Transamination of Aminoalkylphosphonic Acids with Alpha Ketoglutarate

Abstract. *Dialyzed homogenates prepared from* Escherichia coli, Tetrahymena pyriformis, *sea anemone* (Anthopleura elegantissima), *and mouse liver were tested for ability to transaminate 17 aminoalkylphosphonic acids with α-ketoglutarate. 2-Aminoethylphosphonic acid (2-AEP), which occurs naturally in* Tetrahymena *and anemone, was transaminated by these latter organisms more than any of the substances tested, but not by preparations from liver or* E. coli. *3-Aminopropylphosphonic acid was transaminated by all preparations, but much less by* Tetrahymena *or anemone than was 2-AEP. 2-Amino-3-phosphonopropionic acid was transaminated by all preparations. 2-Amino-4-phosphonobutyric acid was transaminated by three of the preparations, but not by liver. Of the other 13 substances tested, the following gave positive results:* DL-*1,2-diaminoethylphosphonic acid with* E. coli, DL-*1,2-diaminoethylphosphonic and aminomethylphosphonic acids with* Tetrahymena, DL-*1-aminopropylphosphonic acid with anemone, and* DL-*1-aminoethylphosphonic and* DL-*1-aminobutylphosphonic acids with liver. The significance of these transaminations is discussed.*

A new field of biochemistry deals with compounds possessing the C–P bond. 2-Aminoethylphosphonic acid (2-AEP) has been found in ciliates from sheep rumen (*1*), the sea anemone *Anthopleura elegantissima (2)*, abalone (*Haliotis midae*) (*3*), the marine bivalves *Mytilus edulis, Crassostrea virginica*, and *Venus mercenaria (4)*, several freshwater bivalves (*5*), *Tetrahymena pyriformis* (*6*), and bovine brain and goat liver (*7*). The latter substance occurs in the free form *(2)*, as a glycerol ester *(2)*, and as a constituent of phospholipids *(2, 3, 5, 8)* and proteins *(4, 9)*. 2-Amino-3-phosphonopropionic acid was found in the zoanthid *Zoanthus sociatus* and in *T. pyriformis* (*10*). Recently the 2-methylamino-, 2-dimethylamino-, and 2-trimethylaminoethylphosphonic acids have been found in the sea anemone *A. xanthogrammica* (*11*).

Microorganisms can utilize the phosphorus of alkyl- (*12*) and aminoalkylphosphonic acids *(13)* for growth; therefore, the C–P bond can be split. However, there is still no experimental evidence of any scheme for biological synthesis or degradation of the naturally occurring phosphonic acids, or of the formation or splitting of the C–P bond itself. Because it was considered possible that transamination reactions might be involved in the metabolism of phosphonic acids with primary amino groups, we have studied the transamination with α-ketoglutarate of 17 aminoalkylphosphonic acids in dialyzed homogenates prepared from *Escherichia coli, T. pyriformis*, the sea anemone *A. elegantissima*, and (Swiss) mouse liver.

A modified procedure for enzyme assay at *p*H 8.3 (*14*) was based on utilization of α-ketoglutarate-1-C^{14} (0.02*M*) as amino acceptor in incubation mixtures (*15*) containing a dialyzed enzyme preparation and ammonia or an aminoalkylphosphonic acid as potential amino donor (0.04*M*). The incubations were performed under completely anaerobic conditions in prepurified N_2. The amount of enzyme activity under standardized conditions was estimated by trapping in Hyamine base the $C^{14}O_2$ liberated quantitatively under anaerobic conditions by a bacterial glutamic decarboxylase preparation from the L-glutamic acid-1-C^{14} formed in the reaction, and determining the radioactivity in a scintillation spectrometer. In all instances "blank" incubations were performed, differing from the experimental samples in containing no added potential amino donors; the values were subtracted from those obtained in the presence of such substances. All determinations were made in duplicate or triplicate. The enzyme preparation employed did not liberate any $C^{14}O_2$ from the labeled α-ketoglutarate under the conditions of the experiment.

All homogenates were made in an aqueous solution containing pyridoxal phosphate, *S*-(2-aminoethyl)isothiouronium bromide (both at $1 \times 10^{-4}M$), and sufficient Triton X-100 to give a final concentration of 0.15 percent. A 5-percent homogenate was made directly from acetone-dried powder of *E. coli*, a 20-percent homogenate (based on packed-cell volume) was made from a 47-hour culture of *T. pyriformis*, a 10-percent homogenate (fresh-weight basis) was made from livers of mice immediately after death by cervical dislocation, and a 15-percent homogenate (fresh-weight basis) was made from anemones. Before the study the anemones were maintained in the laboratory for several weeks in tanks of circulating salt water, during which time they had ejected detritus such as sand, shells, and marine organisms. The anemones were macerated with scissors, minced in a Waring Blendor fitted with special cutting blades, and finally homogenized in an all-glass homogenizer. All homogenates were dialyzed with stirring for 3 hours against a large excess of suspending fluid and rehomogenized after removal from the dialysis sacs; portions were taken for enzyme assays and determinations of protein content (*16*).

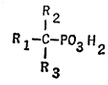
In incubation mixtures containing amino compounds, α-ketoglutarate, and crude enzyme preparations there was a possibility that the nitrogen of the amino compounds might in some way be liberated as ammonia, and that glutamic dehydrogenase could catalyze the formation of glutamic acid from the ammonia and α-ketoglutarate. However, the results (Table 1) show that, even when ammonia was present in a concentration isomolar with that of the aminoalkylphosphonic acids, there was no significant formation of glutamic acid in three of the preparations tested and only slight formation of glutamic acid in the case of the liver preparation. All our reactions were carried out anaerobically. Deamination of amino compounds is usually an oxidative reaction. 2-Aminoethylphosphonic acid (Table 1, No. 2) was transaminated with α-ketoglutarate by the *Tetrahymena* and anemone preparations to a greater extent than was any other of the substances tested, but was not transaminated by the *E. coli* or mouse-liver homogenates. This finding indicates that the preparations from organisms known to contain 2-AEP can probably degrade this substance or form it according to the equation:

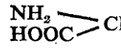
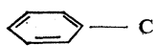
$$NH_2CH_2CH_2PO_3H_2 + HOOCCH_2CH_2C(=O)COOH \rightleftarrows O{=}CH(CH_2PO_3H_2) + HOOCCH_2CH_2CH(NH_2)COOH$$

(*a*)

Although one of the products is probably phosphonoacetaldehyde (*a*), this substance has not yet been isolated and

Table 1. Transaminase activities (μmole per hour per gram of protein). Compound No. 1, NH_3 (ammonium acetate), was transaminated by only the liver homogenate (8 μmole g^{-1} $hour^{-1}$). Compounds 4, 5, and 7 to 18 were DL mixtures. Compounds 15 to 18 were used at concentrations of 0.02*M*; all others, at 0.04*M*. The structure referred to is

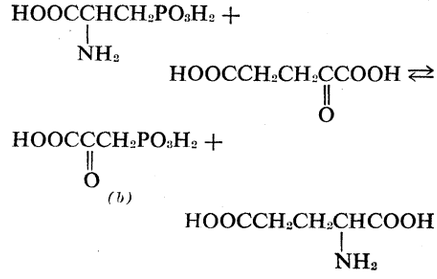


Amino donor				Homogenate			
No.	R_1	R_2	R_3	*E. coli*	*Tetrahymena*	Anemone	Liver
2	NH_2CH_2	H	H	0	135	107	0
3	$NH_2CH_2CH_2$	H	H	44	18	14	16
4		H	H	105	65	10	150
5		H	H	1382	32	45	0
6	H	H	NH_2	0	12	0	0
7	CH_3	H	NH_2	0	0	0	9
8	CH_3CH_2	H	NH_2	0	0	7	0
9	$CH_3CH_2CH_2$	H	NH_2	0	0	0	8
10	$CH_3CH_2CH_2CH_2$	H	NH_2	0	0	0	0
11	CH_3	CH_3	NH_2	0	0	0	0
12	CH_3CH_2	CH_3	NH_2	0	0	0	0
13		H	NH_2	0	0	0	0
14		H	NH_2	0	0	0	0
15	NH_2CH_2	H	NH_2	11	24	0	0
16	$NH_2CH_2CH_2$	H	NH_2	0	0	0	0
17	$NH_2CH_2CH_2CH_2$	H	NH_2	0	0	0	0
18	$NH_2CH_2CH_2CH_2CH_2$	H	NH_2	0	0	0	0

characterized; nor to our knowledge has it been synthesized. A large-scale experiment with the *Tetrahymena* preparation was performed using P^{32}-labeled AEP as substrate and cold α-ketoglutarate. There was no evidence of liberation of P^{32}-orthophosphate during the course of the reaction, but one could detect a P^{32}-labeled 2,4-dinitrophenyl hydrazone in the reaction mixture that was not found in control experiments with a boiled *Tetrahymena* preparation.

When 2-AEP was reacted with ninhydrin at *p*H 5.0 and 96°C, formation of the ninhydrin color and liberation of inorganic phosphate took place in a parallel manner, and acetaldehyde could be isolated from the reaction mixture (*17*). Since phosphonoacetaldehyde probably is the first product formed from 2-AEP in the reaction with ninhydrin, these results suggest that phosphonoacetaldehyde is unstable under the conditions employed and decomposes rapidly to orthophosphate and acetaldehyde. 2-Aminoethylphosphonic acid, itself, is completely stable under these conditions (*p*H 5.0, 96°C); thus, in organisms that can transaminate 2-AEP with α-ketoglutarate (or with other keto acids) there is a mechanism for the labilization of the C–P bond and, therefore, for the potential retrieval of both the carbon skeleton and the phosphate in biologically useful forms. If there were a metabolic source of phosphonoacetaldehyde, it could be transaminated with glutamate (or possibly other amino acids) to form 2-AEP.

A phosphonic acid analog of aspartic acid, DL-2-amino-3-phosphonopropionic acid (Table 1, No. 4), was transaminated to a greater extent by *E. coli* and liver than by the *Tetrahymena* and anemone homogenates (Table 1). Since the latter substance occurs naturally in *Tetrahymena* (*10*), it is possible that *Tetrahymena* may form or degrade it according to the equation:



One of the probable products is phosphonopyruvic acid (*b*), a substance that itself may have a labile C–P bond and which may be expected to be decarboxylated readily to phosphonoacetaldehyde, which in turn may be degraded in the manner discussed. Thus also in organisms that can transaminate 2-amino-3-phosphonopropionic acid there may be a mechanism for the cleavage of the C–P bond. Metabolically formed phosphonopyruvic acid may be a precursor of 2-amino-3-phosphonopropionic acid. A phosphonic acid analog of glutamic acid, DL-2-amino-4-phosphonobutyric acid (Table 1, No. 5), was transaminated by the *E. coli* preparation at the greatest rate of any of the substances tested, to a much smaller extent by homogenates of *Tetrahymena* or sea anemone, and not at all by the liver preparation.

It is interesting that each of the four 2- or 3-aminoalkylphosphonic acids tested (Table 1, Nos. 2 to 5) was transaminated by at least two of the four enzyme preparations, and that all were transaminated to some extent by the *Tetrahymena* and anemone homogenates. Transamination by the *Tetrahymena* and anemone preparations of 3-aminopropylphosphonic acid (Table 1, No. 3) was much less than that of 2-AEP. Of the four diaminoalkylphosphonic acids tested (Table 1, Nos. 15 to 18), only DL-1,2-diaminoethylphosphonic (No. 15) was transaminated to some extent by the *E. coli* and *Tetrahymena* preparations. Several of the 1-aminoalkylphosphonic acids tested (Table 1, Nos. 6 to 9) were transaminated slightly by one or another of the preparations. Catalin models revealed that in 2- and 3-aminoalkylphosphonic acids the amino group and the oxygen atoms of the phosphonic acid groups can be in direct contact, but that this contact is not possible in the case of the 1-aminoalkylphosphonic acids.

A tentative unifying hypothesis for the degradative metabolism of compounds having the C–P bond, consistent with all available data, is that the production of a carbonyl function in positions α or β to the C–P bond may so labilize the latter bond that it may be decomposed spontaneously or by enzymic catalysis to orthophosphate and the corresponding carbonyl compound.

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Cyclic Uridine-3′,5′-Phosphate: Molecular Structure

Abstract. *The crystal structure of the triethylammonium salt of cyclic uridine-3′,5′-phosphate was solved by use of the tangent formula to refine phase angles based upon the positions of six of the atoms. The two independent uracil rings are planar and in the keto form. The base-sugar torsion angles are in the* anti *range. The sugar puckering is C3′*-endo, *and the ribose conformation about the C5′–C4′ bond is* trans-gauche.

Ribonucleic acid is a polynucleotide in which the monomeric units are bonded together through phosphodiester linkages between the C3′ and C5′ atoms of adjacent sugars. Ribonucleotides containing a cyclic phosphate ester are intermediates in the breakdown of RNA by ribonuclease, acid, or alkali (*1*). Moreover, the preparation of cyclic 3′,5′-ribonucleosides was important in the synthesis of the specific 3′,5′-phosphodiester linkage of a dinucleotide (*2*). No detailed molecular structures of cyclic nucleotides have been reported.

I have studied the crystal structure of cyclic uridine-3′,5′-phosphate (3′,5′-UMP) in order to determine the conformation and stereochemistry of the molecule. Precise stereochemical and structural information about nucleosides and nucleotides is of importance in the development and evaluation of hypotheses regarding the structure and reaction mechanisms of nucleic acids and related species. Recent publications (*3–5*) have compared the results of studies of crystal structure of nucleotides and of a 2′,5′-dinucleoside phosphate (*6*) in order to draw conclusions regarding favorable conformations for these molecules. Sundaralingam analyzed the furanose ring conformations in nucleic acids (*7*) and, on the basis of this work and stereochemical considerations, predicted several structural features for the 3′,5′-cyclic nucleotides; his predictions have proved to be quite accurate for 3′,5′-UMP.

Rammler crystallized the nucleotide as the triethylammonium salt from a mixture of alcohol and water. The cell dimensions of the crystals are: a, 15.49 ± 0.03; b, 11.67 ± 0.02; c, 11.01 ± 0.02; β, 98.5 ± 0.2 deg; systematic extinctions indicate that the space group of this optically active molecule is $P2_1$. The measured density, 1.38 g/cm^3, corresponds to two molecules of the 3′,5′-UMP, and two triethylammonium ions in the asymmetric unit, and twice this amount in the unit cell (d_{calc}, 1.38 g/cm^3). The existence of two crystallographically independent molecules in the unit cell provides a useful internal check on the molecular dimensions and the conformation of the nucleotide. The x-ray diffraction data were collected on a G.E. goniostat having a single-crystal orienter using the stationary crystal–stationary counter technique. The preliminary refinement that I report was based upon 2405 nonzero intensities with 2θ less than 125 deg.

One phosphate group and the second unique phosphorus were located in the cell by inspection of a sharpened three-dimensional Patterson synthesis. The phase angles predicted on the basis of these six atoms were refined by use of Karle and Hauptman's tangent formula (*8*), and a Fourier map based upon the refined phases and observed amplitudes revealed the rest of the structure quite clearly. Karle (*9*) reports similar successful applications of the tangent formula. The trial structure was refined by use of a block-diagonal least-squares refinement program, with each of the atoms assigned an isotropic temperature

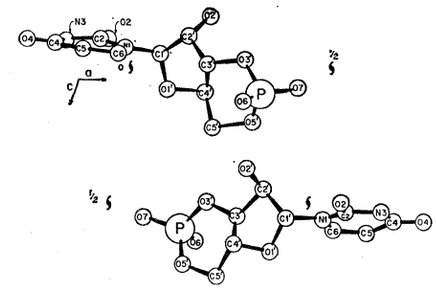

Fig. 1. The molecules viewed down b. No hydrogen atoms or triethylammonium ions are shown; C2 of the upper molecule was moved slightly to avoid overlap in the view.

parameter. Three cycles of refinement reduced the agreement index R to 16 percent; several cycles of anisotropic refinement have further reduced this figure to 8 percent. The molecular structure that I describe is that reflected by the coordinates from the anisotropic refinement. The estimated errors in bond lengths are ± 0.03 Å; in bond angles, ± 2 deg. Additional data are being collected for improvement of the precision of the analysis, but this extension will not alter the conformational features that I describe. The bond distances and angles in the molecules do not differ significantly from the expected values.

Figure 1 is a schematic representation of the molecule and indicates the numbering system used. The uracil rings are planar within the accuracy of the analysis and are in the keto form. The four carbonyl bonds average 1.19 Å. None of these oxygens are involved in hydrogen bonds, and the distances can be compared with the carbonyl distance of 1.201 Å found in cytidylic acid b (*10*). Nitrogen N3 is hydrogen bonded to O6 of a neighboring phosphate group in both unique molecules. The hydrogen bond distances (2.76 and 2.85 Å) are in the expected range, and the P–O6 and P–O7 distances average 1.47 Å. If O6 or O7 were protonated, the P–O distance would be 1.56 Å (*4*). The O7 atoms of the phosphate groups are hydrogen bonded to the nitrogen atom of the triethylammonium ion in both molecules.

The torsion angles of the uracil rings about the beta-glycosidic C1′–N1 bonds are −72 and −56 deg, both in the *anti* range, as is found in most nucleic acid derivatives (*5, 11*). The C3′ atoms of the furanose rings are 0.64 and 0.58 Å out of the planes defined by the other four atoms. The displacements are on the same side of the planes as