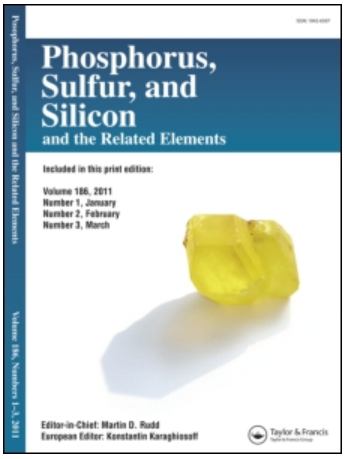


Phosphorus, Sulfur, and Silicon and the Related Elements

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

Pathways of Enzymatic Phosphotransfer Reactions

P. A. Frey[a]; E. Cardemil[a]; R. Iyengar[a]; J. Van Pelt[a]
[a] Institute for Enzyme Research, Graduate School, and Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, Wisconsin, U.S.A.

To cite this Article Frey, P. A. , Cardemil, E. , Iyengar, R. and Van Pelt, J.(1987) 'Pathways of Enzymatic Phosphotransfer Reactions', Phosphorus, Sulfur, and Silicon and the Related Elements, 30: 3, 563 — 566
To link to this Article: DOI: 10.1080/03086648708079127
URL: http://dx.doi.org/10.1080/03086648708079127

Phosphorus and Sulfur, 1987, Vol. 30, pp. 563–566
Photocopying permitted by license only

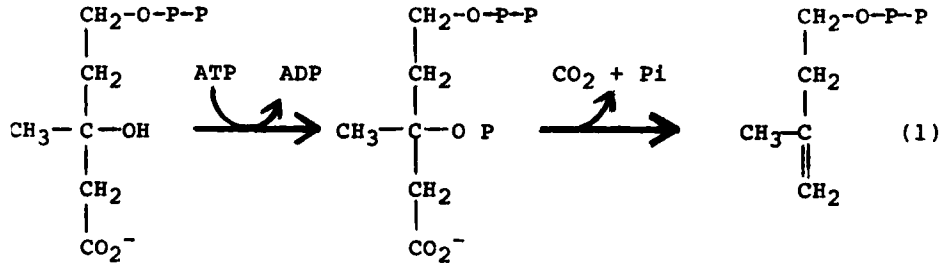
PATHWAYS OF ENZYMATIC PHOSPHOTRANSFER REACTIONS

P.A. FREY, E. CARDEMIL, R. IYENGAR and J. VAN PELT
Institute for Enzyme Research, Graduate School, and
Department of Biochemistry, College of Agricultural and
Life Sciences, University of Wisconsin-Madison, Madison,
Wisconsin 53705 U.S.A.

Abstract Mevalonate 5-pyrophosphate (MVAPP) decarboxylase catalyzes the decarboxylation of MVAPP to isopentenyl pyro-phosphate, an ATP-dependent process in which 3-phospho-MVAPP is a transient intermediate that undergoes concomitant decarboxylation and elimination of phosphate. Reaction of (S_p)-adenosine 5'-O-3-thio [3-$^{17}O_2$,^{18}O] triphosphate in place of ATP produces (R)-[^{17}O,^{18}O] thiophosphate in place of phosphate. Therefore, the phosphotransfer step producing 3-phospho-MVAPP proceeds with inversion of configuration at P. Gentamicin nucleotidyl-transferase catalyzes the reaction of ATP with the C-2" hydroxyl group of aminoglycoside antibiotics to produce AMP-2"-aminoglycosides, thereby inactivating the drugs. Enzymatic reaction of (S_p)-2'-deoxyadenosine 5'-O[$^{\alpha}$-^{17}O] triphosphate with tobramycin produces (R_p)-[$^{\alpha}$-^{17}O]dAMP-2"-tobramycin. Therefore, transfer of the 2'-deoxyadenosine 5'-phosphoryl group proceeds with inversion of configuration. Since both reactions are uncomplicated bisubstrate processes and both proceed with inversion at P, it is likely that both proceed by mechanisms involving direct, single-step phosphotransfer from the phospho-donor substrate to the acceptor, rather than by double-displacement mechanisms involving covalent, phosphoenzyme-intermediates.

The overall stereochemical course of an enzymatic phosphotransfer reaction gives information about the number of steps in the reaction pathway.[1] The stereochemical consequence of each enzymatic phosphotransfer is inversion of cofiguration at P; therefore, mechanisms involving an even number of transfers lead to retention of configuration at P whereas those involving an uneven number lead to overall inversion. In enzymatic phospho-transfers the question of multistep transfers mediated by covalent phosphoenzyme-intermediates invariably arises. In the simplest cases, single step displacements proceed with inversion of configuration at P, whereas the more complex double displace-ments proceed with overall retention.[1,2] In this paper we pre-sent recent experiments dealing with the stereochemical courses of phosphotransfers catalyzed by mevalonate 5-pyrophosphate decarboxylase and gentamicin nucleotidyl transferase.

Enzymatic decarboxylation of mevalonate 5-pyrophosphate (MVAPP) is an ATP-dependent reaction that involves the intermediate formation of 3-phospho-mevalonate 5-pyrophosphate and its

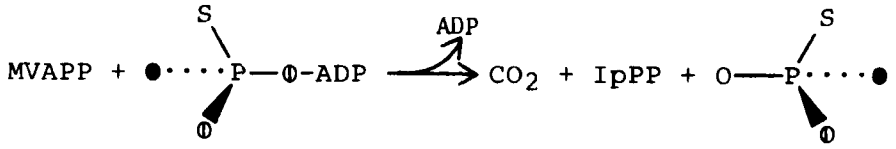


MVAPP IpPP

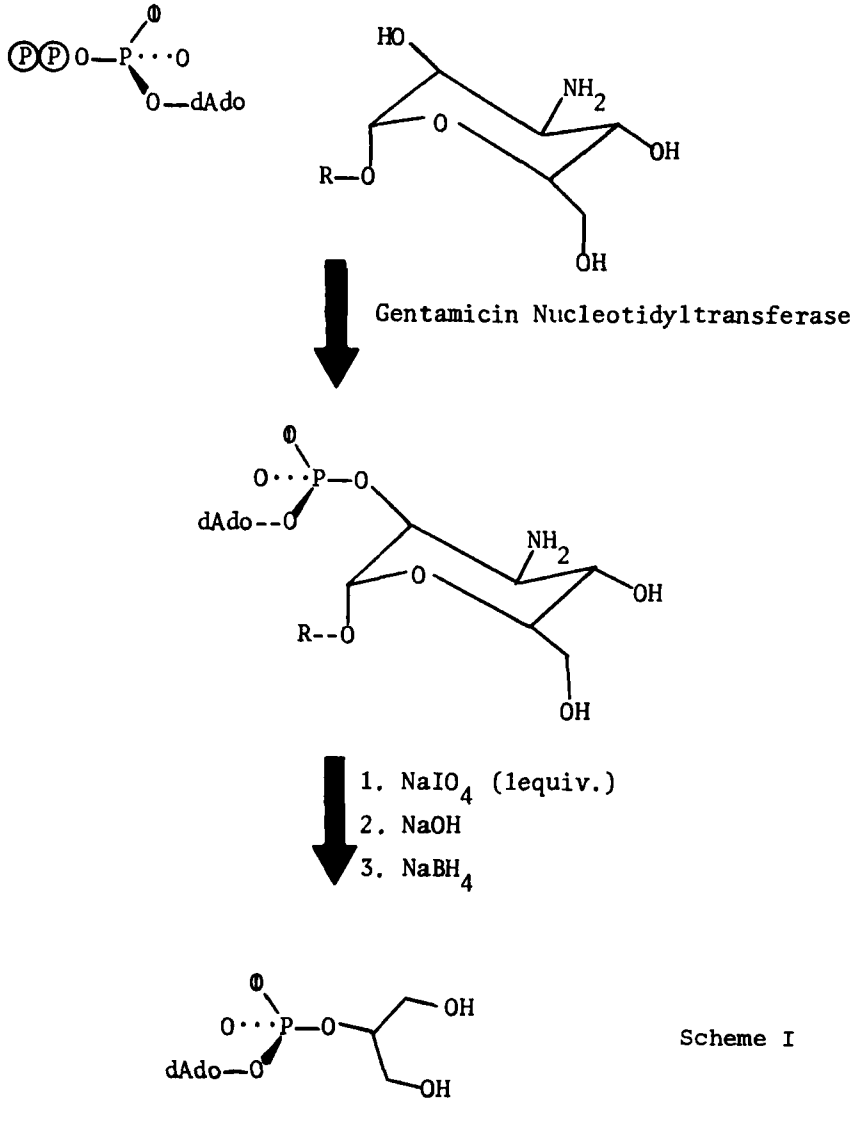
subsequent decarboxylation to carbon dioxide, inorganic phosphate and isopentenyl pyrophysphate (IpPP) (see eq. 1). IpPP and its derived isomer dimethylallyl pyrophosphate are the biological isoprenoid precursors of terpenes, steroids and carotenoids. Phosphorylation of the 3-hydroxyl group facilitates decarboxylation by transforming the hydroxyl group into a stable leaving group as inorganic phosphate. Phosphorylation could proceed either by direct transfer of the terminal phosphoryl group of ATP to the 3-hydroxyl group or by an enzyme mediated, two step pathway in which the phosphoryl group is first transferred to an enzymic nucleophile, forming a covalent phosphoryl-enzyme, and then to MVAPP.

To resolve this issue (Sp)-adenosine 5'-O[3-thio-[3-$^{17}O_2$,^{18}O]]triphosphate ([γ-$^{17}O_2$,^{18}O]ATPγS) was synthesized and used as the substrate in place of ATP. The [^{17}O,^{18}O]PSO_3^{3-} produced was isolated and its configuration at P determined[3], with the results illustrated in eq. 2. The thiophosphate was (R)-[^{17}O,^{18}O]PSO_3^{3-}, the product of inversion at P. This was consistent with a single step, direct transfer of the thiophosphoryl group from (S_p)-[$^{17}O_2$,^{18}O]ATP S to MVAPP. Enzymatic elimination of thiophosphate concomitant with decarboxylation (eq.1) proceeded with C-O bond cleavage and no stereochemical consequences at P.

(2)



Certain drug-inactivating enzymes catalyze transfers of nucleoside 5'-O-phosphoryl groups from nucleoside 5'-O-triphosphates to the 2"-hydroxyl group of aminoglycoside antibiotics. Tobramycin is such an antibiotic that is subject to nucleotidylation by the action of gentamicin nucleotidyl-transferase. Deoxy-ATP (dATP) is an excellent substrate for this enzyme. To determine the stereochemical course of nucleotidyl transfer we synthesized (S_p)-[α-^{17}O]dATP and used it as the substrate with tobramycin to produce [α-^{17}O]dAMP-tobramycin.



Scheme I

This product was chemically degraded to the glyceryl ester of [α-^{17}O]dAMP by a procedure that did not involve bond cleavage at P (Scheme I). Glyceryl-[α-^{17}O]dAMP was subjected to snake venom phosphodiesterase-catalyzed hydrolysis in $H_2{}^{18}O$ to [α-^{17}O,^{18}O]dAMP, which was in turn enzymatically phosphorylated to [α-^{17}O,^{18}O]dATP and cyclized to 2'-deoxyadenosine 3',5'-cyclic (^{17}O,^{18}O] phosphate ([^{17}O,^{18}O]cdAMP). Alkylation of [^{17}O,^{18}O]-cdAMP with diazoethane produced a mixture of axial and equatorial ethyl esters, which were subjected to ^{31}p-NMR analysis to determine the configuration at P. Configurational analysis of [α-^{17}O,^{18}O]dAMP by cyclization and alkylation has been described by Gerlt and coworkers.[4]

According to our stereochemical analysis the product resulting from gentamicin nucleotidyltransferase-catalyzed reaction of tobramycin with (Sp)-[α-^{17}O]dATP is (R_p)-[α-^{17}O]dAMP-tobramycin resulting from inversion of configuration at P. Therefore, the catalytic pathway must involve an uneven number of phosphotransfer steps. Given that the reaction kinetics shows sequential binding of substrates with nucleotidyl-transfer occurring within a ternary complex of enzyme, dATP and tobramycin, the catalytic pathway most likely involves direct transfer of the nucleotidyl moiety from dATP to tobramycin in a single step.

Acknowledgement

This research was supported by Grant No. GM 30480 from the National Institute of General Medical Sciences, USPHS.

References

1. P.A. Frey, in "New Comprehensive Biochemistry" (A. Neuberger and L.L.M. van Deenen, Gen Eds.) Stereochemistry (Ch. Tamm, Vol. Ed.) 3, 201 (1982).

2. P.A. Frey, Tetrahedron, Report Number 127, 38, 1541 (1982).

3. M.R. Webb and D.R. Trentham, J. Biol. Chem. 255, 1775 (1980).

4. J.A. Coderre, S. Mehdi and J.A. Gerlt, J. Am. Chem. Soc., 103, 1872 (1981).