since we have shown that the pentose phosphates exchange with water quite slowly. Conceivably, ^{18}O could be lost by exchange with water from the 2 position of fructose 6-phosphate as it is transferred as an active glycolaldehyde by transketolase. Though this exchange could account for the data, some findings from this laboratory make us reclutant to accept this explanation. Incubation of transketolase with fructose 6-phosphate and thiamine pyrophosphate in $H_2{}^{18}O$ did not lead to incorporation of ^{18}O into the fructose 6-phosphate. Though this experiment is not conclusive, it suggests, from what is known of the mechanism of this enzyme (Krampitz, 1969), that transketolase does not cause the loss of ^{18}O from the 2 position of fructose 6-phosphate when it serves as a glycoaldehyde donor.

If one assumes that ^{18}O would not be lost in the transketolase reaction, then the only way to explain the data is to assume that 90% of the active glycolaldehyde transferred to [2-^{18}O]glyceraldehyde phosphate comes from a 2-keto sugar other than fructose 6-phosphate. Caprioli and Rittenberg (1969) arrived at the same conclusion from their studies on wild-type *E. coli* B. Other keto sugars have been shown (de la Haba *et al.*, 1955) to serve as two carbon donors in the transketolase reaction. These include hydroxypyruvate, L-erythrose, xylulose 5-phosphate, sedoheptulose 7-phosphate, octulose 8-phosphate, and D-xylulose. Most of these would not contain ^{18}O in the 2′-oxygen atom. From the data available, it is not possible to make any definite decision as to which two-carbon donors are playing a major role. The resolution of this question must await further experiments.

E. coli K10 DF1000 (Wild Type). This is the parent strain from which the *zwf*$^-$ and *tkt*$^-$ mutants were isolated and can synthesize pentose phosphate both by the oxidative and nonoxidative pathways. Table II shows that when this organism was grown on [1-^{18}O]glucose, the nucleosides contained 26% ^{18}O in the 5′ position. From our results with the *tkt*$^-$ mutant, it is clear that when the oxidative pathway is used for pentose phosphate synthesis [1-^{18}O]glucose gives unlabeled pentose phosphate and there is no equilibration of oxygen between C-1 and C-6 of glucose *via* the triose phosphates. The *zwf*$^-$ mutant showed that when [1-^{18}O]glucose was converted to

pentose phosphate exclusively by the nonoxidative pathway the pentose phosphate contained 48% of the label in the 5′ position. The finding of 26% ^{18}O in the 5′ position in wild-type cells would be interpreted, according to model I, as showing that in these cells 46% of the ribose phosphate was made *via* the oxidative pathway and 54% *via* the nonoxidative pathway. This model assumes no exchange of label in the pentose phosphates by reversible reactions. Model II could explain the finding of 26% ^{18}O in the 5′ position by proposing that all the pentose phosphate is made *via* the oxidative pathway and is unlabeled. However, the unlabeled xylulose 5-phosphate could undergo reversible transketolase reactions (reactions A and C, Figure 3) and incorporate ^{18}O from labeled glyceraldehyde 3-phosphate. Since the glyceraldehyde 3-phosphate would contain 50% ^{18}O, any pentose phosphate made by exchange with this triose phosphate would contain 50% ^{18}O in the 5 position. The finding of 26% ^{18}O in the 5′ positions of the nucleosides could be explained by assuming that half of the unlabeled xylulose 5-phosphate made by the oxidative pathway undergoes this exchange reaction.

The two models presented are extremes in that one proposes that no pentose phosphate is synthesized by the nonoxidative pathway and accounts for the isotopic data by reversible exchange reactions with labeled triose phosphate, while the other model proposes that pentose phosphate is synthesized by the nonoxidative pathway but does not consider incorporation of label into the pentose phosphates by reversible exchange reactions. In fact, the true model could be a combination of both in which some pentose phosphate could be synthesized by the nonoxidative pathway to give a labeled product but where the unlabeled pentose phosphate made by the oxidative pathway could also become labeled by undergoing exchange reactions with labeled triose phosphate.

Since the results with [1-^{18}O]glucose could be explained in a number of ways, a similar experiment was carried out with the wild-type cells grown on [2-^{18}O]fructose as the sole carbon source. The results are presented in Table II. The ^{18}O was found predominantly in the 2′ (9.7%) and 4′ (24%) positions of the nucleosides, as was the case for the *zwf*$^-$ mutant which utilized the nonoxidative pathway exclusively. According to model I, the concentration of ^{18}O in the 4′ position reflects the contribution of the nonoxidative pathway to pentose phosphate synthesis since the oxidative pathway would produce unlabeled pentose phosphate. If the wild-type *E. coli* makes 54% of its ribose phosphate by the nonoxidative pathway, as was concluded from growth on [1-^{18}O]glucose, one would expect to find 27% ^{18}O in the 4′ position of ribose phosphate when grown on [2-^{18}O]fructose, provided this pathway consists essentially of a single transketolase reaction (reaction A, Figure 3) and no loss of ^{18}O occurs from the dihydroxyacetone phosphate. This predicted value of 27% is close to the 24% ^{18}O found in this position (Table II) and would appear to satisfactorily explain the data.

However, this explanation assumes no loss of ^{18}O from dihydroxyacetone phosphate by exchange with water. The results with the *zwf*$^-$ cells showed that there was a 35% loss of label from position 2 of dihydroxyacetone phosphate. If one assumes in this case only an 11% loss of label, then the predicted value would be 24%, the value actually found. If, on the other hand, it is assumed that the value of 35% loss of label found in the *zwf*$^-$ mutant reflects the loss of label normally taking place in a cell, then one would expect to find only 17% ^{18}O in the 4′ position. This value is considerably less than that found.

In order to explain this discrepancy, certain other assumptions that were made must be reexamined. In comparing the results with [1-^{18}O]glucose and [2-^{18}O]fructose, it was assumed that glucose and fructose can be considered as equivalent hexoses, *i.e.*, that both sugars were transported into the cell by the same mechanism, phosphorylation to hexose 6-phosphate, and rapid equilibration of the fructose 6-phosphate and glucose 6-phosphate *via* the hexose isomerase. However, recent findings cast doubt on this assumption. Fraenkel (1968a,b) has shown that in *E. coli* fructose enters the cells not as fructose 6-phosphate but as fructose 1-phosphate, which is then phosphorylated to fructose 1,6-diphosphate by an inducible kinase. Kundig and Roseman (1971) purified the membrane-bound protein system from *E. coli* which phosphorylates and transports glucose into the cell and found that the system specific for glucose did not transport fructose. Saier *et al.* (1971) showed that in certain photosynthetic bacteria, fructose is transported by an enzyme system that transfers the phosphate from phosphoenolpyruvate to position 1 of fructose.

Since fructose enters the glycolytic pathway as fructose 1,6-diphosphate, it is conceivable that more could be metabolized by the nonoxidative pathway than when glucose is used as the carbon source. In order to be metabolized by the oxidative pathway, the fructose diphosphate must first be dephosphorylated at the 1 position by fructose 1,6-diphosphatase. Therefore, in the wild-type *E. coli*, glucose and fructose cannot be considered as equivalent hexoses. It would not be unreasonable to expect that fructose-grown cells would utilize the nonoxidative pathway more extensively than glucose-grown cells.

In fact, the data of Caprioli and Rittenberg (1969) support this idea, although they did not interpret their findings this way. They compared the ^{18}O distribution in the nucleosides isolated from *E. coli* B when grown on [2-^{18}O]glucose 6-phosphate and [2-^{18}O]fructose. In this case, one can compare directly glucose and fructose since both contain the ^{18}O in the same position. In both cases, only the 2′- and 4′-oxygen atoms were labeled with 14 atom % excess ^{18}O (normalized value) in the 2′ position. The 4′-oxygen atom contained 19% ^{18}O (normalized) when grown on [2-^{18}O]glucose 6-phosphate and 25% ^{18}O (normalized) when grown on [2-^{18}O]fructose. Though Caprioli and Rittenberg (1969) did not interpret this difference, we consider it evidence that cells grown on fructose utilize the nonoxidative pathway more extensively than cells grown on glucose.

Using the data of Caprioli and Rittenberg (1969), one can calculate the ^{18}O content in the 4′-oxygen atom of the nucleosides if [2-^{18}O]glucose were used as the carbon source in the present experiments instead of [2-^{18}O]fructose, and compare that result to the one obtained with [1-^{18}O]glucose. This is done by multiplying the ^{18}O content in the 4′ position for wild-type cells grown on [2-^{18}O]fructose by $^{19}/_{25}$ (the values from Caprioli and Rittenberg). These values are listed in parentheses in Table II. For *E. coli* K10 DF1000, one calculates 18% ^{18}O in the 4′ position, whereas 17% ^{18}O was predicted on the basis of the [1-^{18}O]glucose results. Therefore, for the wild-type *E. coli* K10, pentose is synthesized 46% *via* the oxidative pathway and 54% *via* the nonoxidative pathway. In *E. coli* B, Caprioli and Rittenberg (1969) interpreted their results to indicate that pentose is synthesized 28% *via* the oxidative pathway and 72% *via* the nonoxidative pathway. The low ^{18}O in the 2′ position when grown on [2-^{18}O]fructose can be explained in the same manner as was done for the *zwf*$^-$ mutant.

Now one should consider how the [2-^{18}O]fructose results in wild-type cells could be explained according to model II. It

was concluded from the [1-^{18}O]glucose experiment that all the pentose phosphate could be made by the oxidative pathway if it is assumed that half the pentose phosphate incorporates ^{18}O by reversible transketolase exchange with labeled triose phosphate. With [2-^{18}O]fructose, the pentose phosphate made by the oxidative pathway would be unlabeled. If it is assumed that glyceraldehyde 3-phosphate contains 50 atom % excess ^{18}O in the 2 position (*i.e.*, no loss of ^{18}O by exchange in dihydroxyacetone phosphate) and that half of the unlabeled pentose phosphate undergoes reversible transketolase reactions (reactions A and C, Figure 3), then one would predict 25% ^{18}O in the 4′ position. This compares favorably with the 24% found.

Exchange by reaction A (Figure 3) would incorporate ^{18}O in the 2′ position while exchange by reaction C would not. Therefore, if we further assume that half of the pentose phosphate which exchanges does so 20% *via* reaction A and 80% *via* reaction C, we would predict 10% ^{18}O in the 2′ position. This compares favorably with the 9.7% found.

Though the [2-^{18}O]fructose results in wild-type *E. coli* can be explained according to model II as outlined above, there are a number of objections to this explanation. First we must assume that any glyceraldehyde 3-phosphate formed from fructose 6-phosphate contains 50% ^{18}O and that there is no loss of label from [2-^{18}O]dihydroxyacetone phosphate. Our results with the *zwf*$^-$ mutant, as well as those of others mentioned above, argue against this assumption and strongly suggest a loss of ^{18}O from the 2 position of dihydroxyacetone phosphate. Of course, if one assumes a 50% loss of label and complete equilibration of labeled and unlabeled pentose phosphates, then one would obtain the result found (24% in the 4′ position). However, the experiments with [1-^{18}O]glucose rule out complete equilibration. Secondly, this explanation assumes that glucose and fructose are equivalent hexoses, whereas the data of Caprioli and Rittenberg (1969) show a considerable difference in their conversion to pentose phosphate.

E. coli K12 W6. This organism is genetically similar to the K10 strain except that it lacks the Hfr factor. When grown in log phase (with methionine and biotin) on [1-^{18}O]glucose, the ^{18}O was found predominantly in the 5′ position (Table II) and from the ^{18}O content of 24%, one would conclude from model I that 51% of the ribose was made by the oxidative pathway and 49% by the nonoxidative pathway. These values are quite close to those found in *E. coli* K10 DF1000. These results would be explained according to model II in the same manner as was done from *E. coli* K10 DF1000, *i.e.*, all pentose phosphate made *via* the oxidative pathway with 47% exchange *via* transketolase.

When grown on [2-^{18}O]fructose, the results in Table II were obtained which were used to calculate the expected values for growth on [2-^{18}O]glucose according to model I (Table II, parentheses). The label was found only in the 2′ and 4′ positions. If one makes the same assumptions as in the case of *E. coli* K10, *i.e.*, 35% loss of label in dihydroxyacetone phosphate and nonequivalence of glucose and fructose, only 15% ^{18}O would be predicted for the 4′ position, if 49% of the ribose phosphate was made by the nonoxidative pathway. If no ^{18}O were lost by exchange in dihydroxyacetone phosphate, 24% ^{18}O would be expected in the 4′ position. The experimental value of 22% could be due to less ^{18}O exchange (~10%) from the triose phosphate in this strain of *E. coli*. There are, of course, other explanations to account for this discrepancy, none of which can be proven at present.

According to model II, the [2-^{18}O]fructose result would be interpreted as being due to 57% isotopic exchange between the pentose phosphates *via* transketolase. This should be compared with the 47% exchange deduced from the [1-^{18}O]glucose experiment with the same organism.

The interest in using this relaxed strain was that it enabled the study of pentose synthesis during starvation for methionine when RNA synthesis continues in the absence of protein and DNA synthesis. Cells were, therefore, suspended in a medium devoid of methionine but containing [1-^{18}O]glucose or [2-^{18}O]-fructose as sole carbon sources. The normalized values, corrected for the unlabeled RNA present before the addition of labeled hexose, are listed in the last column of Table II. When utilizing [1-^{18}O]glucose as a carbon source, the ribose phosphate had label only in the 5′ position and from the ^{18}O content (26.7%) it would appear that 42% of the ribose was made by the oxidative pathway and 58% by the nonoxidative pathway according to model I. These values are not very different (51 and 49%) from those found when these cells were growing in the log phase, suggesting that pentose synthesis is approximately the same under both conditions, except that under starvation conditions the nonoxidative pathway is slightly favored.

The calculated values for growth on [2-^{18}O]glucose obtained from [2-^{18}O]fructose results are given in Table II (parentheses). These are similar to those obtained during log phase growth and also suggest that pentose phosphate synthesis during starvation is similar to that during growth, but that the nonoxidative pathway is utilized to a slightly greater extent.

According to model II, the [1-^{18}O]glucose result would suggest 53% isotopic exchange among the pentose phosphates while the [2-^{18}O]fructose result would suggest 61% exchange.

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Use of Photochemically Induced Cross-Linking as a Conformational Probe of the Tertiary Structure of Certain Regions in Transfer Ribonucleic Acid†

James Ofengand* and Joan Bierbaum

ABSTRACT: Photochemically induced cross-linking between $^4Srd_8$ and Cyd_{13} in unfractionated tRNA, $tRNA^{fMet}$, and $tRNA^{Val}$ of *Escherichia coli* was studied in terms of its use as a probe of the tertiary structure of tRNA. The rate of cross-linking, a measure of the proximity of the ^{4}Srd and Cyd residues to each other in a tRNA, was influenced by the nature of the tRNA and required Mg^{2+} for optimal cross-linking. The $T_{1/2}$ for cross-linking of $tRNA^{fMet}$ was 1.31 min, and for $tRNA^{Val}$ 2.35 min. Cross-link formation was measured by reduction of the binucleotide product with $NaBH_4$ to the dihydro derivative, Pdo(4–5)hCyd, which has an ultraviolet absorbance band at 377 to 385 nm, and is strongly fluorescent. Both spectral parameters were influenced by tRNA conformation. Fluorescence was markedly dependent on the surrounding tRNA structure. Removal of Mg^{2+} had a quenching effect, nuclease digestion to various limit products quenched the fluorescence to a degree defined by the nuclease–tRNA combination studied, and a transient increase in fluorescence was observed upon hydrolysis of $tRNA^{fMet}$ with pancreatic RNase. The absorption spectra, on the other hand, showed only small changes after complete hydrolysis with pancreatic, T_1, or T_2 RNase and Mg^{2+} depletion had almost no effect. To bypass the influence of structure on the measurement of cross-link formation, ultraviolet absorbance after complete nuclease digestion was adopted as the method of choice. Under appropriate conditions, ultraviolet absorbance without prior hydrolysis, and fluorescence measurements were also shown to be valid measures of cross-link formation.

Irradiation of *Escherichia coli* tRNA at 335 nm induces a specific and quantitative photochemical reaction which results in the covalent cross-linking of two arms of the tRNA cloverleaf *via* $^4Srd_8$ and Cyd_{13} (Favre *et al.*, 1969; Yaniv *et al.*, 1969) due to the formation of the binucleotide I (Figure 1) which can subsequently be converted into the strongly fluorescent compound II (Figure 1) by reduction with $NaBH_4$ under mild conditions while still part of the tRNA (Favre and Yaniv, 1971; Krauskopf *et al.*, 1972). These two nonadjacent positions are within bonding distance of each other in the X-ray structure for yeast $tRNA^{Phe}$ recently proposed by Kim *et al.* (1973).

Formation of the cross-link has little functional effect. The affinity for Val-, Arg-, and Phe-tRNA synthetases is not greatly reduced (Yaniv *et al.*, 1971; Chaffin *et al.*, 1971) although heterologous aminoacylation of cross-linked Val-tRNA by yeast Phe-tRNA synthetase is blocked (Kumar *et al.*, in preparation), ternary complex formation with EFTu-GTP is not detectably altered (Krauskopf *et al.*, 1972), and overall protein synthesis is not markedly inhibited (Yaniv *et al.*, 1971; Chaffin *et al.*, 1971). Because of these facts and additional evidence that a denatured tRNA structure cannot be cross-linked (Favre *et al.*, 1971) it is believed that cross-linking requires the existence of a structure similar to or identical with the biologically active one, at least in the ^{4}Srd-containing region of the molecule.

The usefulness of this reaction for probing the conformation of tRNA lies in the fact that (a) the ability to form the cross-link is dependent on the conformational state of the tRNA being irradiated due to the requirement for proper juxtaposition of the two residues in space before reaction can take place (Favre *et al.*, 1971; Krauskopf and Ofengand, 1971; Siddiqui and Ofengand, 1971; Bergstrom and Leonard, 1972a) and (b) the intensity of fluorescence of the cross-linked and reduced tRNA is highly dependent on its environment (Favre and Yaniv, 1971).

An additional feature of the reduction product II that permits direct measurement of the amount of reduced binucleotide in tRNA independent of its fluorescence properties is its ultraviolet absorption maximum at 380–385 nm (Favre and Yaniv, 1971; Krauskopf *et al.*, 1972) well removed from the main 260-nm absorption band of tRNA.

† From the Roche Institute of Molecular Biology, Nutley, New Jersey 07110. *Received December 13, 1972.*