Studies on the Mechanism of *O*-Alkyl Lipid Synthesis*

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ABSTRACT: Recent studies from this laboratory have indicated that there is a loss of hydrogen from carbon-3 of dihydroxyacetone phosphate (nonphosphorus-linked carbon) in the course of its incorporation into glyceryl ethers. In order to obtain further information on the mechanism of glyceryl ether synthesis a study was undertaken to determine which of the two hydrogens of carbon-3 of dihydroxyacetone phosphate is labilized. Accordingly a mixture of [1,3-^{3}H]- and [1,3-^{14}C]-dihydroxyacetone phosphate was treated with isomerase and incubated in a microsomal system from *Tetrahymena pyriformis* which forms glyceryl ethers. In a similar experiment the effect of fructose 1,6-diphosphate aldolase was evaluated under conditions in which microsomal isomerase was inhibited. It was determined that the hydrogen lost during glyceryl ether synthesis is the one labilized by isomerase. The hydrogen labilized by aldolase is retained.

Glyceryl ethers can be synthesized from long-chain alcohols and dihydroxyacetone phosphate by microsomes obtained from a number of sources. ATP, magnesium, and coenzyme A are required for the formation of *O*-alkyldihydroxyacetone phosphate and NADPH for the transformation of the latter to glyceryl ethers (Kapoulas and Thompson, 1969; Snyder *et al.*, 1970a,b; Wykle and Snyder, 1970).

Currently, the mechanism of the reaction is under investigation in several laboratories. Snyder *et al.* (1970c) have found that the oxygen of [^{18}O]hexadecanol is incorporated into *O*-alkyllipids. Hajra (1970) presented data suggesting that acyldihydroxyacetone phosphate may be a precursor of glyceryl ethers. When acyldihydroxyacetone phosphate was used in glyceryl ether synthesis there was no CoA requirement.

We have recently found that one of the hydrogens of carbon-3[1] of dihydroxyacetone phosphate is labilized in the course of *O*-alkyl lipid formation. This labilization was demonstrated in two ways. First, in the presence of 1-hydroxy-3-chloro-2-propanone phosphate, and inhibitor of triose phosphate isomerase, one tritium from carbon-3 of [1,3-^{3}H]DHAP[2] is lost in the course of glyceryl ether synthesis (Friedberg *et al.*, 1971). Second, in the presence of sufficiently high concentrations of [^{3}H]H_2O, the ether-linked carbon of glyceryl ether glycerol becomes tritiated (S. J. Friedberg, 1971, unpublished data).

Previous studies on the mechanism of action of triosephosphate isomerase and fructose 1,6-diphosphate aldolase have shown that these two enzymes stereospecifically labilize one of the hydrogens of C-3 of dihydroxyacetone phosphate and exchange these with hydrogen from the aqueous environment (Bloom and Topper, 1956; Rose and Rieder, 1958; Rieder and Rose, 1959). Isomerase labilizes one of the hydrogens and aldolase the other.

In the present instance, we have used this information to study the formation of the *O*-alkyl lipid bond with specifically labeled dihydroxyacetone phosphate. That is, by alternately removing one hydrogen of C-3 with isomerase and the other with aldolase it should be possible to detect which hydrogen is removed in the course of *O*-alkyl lipid synthesis. The results, in fact, indicate that this is the case and add further support to our recent finding that one hydrogen is lost in the course of *O*-alkyl lipid formation. This hydrogen is the same hydrogen labilized by isomerase and is different from the one labilized by aldolase. Thus, the data indicate that a stereospecific enzyme reaction takes place in the course of glyceryl ether synthesis in which one hydrogen is exchanged.

Materials and Methods

Materials

Dihydroxyacetone phosphate, 3-phosphoglyceraldehyde, NADPH, ATP, NADH, NAD, triosephosphate isomerase (10 mg/ml), α-glycerophosphate dehydrogenase (10 mg/ml), glycerokinase (2 mg/ml), and triethanolamine were obtained from the Sigma Chemical Co. [1-^{14}C]Hexadecanol (specific activity 25.5 mCi/mmole) was obtained from Tracerlab. [1,3-^{3}H]Glycerol (specific activity 1.58 Ci/mmole, prepared by the reduction of glyceraldehyde with tritium) and [1,3-^{14}C]glycerol (specific activity 15.2 mCi/mmole) were obtained from Amersham–Searle. The purity of labeled glycerol was established by thin-layer chromatography on cellulose using isopropyl alcohol–acetic acid–water (3:1:1, v/v) as the developing solvent. Hydrazine hydrate was obtained from Mallinckrodt. CAP, an inhibitor of triosephosphate isomerase, was prepared by the method of Hartman (Hartman, 1970). CAP completely inhibited triosephosphate isomerase, both from a commercial source (Sigma) and in microsomes. Phosphorus analysis revealed that the compound contained 1 atom of phosphorus/mole of CAP.

Methods

Preparation of [1,3-^{3}H]Dihydroxyacetone Phosphate and [1,3-^{14}C]Dihydroxyacetone Phosphate. The method is a modi-

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[1] The unphosphorylated primary carbinol carbon is designated as carbon-3 of dihydroxyacetone 1-phosphate. Carbon-3 of dihydroxyacetone 1-phosphate corresponds to carbon-1 of the precursor *sn*-glyceryl 3-phosphate and carbon-1 of 3-phospho-D-glyceraldehyde.

[2] Abbreviations used are: DHAP, dihydroxyacetone phosphate; CAP (chloroacetol phosphate), 1-hydroxy-3-chloro-2-propanone phosphate.

fication of a procedure described by Hajra (1968) for the synthesis of [^{32}P]dihydroxyacetone phosphate (Friedberg *et al.*, 1971; Hajra, 1968). The two compounds were prepared separately. In a typical preparation, 200 μCi of [1,3-^{14}C]glycerol (12 μmoles) was dried in a stream of nitrogen at room temperature. Five milliliters of 0.2 M glycine containing 2.06 g of hydrazine hydrate was adjusted to pH 9.8 with concentrated HCl and added to the glycerol together with ATP (60 μmoles), $MgCl_2$ (12 μmoles), NAD (22 μmoles), α-glycerophosphate dehydrogenase (0.3 mg), and glycerokinase (0.04 mg). Incubation was carried out for 2 hr at 37° with shaking. The reaction mixture was then immediately cooled to 0–3° in an ice bath. The hydrazine was removed in the cold by extracting the incubation mixture three times with benzaldehyde and then three times with ethyl ether. One drop of 5% human serum albumin was added and the protein precipitated with 0.5 ml of 50% trichloroacetic acid and removed by centrifugation. The mixture was then treated with two 0.5-g portions of charcoal. The charcoal was washed with a small amount of water. The incubation mixture and water were combined and filtered. The mixture was then adjusted to pH 6 using phenol red as an indicator and 0.5 ml of 1 M calcium chloride was added. A small amount of precipitate which formed immediately was removed by centrifugation. Ten volumes of acetone–ethanol (1:1, v/v) was added, and the mixture was kept at −20° overnight. The precipitate was removed by centrifugation, dried with nitrogen, and mixed with 1 ml (1.9 mequiv/ml of wet resin) of a slurry of Dowex AG 50W-X8 (100–200 mesh), hydrogen form. This was added to a 1-ml column of the same resin in the sodium form and eluted with water. The eluate was lyophilized and dissolved in 5 ml of water. The yield was approximately 60%. The composition of the mixture was examined by thin-layer chromatography on cellulose layers 0.1 mm thick and developed in a mixture of *tert*-butyl alcohol (60 ml), water (30 ml), and *p*-toluenesulfonic acid (2 g). The mixture was also examined by high-voltage electrophoresis at pH 1.5 using either 0.1 M oxalate buffer or 8% aqueous formic acid. The labeled DHAP gave a single radioactive peak in both systems. The specific activity of the materials was then determined and [^{3}H]DHAP and [^{14}C]DHAP were mixed in a ratio of approximately 15:1 and the exact composition was determined by the use of appropriate internal standards. The tritium distribution and ^{3}H:^{14}C ratios of the substrate double-labeled DHAP and glyceryl ethers were measured by periodate cleavage, precipitation of formaldehyde with dimedon, and determination of the activity in formaldehydodimedon and residual water as previously described (Friedberg *et al.*, 1971). Ultimately, these activities were determined by combustion of the intact [^{3}H,^{14}C]DHAP and of the products of periodate treatment in a Packard Tri-Carb Oxidizer which separates ^{3}H and ^{14}C and measures these separately as [^{3}H]H_2O and [^{14}C]CO_2. Initially, in using conventional counting procedures an anomaly was found in the ^{3}H:^{14}C ratios of the substrate DHAP *vs.* the formaldehydodimedon. The latter, a substance soluble in organic solvents, always indicated a higher ratio than the former. This was noted in spite of attempts to determine absolute activity with internal standards and with a variety of systems for counting water-soluble substances. The problem was resolved by using the combustion procedure. The explanation for the tritium-counting problem in the aqueous counting systems is not certain since the DHAP appeared to be soluble in all systems tested. It was later determined that addition of 10% water to the Triton–phosphor appeared to correct the problem (see below). The identity of the labeled DHAP was established enzymatically with α-glycerophosphate dehydrogenase and NADH and high-voltage electrophoresis (Friedberg *et al.*, 1971).

Other Methods

Liquid scintillation counting was done as previously described by the method of Patterson and Greene (1965) using two parts of toluene–phosphor and one part of Triton X-100. In order to ensure reproducibility, the amount of aqueous material to be counted was maintained at 10% by volume of the total counting solution. Absolute activities were determined by the use of internal standards. The preparation of microsomes, incubations, chromatographic procedures, and other chemical procedures have been previously described (Friedberg *et al.*, 1971).

Results

In order to determine which hydrogen of C-3 of DHAP is exchanged during the course of glyceryl ether synthesis, [1,3-^{3}H,1,3-^{14}C]DHAP was first treated with either 20 μg of triosephosphate isomerase (48 μM units) or with 10–50 μg of fructose 1,6-diphosphate aldolase (0.14–0.7 μM unit). It was found that each enzyme removed 25% of the tritium and that both together removed 50% of the tritium from [^{3}H,^{14}C]DHAP. This was determined by reisolation of aliquots of the treated substrate on 1 × 0.5 cm columns of Dowex 1-8X chloride (200–400 mesh). Tritiated water was first removed with 5 ml of water and DHAP with approximately 5 ml of 0.03 N HCl. Although the aldolase appeared to be free of isomerase by the glyceraldehyde 3-phosphate dehydrogenase reaction, excessive quantities of aldolase produced greater than 25% loss of tritium suggesting the presence of trace quantities of isomerase. This problem was resolved by using only 10–50 μg of aldolase and at least 0.1–0.2 μmole of [^{3}H,^{14}C]DHAP. The removal of tritium from DHAP was also examined by treatment of the substrate with periodate and examining the amount of tritium remaining on C-3. The results indicated loss of half the tritium from C-3 after treatment with aldolase.

The effect of aldolase treatment on glyceryl ether synthesis from [1,3-^{3}H,1,3-^{14}C]DHAP was evaluated by several slightly different procedures. The results proved to be the same in each instance and the following experiments were selected as representative.

The control experiments, showing loss of approximately half the tritium from C-3 of DHAP, consisted in incubating 1 ml of CAP-treated microsomes with untreated [1,3-^{3}H,1,3-^{14}C]DHAP, the necessary cofactors, and hexadecanol (Table I). Lipids were extracted and the glyceryl ethers were isolated by thin-layer chromatography as detailed in Table I and counted. Aliquots of the glyceryl ethers were treated with periodate to cleave the glyceryl ethers between vicinal hydroxyl groups (Thompson and Hanahan, 1963) and the long-chain aldehydes (C-3 of DHAP) formed from the cleavage were extracted with petroleum ether, dried, and counted. The formaldehyde liberated from the periodate reaction (C-1 of DHAP) was precipitated as formaldehydodimedon. The results reveal a loss of tritium in intact glyceryl ethers formed from [^{3}H,^{14}C]DHAP consistent with the exchange of one C-3 hydrogen. There was approximately a 50% loss of tritium from C-3 as determined by the activity in long-chain aldehydes confirming the exchange of one hydrogen from C-3. The ^{3}H:^{14}C ratio of C-1 (formaldehydodimedon) remained essentially unchanged.

The effect of aldolase on the synthesis of glyceryl ethers

TABLE I: Effect of Aldolase Treatment of [1,3-^{3}H,1,3-^{14}C]-DHAP on ^{3}H Loss During Glyceryl Ether Synthesis.

Treatment	Ratio ^{3}H:^{14}C DHAP, Substrate	Glyceryl Ethers ^{3}H:^{14}C	^{3}H:^{14}C of C-3 (Long-Chain Aldehydes)	^{3}H:^{14}C of C-1 (Formaldehydodimedon)
Experiment I				
None[a]	15.1	9.95	5.3	15.5
[^{3}H,^{14}C]-DHAP pretreated with aldolase[b]		7.64	0.1	14.7
Aldolase added to incubation mixture[c]		7.59	0.09	14.0
Experiment II				
None[a]	15.1	10.6	6.8	17.5
[^{3}H,^{14}C]-DHAP pretreated with aldolase[b]	11.05	7.4	0.39	16.3

[a] One milliliter of microsomes (2 mg of protein) in 0.1 M phosphate buffer (pH 7.2) was first incubated with 0.2 ml of 0.05 M CAP for 30 min at 30° to inhibit isomerase activity. The microsomes were then incubated with 15 μmoles of ATP, 4 μmoles of magnesium, 0.1 μmole of CoA, 0.1 μmole of hexadecanol in 1% Tween 80 and 0.1 μmole of [1,3-^{3}H,1,3-^{14}C]DHAP (approximately 7 × 10^6 dpm of ^{3}H and 6.3 × 10^5 dpm of ^{14}C). The exact ratios are indicated above. Incubation was carried out at 30° for 2 hr. NADPH was then added and incubation continued for another hour after which the lipids were extracted and the glyceryl ethers isolated by thin-layer chromatography on silica gel G (developed with ligroine–ethyl ether–acetic acid, 20:80:1, v/v). The glyceryl ethers were eluted with ethyl ether and a portion, in 1 ml of ethanol, was treated with 0.6 ml of 0.05 M periodate in 25% acetic acid with 1 mg added tetradecylglyceryl ether and 18 μmoles (2 μl) of formaldehyde, at room temperature, for 30 min, in the dark. Then 0.6 ml of 0.5 N arsenite was added and the long-chain aldehydes were extracted with petroleum ether (bp 30–60°). The water phase was brought to pH 5.5 with KOH. Dimedon, 0.5 ml of 4% solution in 50% ethanol, was added. The formaldehydodimedon was allowed to precipitate overnight at 4°, and then extracted with petroleum ether and counted in phosphor–Triton (2:1, v/v). [b] [^{3}H,^{14}C]-DHAP was first treated with 10 μg of fructose 1,6-diphosphate aldolase for 20 min at 30° and then incubated as indicated above. [c] One milliliter of CAP-treated microsomes was incubated with [^{3}H,^{14}C]DHAP, 50 μg of aldolase, 15 μmoles of ATP, 0.1 μmole of CoA, and 4 μmoles of magnesium for 5 min at 30°. The reaction was initiated by the addition of 0.1 μmole of hexadecanol in 1% Tween 80.

from [^{3}H,^{14}C]DHAP (Table I) was determined simultaneously with the control experiments. In one experiment [^{3}H,^{14}C]-DHAP was first treated with aldolase. In another, the aldolase was added directly to the complete incubation system. In a

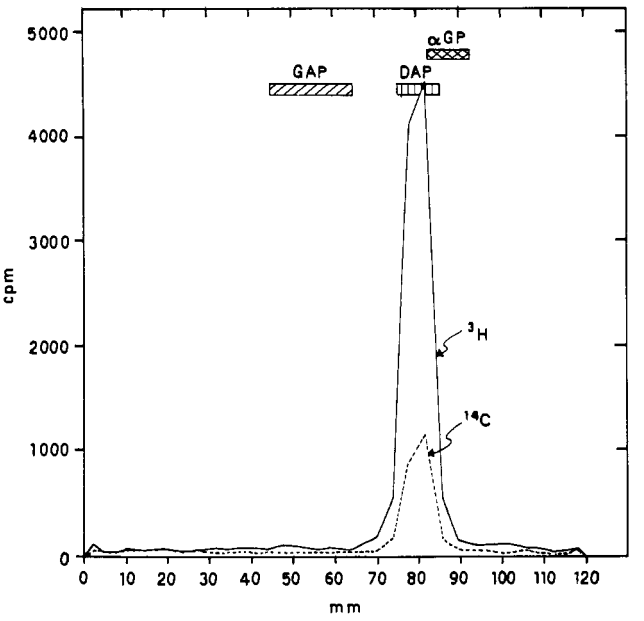

FIGURE 1: Radioactive profile of cellulose thin-layer chromatography of DHAP remaining in the medium after glyceryl ether synthesis. Following incubation of [^{3}H,^{14}C]DHAP in the microsomal enzyme system which forms glyceryl ethers, the DHAP in the water phase remaining after lipid extraction was recovered and isolated by column chromatography on Dowex 1-8X chloride followed by high-voltage electrophoresis. An aliquot was taken for thin-layer chromatography to determine the purity. The reisolated [^{3}H,^{14}C]DHAP was subsequently treated with periodate to determine the distribution of the radioactivity on the molecule. Details are given in the text (GAP, 3-phosphoglyceraldehyde; DAP, dihydroxyacetone phosphate; αGP, α glycerophosphate).

third [^{3}H,^{14}C]DHAP was first treated with aldolase, and the aldolase was precipitated by adding an equal volume of ethanol. Using aldolase treated [^{3}H,^{14}C]DHAP, the glyceryl ethers isolated after incubation showed loss of half the tritium. Treatment of the glyceryl ethers with periodate revealed no loss of tritium from C-1 (formaldehydodimedon) and loss of virtually all of the tritium from C-3 (long-chain aldehydes). These experiments show the loss of one tritium of C-3 of DHAP in the control experiment and loss of both after aldolase, indicating that each process labilizes a different hydrogen.

Since isomerase is known to labilize a different hydrogen from C-3 than aldolase, isomerase treated DHAP would be expected to show no further loss of tritium after glyceryl ether synthesis. Thus, experiments using isomerase treated [1,3 ^{3}H,-1,3 ^{14}C]DHAP show no loss of tritium (Table II) after glyceryl ether synthesis.

The validity of the data and their interpretation hinge on the demonstration that there was no residual microsomal isomerase activity which could have accounted for the removal of the tritium. The absence of isomerase activity was demonstrated by the fact that CAP completely inhibited the activity of commercial triosephosphate isomerase as well as that of isomerase present in microsomes. In addition, there was no loss of tritium in DHAP remaining in the water phase after incubation when neither isomerase nor aldolase was added. Furthermore, labeled 3-phosphoglyceraldehyde was not detected either in the medium after thin-layer chromatography or after high-voltage electrophoresis.

Although the data discussed in the previous paragraph indicated that DHAP remaining after incubation had lost no tri-

TABLE II: Effect of Isomerase Treatment of [1,3-^{3}H,1,3-^{14}C]DHAP on ^{3}H Loss During Glyceryl Ether Synthesis.

Treatment	Ratio ^{3}H:^{14}C DHAP Substrate	Glyceryl Ethers ^{3}H:^{14}C	^{3}H:^{14}C of C-3 (Long-Chain Aldehydes)	^{3}H:^{14}C of C-1 (Formaldehydo-dimedon)
Experiment I				
None[a]	15.1	10.6	6.8	17.5
[^{3}H,^{14}C]DHAP pretreated with isomerase[b]	11.3	10.8	6.3	16.3
Experiment II				
None[a]	13.0	10.2	5.8	13.5
[^{3}H,^{14}C]DHAP pretreated with isomerase[b]	9.8	10.2	5.8	11.7

[a] The conditions for incubation were the same as those described in Table III. [b] The conditions for incubation were the same as for "*a*" except that the substrate was pretreated with triosephosphate isomerase.

tium, further evidence for the absence of isomerase was considered necessary. Proof of this would be the demonstration of a total lack of tritium loss from C-3 of DHAP remaining after incubation. Accordingly, 6 ml of CAP-treated microsomes was incubated with [^{3}H,^{14}C]DHAP (0.6 μmole, 55,500,000 dpm ^{3}H and 4,310,000 of dpm ^{14}C), 90 μmoles of ATP, 3 μmoles of CoA, 24 μmoles of magnesium, and 0.6 μmole of hexadecanol in 0.03 ml of 1% Tween 80. The lipids were extracted by the method of Folch (Folch *et al.*, 1957). The water–methanol phase was filtered and dried in a rotary evaporator and dissolved in water to a concentration of 0.01 M anion concentration. The material was applied to a 2 × 1 cm column of Dowex 1-X8 chloride (200–400 mesh). The column was first eluted with water and then with 0.03 M HCl to elute the DHAP. The fractions containing DHAP were lyophilized, combined, and dissolved in 1 ml of H_2O. DHAP was reisolated by high-voltage electrophoresis on Whatman No. 3MM paper at 3000 V for 90 min in 8% formic acid. The DHAP was located with standards and eluted with 1 ml of water. The purity of the eluted DHAP was checked by thin-layer chromatography on cellulose. The developing solvent was *tert*-butyl alcohol–water–*p*-toluenesulfonic acid (60:15:2, v/v/w). A single peak was obtained (Figure 1). Aliquots of the reisolated DHAP were then treated with periodate. The formaldehyde released (carbon-3 of DHAP) and the water (carbons-1 and -2) were assayed for radioactivity (Table III) and it was evident that no tritium had been lost from carbon-3 due to triosephosphatase isomerase activity.

TABLE III: ^{3}H and ^{14}C Distribution and Ratios of [^{3}H,^{14}C]-DHAP Remaining after Incubation in Glyceryl Ether Synthesizing System.[a]

	Act. ^{3}H (dpm)	Act. ^{14}C (dpm)	Ratio ^{3}H:^{14}C
Reisolated [^{3}H,^{14}C]-DHAP aliquot	165,500	10,600	15.6
Formaldehydodimedon	83,000	4,980	16.7
(carbon-3)	83,700	4,920	17.0
Water remaining	84,100	4,760	17.7
(carbon-1)	83,200	4,950	16.8

[a] Two aliquots of [^{3}H,^{14}C]DHAP were treated with 0.6 ml of 0.05 M sodium periodate in 25% acetic acid with 0.5 μmole of added DHAP and 18 μmoles (2 μl) of formaldehyde, at room temperature for 30 min in the dark. Then 0.6 ml of 0.5 N sodium arsenite was added and the pH was brought to 5.5 with potassium hydroxide. One-half milliliter of 4% dimedon in 50% ethanol was added. Formaldehydodimedon was allowed to precipitate overnight at 4°. The formaldehydodimedon was extracted with petroleum ether, dried, and dissolved in 2:1 phosphor–Triton X100. Aliquots were taken for counting in a total of 20 ml of the same counting solution plus 0.1 ml of water. One-tenth-milliliter aliquots of the water phase remaining were also counted in 20 ml of 2:1 phosphor–Triton containing 10% water.

Discussion

The present investigation was undertaken to provide further evidence that the enzymatic synthesis of *O*-alkyl lipids involves the labilization of one hydrogen from carbon-3 of DHAP. We have shown that this hydrogen is the same hydrogen labilized by triosephosphate isomerase. We consider the finding, that the loss of hydrogen from carbon-3 of DHAP involves a specific hydrogen, more conclusive evidence of an enzymatic process involved in *O*-alkyl lipid synthesis. Although we favor the concept that the loss of hydrogen indicates the formation of an enediol as an intermediate, the data themselves do not establish this. It is possible that an exchange reaction, such as that which is believed to occur with aldolase, may take place.

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Possible Anticodon Sequences of $tRNA^{His}$, $tRNA^{Asn}$, and $tRNA^{Asp}$ from *Escherichia coli* B. Universal Presence of Nucleoside Q in the First Position of the Anticondons of These Transfer Ribonucleic Acids*

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ABSTRACT: An unidentified nucleoside, Q, was isolated from *Escherichia coli* $tRNA_1^{His}$, $tRNA^{Asn}$, and $tRNA_1^{Asp}$ as well as from $tRNA^{Tyr}$. The nucleotide sequences of oligonucleotides containing Q, obtained by RNase T_1 digestion of these three tRNAs, were determined by conventional techniques. The sequences of these oligonucleotides are A-U-U-Q-U-Gp for $tRNA_1^{His}$, A-C-U-Q-U-U-N*-A-ψ-C-C-Gp for $tRNA^{Asn}$, and C-C-U-Q-U-C-m^2A-C-Gp for $tRNA_1^{Asp}$. From these results and other supporting evidence, Q seems to be located in the first position of the anticodons of all these tRNAs. Q was not found in other *E. coli* tRNAs. Thus there is a strict relation between the presence of Q and codon recognition of *E. coli* tRNA. *E. coli* tRNAs which recognize U and C in the third position and A in the second position of code words always contain Q. It was shown that Q has more affinity for U than for C in codon–anticodon base pairing, since among the trinucleotides corresponding to code words, those ending with U always cause most stimulation of the binding of tRNAs containing Q to ribosomes.

Elucidation of the primary sequences of numerous tRNAs has clearly established the cloverleaf structure of tRNA molecule which was first proposed by Holley *et al.* (1965a). One of the most important contributions of this model is the establishment of the anticodon of tRNA which pairs with the codon of mRNA. As proposed first by Crick (1966a) and Nirenberg *et al.* (1966), the first nucleotide in the anticodon has specific wobbling characteristics to recognize multiple codons. The wobble theory (Crick, 1966b), proposed to explain the mechanism of recognition of the third letter of the codon, postulated that G in the first position of the anticodon will pair with U or C in the codon. In fact, elucidation of the primary sequences of tRNAs which recognize XY_C^U-type codons, such as yeast $tRNA^{Tyr}$ (Madison *et al.*, 1966; Madison and Kung, 1967), yeast $tRNA^{Phe}$ (RajBhandary *et al.*, 1967; RajBhandary and Chang, 1968), yeast $tRNA^{Asp}$ (Gangloff *et al.*, 1971), Torulopsis $tRNA^{Tyr}$ (Hashimoto *et al.*, 1969), wheat germ $tRNA^{Phe}$ (Dudock *et al.*, 1969; Dudock and Katz, 1969), *Escherichia coli* $tRNA^{Phe}$ (Barrell and Sanger, 1969), *E. coli* $tRNA_2^{Leu}$ (Blank and Söll, 1971), *E. coli* $tRNA^{Ile}$ (Yarus and Barrell, 1971), *E. coli* $tRNA_3^{Gly}$ (Squires and Carbon, 1971), *E. coli* $tRNA_2^{Val}$ (Yaniv and Barrell, 1971), and *E. coli* $tRNA_3^{Ser}$ (H. Ishikura, Y. Yamada, and S. Nishimura, unpublished results), actually showed that the first letter of the anticodons of these tRNAs were occupied either by G or 2′-*O*-methylguanosine, and proved the wobble theory. However, in the case of *E. coli* $tRNA^{Tyr}$, the first nucleoside of the anticodon is not normal guanosine, but modified guanosine designated as either Q^1 (RajBhandary *et al.*, 1969), G* (Goodman *et al.*, 1968, 1970), or R (Doctor *et al.*, 1969) is located in that position. The structure of Q is not known yet but it is reasonable to imagine that such modification of G to Q must have a specific function in the decoding process by *E. coli* $tRNA^{Tyr}$. As an approach to understand the function of Q, we examined most of the purified *E. coli* tRNAs which were available in our laboratory to see whether Q was present in other *E. coli* tRNAs besides *E. coli* $tRNA^{Tyr}$. As reported in this communication, we could show a relation between the presence of Q and codon recognition of *E. coli* tRNA. Thus in addition to $tRNA^{Tyr}$ other *E. coli* tRNAs which recognize U and C in the third position,

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[1] The abbreviations used are: Q, unidentified minor nucleoside; Q*, Q modified with periodate; s^4U, 4-thiouridine; Gm, 2′-*O*-methylguanosine; N, 2-methylthio-N^6-(Δ^2-isopentenyl)adenosine; m^7G, 7-methylguanosine; m^2A, 2-methyladenosine; N*, *N*-[9-(β-D-ribofuranosyl)purin-6-ylcarbamoyl]threonine; PDase, snake venom phosphodiesterase; pancRNase, bovine pancreatic RNase; PNPase, *E. coli* polynucleotide phosphorylase; Tyr, *E. coli* $tRNA^{Tyr}$; Tyr 2, *E. coli* $tRNA_2^{Tyr}$; His 1, *E. coli* $tRNA_1^{His}$; Asn, *E. coli* $tRNA^{Asn}$; Asp 1, *E. coli* $tRNA_1^{Asp}$; optical density unit (ODU), an amount of material with an absorbance of 1.0 at 260 mμ when dissolved in 1 ml of water and measured with a 1-cm light path.