Carbon-Carbon Bond formation

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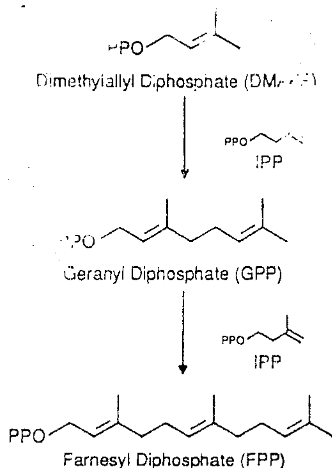
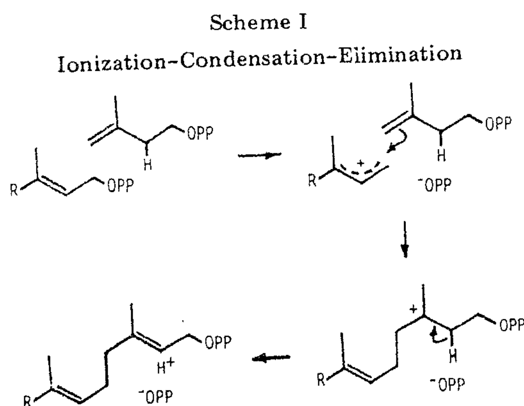
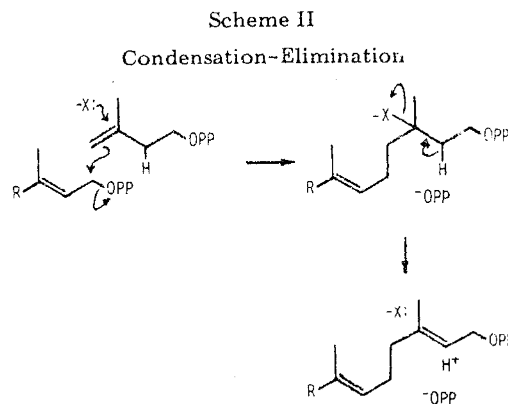
Generic Mechanism for Trans-Prenyl Transferases.



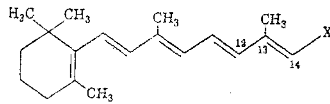




Correct mechanism

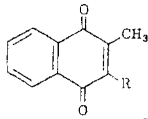
Retinoic Acid Is a Vertebrate Morphogen

Retinoic acid (RA), a derivative of vitamin A (retinol),



X=COOH: **Retinoic acid (RA)**

X=CHO: **Retinal (Vitamin A)**

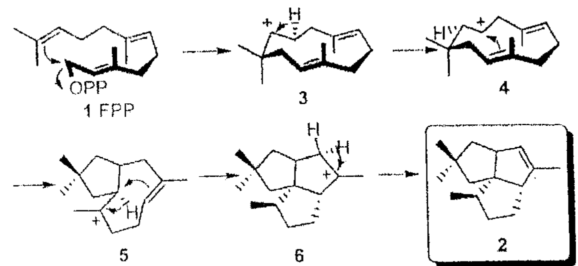


R = $-CH_2-CH=C(CH_3)-CH_2-(CH_2-CH_2-CH(CH_3)-CH_2)_3-H$

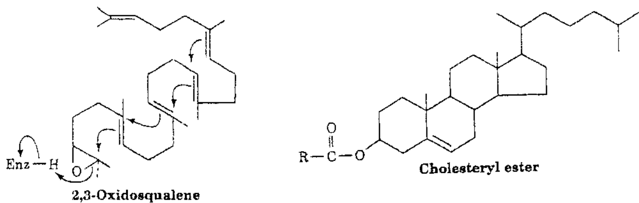
Vitamin K_1 (Phylloquinone)

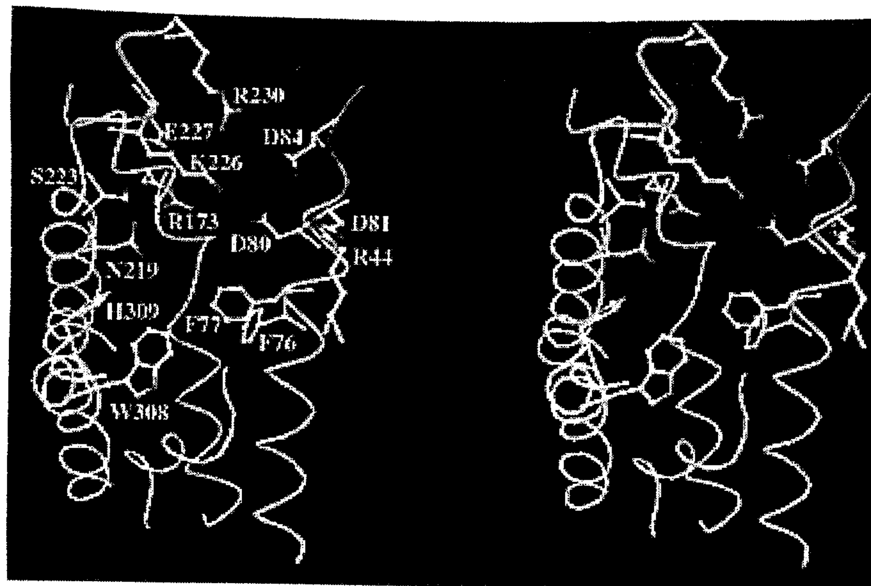
R = $-(CH_2-CH_2=C(CH_3)-CH_2)_6-H$

Vitamin K_2 (Menaquinone)

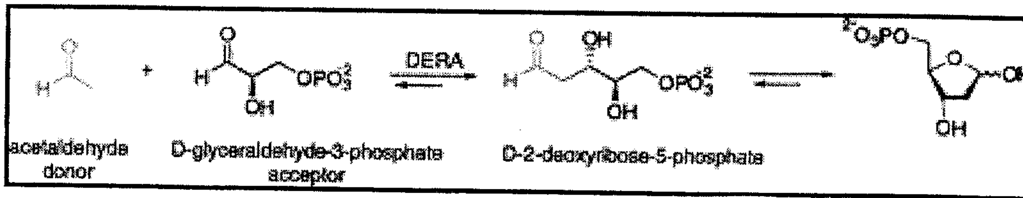


Scheme 1

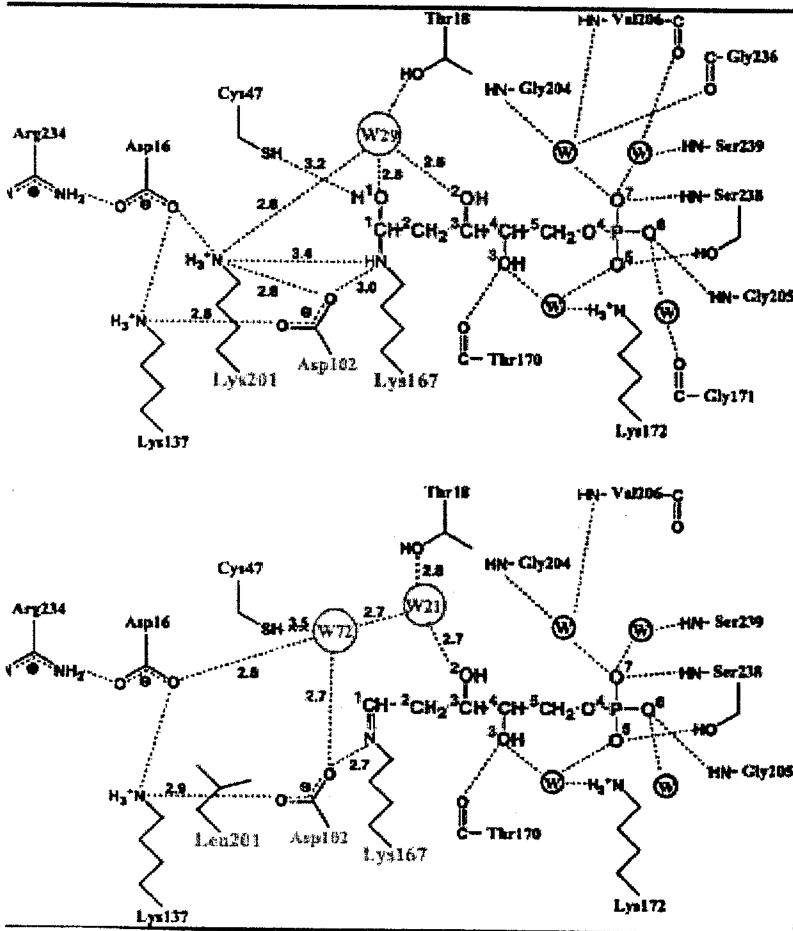


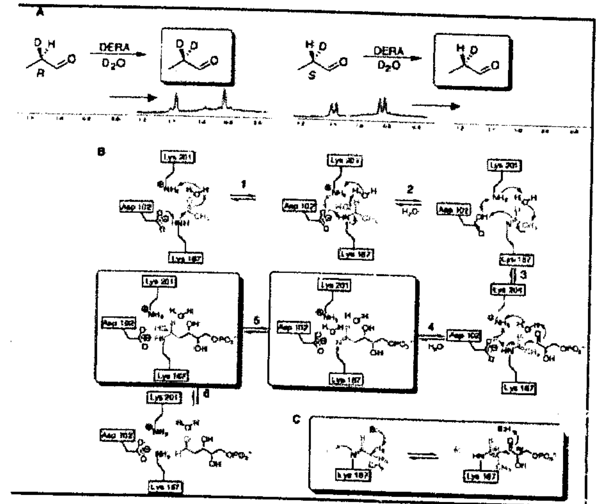


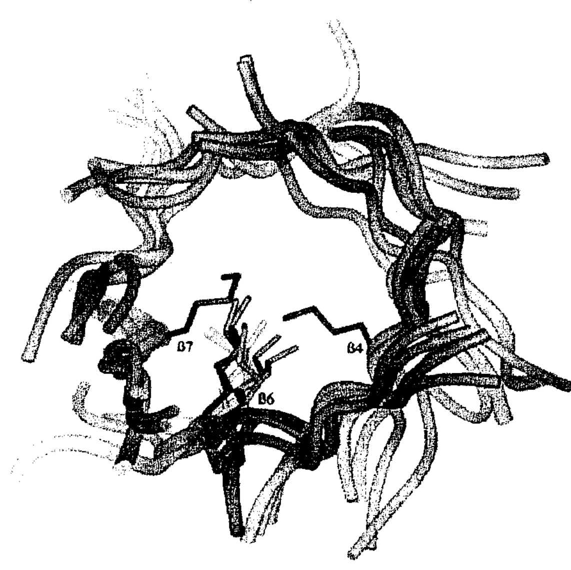
Crossed-eye stereo view of pentalenene synthase active site, illustrating key active site residues. Polar residues at the top are believed to be involved in binding of Mg2+ and pyrophosphate moiety of FPP, while aromatic residues deeper in the active site cleft provide a hydrophobic surface for the farnesyl residue and stabilize the putative carbocation intermediates. Opening of the active site is at the top.



Sciene 294 369-374(2001) Wilson, Wong
Aldolase







From J. Mol Biol (1996) 259 458-466.

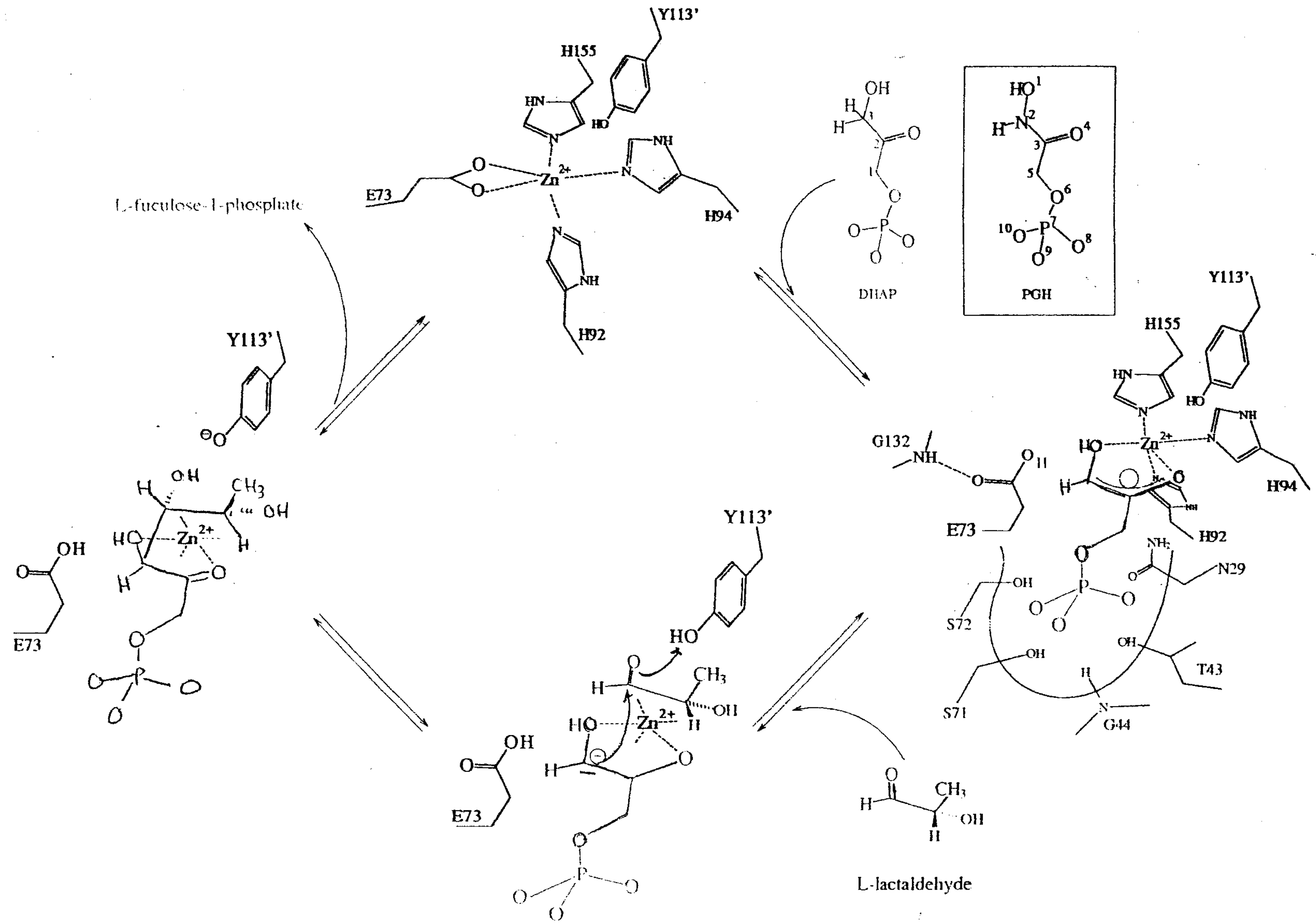
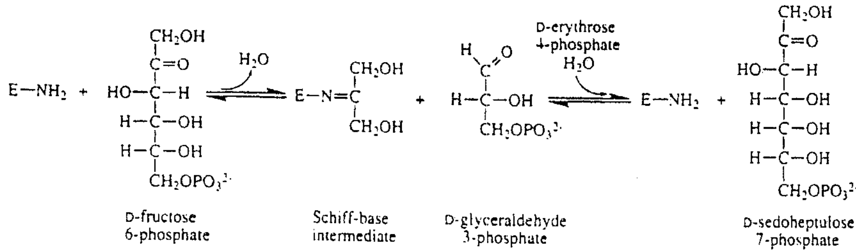
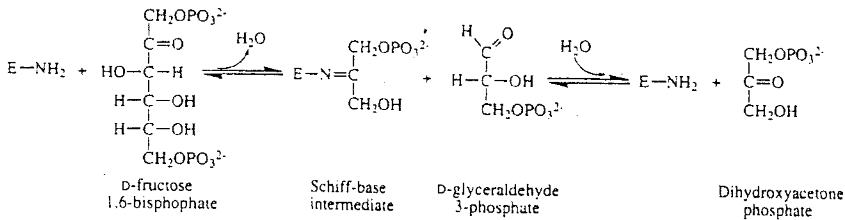


Figure 6. Scheme illustrating the catalytic cycle of FucA. The inhibitor phosphoglycolohydroxamate (PGH) is depicted in an inset beside DHAP in an equivalent conformation. The reaction is described as an aldol addition (clockwise) starting with the substrate-free structure at the top where Glu73 is in the Zn^{2+} coordination sphere. The main steps are: deprotonation of the DHAP C^1-atom by Glu73 and stabilization of the intermediate enediolate by Zn^{2+} (right); binding of the aldehyde with the carbonyl group near Tyr113' pointing with its *si*-face to DHAP (bottom); C–C bond formation and protonation of the carbonyl oxygen by Tyr113' (left); dissociation of the product and resetting of Glu73 and the protonation states at the active center (top).

Reactions catalyzed by (a) transaldolase and (b) fructose-1,6-bisphosphate aldolase.





Citrate Synthase

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FIGURE 4: Schematic of the proposed reaction sequence of the condensation reaction of acetyl coenzyme A and oxaloacetate to form the intermediate citryl coenzyme A. The reaction is proposed to proceed via simultaneous general acid–base catalysis (top) to form a neutral enol intermediate (center) which then attacks the carbonyl carbon of oxaloacetate (bottom). The experimentally observed inversion of configuration of the protons on the methyl group of acetyl-CoA is indicated by the labeling of the hydrogen atoms. Solely for the purposes of discussion (see text), the two carbons of the enol are labeled 1 and 2 as shown.

The (Z)-3-[^{3}H]-PEP yields 98% of its tritium as ^3H_2O after fumarase equilibration, establishing that, with protio-PEP, the carboxytransphosphorylase catalyzes addition of the electrophilic CO_2 to the *si–si* face. The complementary experiment with (E)-3-[^{3}H]-PEP yields the same conclusion, based on the retention of 98% of the radioactivity in fumarate after a similar incubation (Willard and Rose, 1973). By essentially identical experiments, it was possible to show that the other two enzymes, PEP carboxylase and PEP carboxykinase, also catalyze addition of CO_2 to the 2-*si*–3-*si* face of PEP, indicating that they all bind PEP such that the *re–re* face is inaccessible to incoming CO_2 (Rose et al., 1969). This stereochemical unanimity suggests a possible close common evolutionary origin for the active sites of these proteins.

2.A.4 Stereochemical Analysis of Chiral Methyl Groups

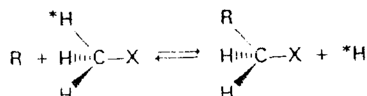
Rose next turned his attention to the carboxytransphosphorylase-dependent conversion of PEP to pyruvate in the absence of CO_2 (Willard and Rose, 1973). This problem is considerably more complex to solve because C-3 of pyruvate is a methyl group, not a methylene prochiral center as in oxaloacetate. Until very recently, it has been impossible to determine the stereochemistry of formation of a methyl group because there was no way of distinguishing the three hydrogens on it. Because the methyl group can freely rotate ($T_{1/2} \approx 10^{-12}$ sec), the three hydrogens are sterically equivalent. The methyl is said to be *torsiosymmetric*.

Ingenious recent experiments have resulted in preparations of chiral acetates and chiral pyruvates, samples where the methyl groups contain all three isotopes of hydrogen (^{1}H, ^{2}H, ^{3}H) and thus are chiral.

chiral *R*-isomer chiral *S*-isomer

With these substrates it is possible, in principle, to determine the stereochemistry of methyl–methylene interconversions. If, during a chemical reaction at a chiral methyl group, the incoming R group is introduced such that it has the same relative stereochemical orientation as the *H it replaces, then the reaction proceeds with retention of configuration at that center.

Welch's Book



If the new carbon–carbon bond has the opposite stereochemical orientation, the transformation will involve inversion. To determine the actual result, one must—

1. know the starting chirality (and know the degree of chiral purity);

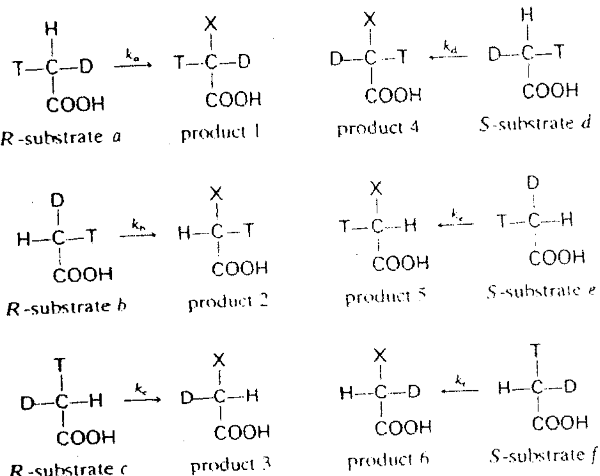
2. have a method of analysis for the chirality generated in the methylene of the product;

3. know that the removal of a hydrogen species at the chiral center proceeds with a kinetic isotope discrimination against deuterium and tritium.

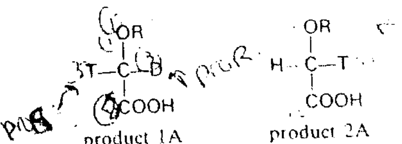
We shall examine each of these conditions before discussing the actual carboxytransphosphorylase data.

The groups of Cornforth and Eggerer and of Arigoni have succeeded in the preparation of chiral 2*R* and 2*S* (^{1}H,^{2}H,^{3}H)-acetic acids where the syntheses lead unambiguously to each isomer (Alworth, 1972, pp. 193–211, 234–240; Cornforth et al., 1969; Luthi et al., 1969; Retey, Luthi, and Arigoni, 1970). As we have noted several times, tritium is normally employed at tracer levels, whereas deuterium is present in all the molecules. These syntheses represented no exception, and only a small percentage (about 1 molecule in 10^9) contained tritium and was chiral; all the others were prochiral (—CH_2D). This problem is obviated however, by the fact that, in all these experiments, *one assays for radioactivity*, measuring only those molecules that contain tritium, and thus selectively examining the behavior of the chiral molecules in a sea of unlabeled, achiral species. Every molecule containing tritium must also contain deuterium in these preparations. We shall now see how measurement of radioactivity can be used to define stereochemistry *with the explicit provision that the interconversion show a normal kinetic isotope effect*. The following explanation is essentially that offered by Luthi et al. (1969; Retey, Luthi, and Arigoni, 1970) and by Alworth (1972, pp. 193–211, 234–240).

Consider a conversion of the *R*- or *S*-acetate into a methylene group, where we specify that the incoming X replaces the removed hydrogen species with retention. Given no steric discrimination between H, D, and T, all six conformations (*a* through *f* in the following expressions) should be equally probable at the active site of an enzyme carrying out the replacement as indicated.



w, if all six bound conformations react *at equal rates* to form product, the reochemical course (retention) *will not be determinate* even from acetate nples of 100% chiral purity. (Reaction at equal rates means that inherent ferences in the rates of C—H, C—D, and C—T bond breakage do not show up V_{max}—that is, these bond-breakage elementary steps are not rate-determining.) iis indeterminacy is easily demonstrated by considering the products from one omer—*R*-acetate, for example. Product molecules 1, 2, and 3 will form at equal tes, but 3 will not be detectable because it bears no tritium, so only 1 and 2 are talogued. Suppose X is an OR group. Then 1 and 2 have the forms shown here 1A and 2A.

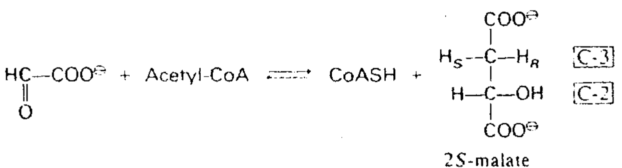


he product 1A has tritium in the *S*-position, whereas 2A has tritium in the :-position. Thus, the 2*R*-[^{1}H,^{2}H,^{3}H]-acetate will generate products whose iethylene groups have *equal amounts* of tritium at 2*R* and 2*S* positions. Exactly ie same conclusion will derive from the 2*S*-chiral acetate, where products 4 and are formed. As Alworth has pointed out, this result cannot then distinguish mong inversion, retention, or a random, nonstereospecific process.

On the other hand, suppose that, in the above reaction of acetate, a kinetic sotope effect is manifested with $k_H > k_D > k_T$. Again we can monitor formation of tritium-containing products 1 and 2 from *R*-acetate. But note that formation of

1 involves C—H cleavage and formation of 2 involves C—D cleavage, so 2 must form more slowly than 1. Product 1 should accumulate preferentially under V_m conditions. Note that the amount of enrichment is directly related to the intramolecular k_H/k_D for the reaction. For example, in formation of products 1A and 2A with an observed k_H/k_D of 4.0 in this transformation, use of *R*-acetate will produce an 80:20 mixture of 1A:2A: that is, 80% of the tritiated product molecules have tritium in the *S*-position of the methylene group. Now, if a method exists for evaluating tritium content at the *S*-position at the methylene C-2 of the products 1A and 2A, the stereochemistry of methyl→methylene conversion is solved. This analytical methodology is of the type we noted earlier in the fumarase reaction (Willard and Rose, 1973; Alworth, 1972, pp. 146–148).

In the first reported example of this technique, the separated chiral *R*- and *S*-acetates were converted via acetate kinase and phosphotransacetylase to *R*- and *S*-acetyl-CoA samples (Cornforth et al., 1969; Luthi et al., 1969; Retey, Luthi, and Arigoni, 1970). These samples then were incubated one at a time with glyoxalate and the enzyme malate synthase (an enzyme we shall discuss in more detail in the following chapter on enzymatic Claisen condensations). This synthase was used because of its availability and because of the known existence of an isotope effect ($k_H/k_T = 2.7$) with [^{3}H]-acetyl-CoA in the rate of *S*-malate formation (Eggerer and Klette, 1967).

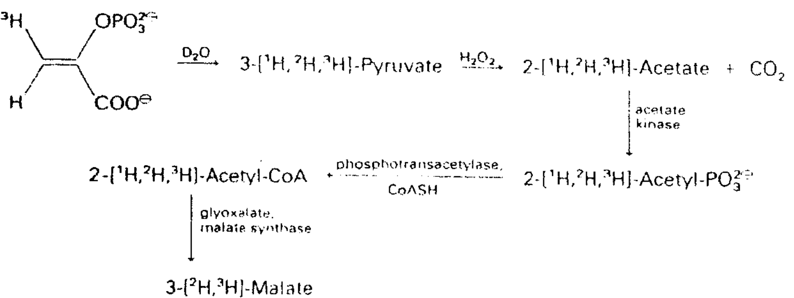


The other reason for choosing this enzyme is that the *S*-malate can then be submitted to the action of fumarase, which removes the hydrogen species at the 3*R*-position of 2*S*-malate. Monitoring of the amount of ^3H_2O formed indicates what percentage of the tritium ends up in the *S*-position at C-3 of the malate. As a control, it was established that achiral [^{3}H]-acetate yields 50% of the tritium as ^3H_2O after equilibration by fumarase. Finally, the 2*S*-acetyl-CoA yields a malate that loses 76.5% of its tritium into water in the presence of fumarase. The malate from *R*-acetyl-CoA loses only 23.3% of its tritium. This result indicates that the major product from *S*-acetyl-CoA is 2*S*,3*R*-[^{3}H]-malate, whereas this is the minor product from *R*-acetyl-CoA. The reader should confirm that these results indicate that the glyoxylate fragment adds in a stereochemical sense opposite to that of the departing proton—*condensation proceeds with inversion at the methyl group.* (For detailed structural presentation of these results, see pp. 193–211 and 234–240 of the book by Alworth, 1972.)

Given the knowledge that malate synthetase shows a kinetic isotope effect and catalyzes condensation with inversion, the malate synthase–fumarase couple can be (and is) used as a convenient analytical system to determine the absolute configuration of acetate molecules of unknown chirality. Two cautions are worth voicing. Whenever possible, the experiments should be done with both isomers of chiral acetate and complementary results obtained. Note further that the preferential accumulation of one isomer of malate is related not only to the kinetic isotope effect* but also to the chiral purity of the acetate samples. Less than absolute chiral purity will diminish the real differentials between rates of formation of the major and minor isomers of tritiated malate.

At this point one can see that the methodology can also be applied for enzymatic methylene→methyl conversions, the reverse of the methyl→methylene transformation. Given a methylene group stereospecifically labeled with two isotopes of hydrogen, if the enzyme cleaving the methylene group to a methyl moiety introduces the third hydrogen isotope in a stereospecific manner, the resultant product will have a chiral methyl group. It is worth noting explicitly that *in this direction (methylene → methyl), an isotope effect is not required for accumulation of a chiral product species.*

With this explanation of the principles and methodology of chiral methyl-group determination, we can return to the carboxytransphosphorylase reaction and the CO_2-independent production of pyruvate from PEP. Rose first used (*Z*)-[^{3}H]-PEP and P_i in D_2O with the transphosphorylase to generate molecules of pyruvate with all three hydrogen isotopes in the methyl group (Willard and Rose, 1973).



The pyruvate sample was decarboxylated with H_2O_2 to acetate, which then was subjected sequentially to acetate kinase, phosphotransacetylase, malate synthase, and fumarase. Surprisingly, fumarase treatment liberated only 50% of the

* The relevant kinetic isotope effect is the one describing *intramolecular* competition between H, D, or T removal from a *given* chiral methyl group (Eggerer and Klette, 1967).