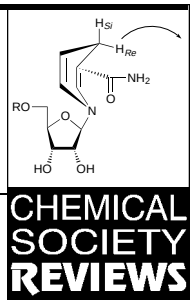
The mechanistic and evolutionary basis of stereospecificity for hydrogen transfers in enzyme-catalysed processes



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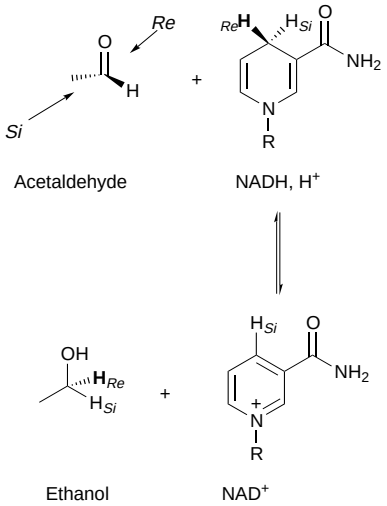
The origin of stereospecificity in enzyme-catalysed processes is attributed to either mechanistic imperatives or to the evolutionary origin of an enzyme. Recent analysis of the *syn–anti* dichotomy for hydratase–dehydratase enzymes has clearly demonstrated that it is a historical contingency that has played a significant role in determination of stereospecificity. Historical contingencies also appear to play a significant role in (a) the selection of one out of a possible eight different stereochemical courses available for an enoyl thioester reductase, and (b) the discrimination between diastereotopic hydrogens in coenzyme B_{12}-dependent rearrangements.

1 Introduction

Enzymes catalyse reactions with remarkable degrees of regiospecificity and stereospecificity. Often enzyme-catalysed processes contain some cryptic stereospecificity that can only be solved by either stable or radioactive isotope labelling experiments. Experiments carried out by Frank Westheimer and Birgit Vennesland in the 1950s are now used as the biochemistry textbook example of the use of such an approach to investigate the stereospecificity of the reaction catalysed by yeast alcohol dehydrogenase (YADH).[1] In this study it was demonstrated that a hydrogen from the *pro*-4*R* position of NADH was transferred to the *Re* face of acetaldehyde. In the reverse reaction the *pro-R* hydrogen at C1 position in ethanol is transferred to the *Re* face of NAD^+ (Scheme 1). In one direction of the reaction the enzyme discriminates between the diastereotopic hydrogens attached at a C4 of the cofactor. In the opposite reaction direction the YADH discriminates between two diastereotopic hydrogens attached to C1 of the substrate ethanol.

The basis for the high degree of stereospecificity in the YADH case is the particular arrangement of the substrate and cofactor at the active site. The X-ray crystal structure of YADH



Scheme 1 Stereochemical course of the reaction catalysed by the yeast alcohol dehydrogenase

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Koren Holland was born in Massachusetts in 1963. After taking her chemistry degree at Skidmore College in 1985, she moved to the University of Maryland at College Park. Under the direction of John Kozarich she obtained her PhD in bioorganic chemistry in 1990. She received a postdoctoral research fellowship from the National Institutes of Health and moved to the Johns Hopkins University, Department of Chemistry, and worked under the direction of Craig Townsend until 1992. She is currently an Assistant Professor of Chemistry at Gettysburg College with research interests in secondary metabolic pathways.

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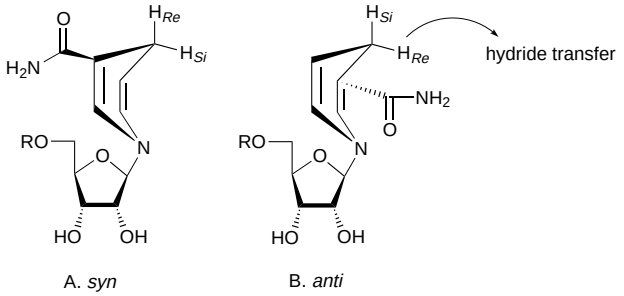
with ethanol and NAD^+ at the active site is clearly consistent with the observed stereospecific course of the enzyme-catalysed reaction.[2]

2 Interpretation of stereochemical diversity

Intriguingly, other alcohol dehydrogenases differ from the YADH and catalyse reactions by transferring the opposite diastereotopic hydrogen of NAD(P)H. Furthermore, an analysis of all dehydrogenases has revealed that almost a 50/50 mix of enzymes use the *pro*-4*R versus* the *pro*-4*S* of NAD(P)H. Clearly in approximately half of the cases studied the orientation of the $NAD(P)^+$ ring with respect to substrate is different to that observed for the YADH (Scheme 2).[3] Mechanistic and historical theories concerning the origin of this stereochemical diversity for dehydrogenases have been presented.[4–6] These have been extensively reviewed previously and are summarized below.



Scheme 2 The conformation of *syn* and *anti* NAD(P)H

The mechanistic model holds that the two heterotopic hydrogens of NAD(P)H have a slightly different redox potential depending upon the *syn* and *anti* orientation (Scheme 2) and that this potential should closely match that of the substrate in order for the enzyme to be a good catalyst.[4] This argument suggests that the differing stereospecificities of dehydrogenases have evolved in order to meet a mechanistic imperative associated with the different substrates. Thus, this model is consistent with Bentley's first rule that enzymes accepting the same substrates generally have the same stereospecificity.[7] However, the mechanistic model presents no clear explanation for exceptions to this rule (the stereospecificity of 3-hydroxy-3-methylglutaryl CoA reductases from *Acholeplasma laidlawii* and yeast for example are different).[6] An additional weakness of the mechanistic model is that it hinges on the hypotheses that evolutionary selection pressures have produced enzymes with catalytic optimality. It has been argued more recently, however, that modern protein diversity only represents a very limited exploration of different amino acid sequences space and that this exploration is limited by the success of earlier motifs.[8] Therefore, the reaction pathways of many enzyme-catalysed processes may represent a local rather than a global local optimum.

The suggestion that some extant proteins do not utilize the most efficient reaction pathway is consistent with an historical interpretation of dehydrogenase stereospecificity. This model proposes that stereospecificity is not a functional trait but is a vestige of an arbitrary choice made early and then retained during the evolutionary process. The stereospecificity of an enzyme-catalysed process is, therefore, maintained during evolution and is a reflection of an enzyme's heritage. An historical model, like the mechanistic model, can rationalize Bentley's first rule with the presumption that all enzymes from all organisms acting on a particular substrate have descended from a single ancestral enzyme. Such a presumption now appears to be quite reasonable; malate dehydrogenase is an example of an enzyme which has both a highly conserved stereospecificity and amino acid sequence (greater than 50% amino acid sequence identity is shared between malate dehydrogenase cloned and sequenced from mammals, plants and microorganisms).[9] For exceptions to Bentley's first rule (such as HMG CoA reductase) the historical model assumes that either (a) these enzymes have independent pedigrees, or (b) the stereospecificity of these dehydrogenases has drifted (whereas for instance it is conserved for the malate dehydrogenases).[6]

The discussions surrounding functional and historical models have been extended to other enzyme-catalysed processes which discriminate between diastereotopic hydrogens. It has been suggested that some of these reactions may also be controlled by mechanistic imperatives and that any historical model to explain all of the observed data would be extremely complex.[6] In this review article we analyse recent stereochemical and protein sequence analyses for three enzyme classes; dehydratase–hydratases, enoyl thioester reductases, and coenzyme B_{12}-dependent mutases. The data presented clearly show that in all three cases the stereospecificity of the enzyme class appears to be based on an historical contingency. We aim to stimulate the reader to consider the ramifications of these results for the controversy surrounding the basis of dehydrogenase stereospecificity.

3 Hydratase–dehydratase enzymes

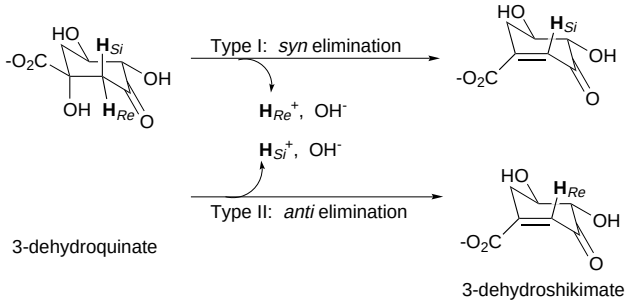
There are two classes of hydratase–dehydratase enzymes; those that catalyse the addition of water in a *syn* fashion and those that do so in an *anti* fashion. This dichotomy could reflect functional advantages. All hydratase–dehydratase reactions where a proton is abstracted α to a carboxylate group proceed with *anti* chemistry (seven examples, including fumarate hydratase, aconitate hydratase and enolase are known).[10] In nonenzymatic reactions the *anti* elimination is favoured over the *syn* elimination as the latter requires an eclipsed geometry in the transition state. Accordingly, the stereospecificity of an *anti* elimination reaction may serve as a selected function which either constrains divergent evolution or encourages convergent evolution. However, there is a group of enzymes that produce *syn* elimination reactions of water (including enoyl coenzyme A hydratases, fatty acid synthases and β-hydroxydecanoyl thioester dehydratases). A functional theory for determining stereospecificity for hydratase–dehydratases would require that a two step *syn* elimination is specifically favoured for the substrates used by these enzymes. In this class of enzymes the proton is abstracted α to a carbonyl group of a thioester or a ketone, and it is suggested that acidity of this proton may alter the preferred pathway; the α-protons of thioesters are more acidic than those of either carboxylate salts or free acids and may tilt the stereochemistry towards *syn* elimination in which an economical single acid–base group would interact with both the α-proton and the β-leaving group.[3,11] Thus a functional theory exists for the stereochemical dichotomy of the hydratase–dehydratases as well as the dehydrogenases.

This functional theory has been investigated by comparing the chemical stereoselectivity for a non-enzymatically catalysed reaction with the analogous enzyme-catalysed process.[10] It was shown that conjugate addition of water or fumarate was slightly biased towards *anti* addition (1.3 : 1). In contrast, addition of water to the α,β-unsaturated thioester of crotonic acid produces a substantial 4.3 : 1 bias towards *anti* addition. These observations are opposite to the enzymatic *syn–anti* dichotomy, where fumarate hydratase and enoyl CoA hydratase give *anti* and *syn* addition–elimination pathways, respectively. Therefore, the enoyl CoA hydratase utilizes the *syn* pathway for reasons other than a mechanistic imperative.

A historical theory would argue that each stereochemical class of hydratase–dehydratase has originated from at least two different ancestral progenitors; enzymes catalysing *syn* elimination pathways are not related to those which catalyse *anti* eliminations. This theory would also predict that once a *syn* elimination pathway was adopted it would be subsequently conserved during the evolution. An enzyme such as the enoyl

CoA hydratase would, therefore, have optimized this *syn* pathway within the confines of a conserved active site structure. The difference between *syn* and *anti* elimination pathways involving unhindered acyclic substrates is relatively small (less than 3 kcal mol^{-1}) and thus may not be sufficient to deter an enzyme from adopting a *syn* elimination pathway. Interestingly, the difference in reduction potential of the NAD(P)H in the *syn* and *anti* form is even less, approximately 1.3 kcal mol^{-1} (1 cal = 4.184 J).[2]

The dehydroquinate dehydratases (DHQase) provide additional compelling evidence for a historical contingency in the stereochemistry of hydratase–dehydratase enzymes. This enzyme catalyses a dehydration reaction of dehydroquinic acid to dehydroshikimic acid (Scheme 3). The type I DHQases catalyse this reaction with removal of the *pro*-2*R* hydrogen, whereas the type II DHQases remove the *pro*-2*S* hydrogen.[12] This stereochemical dichotomy for the same substrate cannot be readily explained by a mechanistic imperative (the substrate is the same for both types of DHQase). An historical contingency would argue that the two enzyme types are unrelated. Indeed, there is no clear amino acid sequence homology between the type I and the type II dehydratases.[13] All type II dehydroquinate dehydratases that have been cloned and sequenced show strong homology at the level of the predicted amino acid sequence. The same is true for all of the type I dehydroquinate dehydratases. Two distinct families of dehydroquinate dehydratases have evolved that catalyse a reaction with a different mechanism and a different stereospecificity.[13] The stereospecificity of the DHQases reflects the evolutionary origins, or pedigree, of the enzyme.

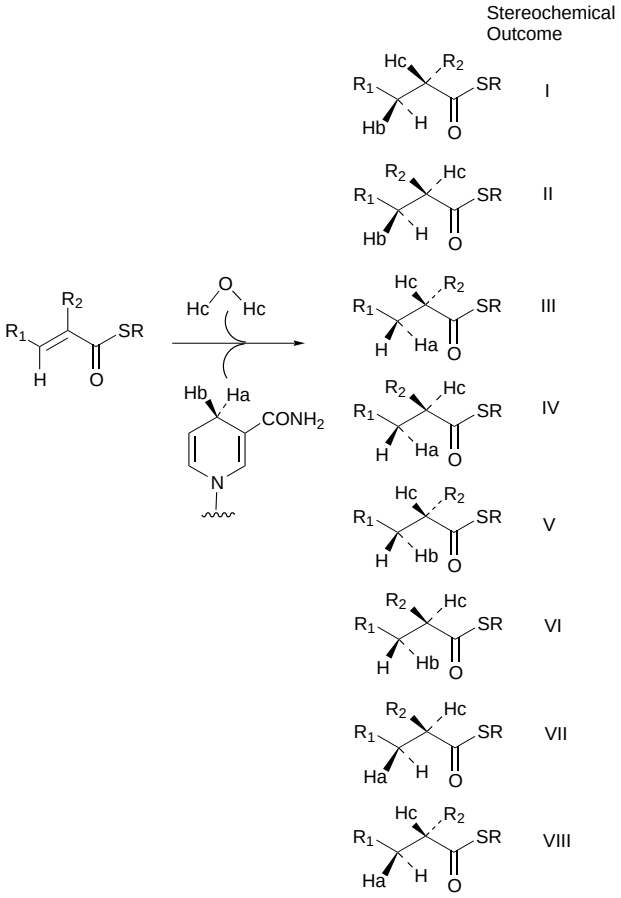


Scheme 3 Stereochemical course of the reaction catalysed by Type I and Type II dehydroquinate dehydratases

4 The enoyl thioester reductases

This class of enzymes is involved in the conversion of an enoyl thioester to an acyl thioester (Scheme 4).[14] There are three cryptic stereochemical details for these reactions: (a) the transfer of either the *pro*-4*R* or *pro*-4*S* hydrogen of NADPH to the substrate, (b) addition of this hydrogen to either the *Re* or *Si* face of the β-carbon of the substrate, and (c) the addition of a solvent hydrogen to either the *Re* or *Si* face of the α-carbon of the substrate. Accordingly, there are a total of eight different stereospecific courses that an enoyl thioester reductase can follow (assuming that the regiospecificity of the process is always addition of the solvent hydrogen at the α-carbon) (Scheme 4). Until recently, only four of these stereochemical outcomes had been observed with the enoyl thioester reductases (Table 1). A general pattern had emerged in which the nucleotide specificity, either *pro*-4*R* or *pro*-4*S*, appeared to determine the stereospecificity of the hydrogen addition at the β-carbon of the fatty acid, *pro*-3*R* or *pro*-3*S* respectively.[15] If this observation were applicable for all enoyl thioester reductases, no additional stereochemical outcomes would have been observed.

Recently, however, it has been shown that a crotonyl CoA reductase (CCR) from *Streptomyces collinus* converts crotonyl CoA to butyryl CoA with addition of the *pro*-4*S* hydrogen of the



Scheme 4 Eight possible stereochemical outcomes for a reaction catalysed by an enoyl thioester reductase. In all outcomes the solvent hydrogen is added to the α-carbon (an additional eight stereochemical outcomes would be possible if addition to the β-carbon was allowed. SR represents either coenzyme A (SCoA) or an acyl carrier protein (SACP).

nucleotide to the *Re* face of the β-carbon and solvent hydrogen to the *Re* face of the α-carbon.[14] With the inclusion of this novel stereochemical course five of the eight stereochemical outcomes possible for enoyl CoA thioester reductases have now been observed (Table 1). As the nucleotide stereospecificity does not determine the stereospecificity of hydrogen addition at the β-carbon, it has been suggested that eventually examples of all eight possible stereochemical outcomes may be observed for these enzymes.[14]

Enoyl thioester reductases represent an unusual case where both a functional and a historical model must be invoked to interpret the observed regio- and stereo-specificities.[14] In all cases examined to date the solvent hydrogen is added to the α-carbon. This observation is associated with a mechanistic imperative associated with the polarization of the α,β-double bond. In contrast, there is no clear mechanistic imperative for the tremendous diversity in the stereospecifity of these enzymes. As the reduction potential of acyclic enoyl thioesters is unlikely to vary significantly, the dichotomy in nucleotide specificity is likely determined by a historical contingency. This argument can be extended to include the additional stereochemical details of enoyl thioester reductases; for instance the addition of the nucleotide hydrogen to the *Re* or *Si* face of the β-carbon of the substrate has no clear mechanistic advantage. Thus, the five different stereospecificities of the enoyl thioester reductases may correlate with a minimum of five different evolutionary origins (*i.e.* the enzymes that exhibit different stereospecificities are not related, while those exhibiting the same stereospecificities may or may not be related). An alternative interpretation is that the enoyl thioester reductases share a common evolutionary origin, but that their ster-

Table 1 Stereochemical outcome of the reaction catalysed by various enoyl reductases[a]

Enzyme source	Stereospecificity of NAD(P)H	Attack of hydrogen		Type of addition	Stereochemical outcome
		C-3	C-2		
Brevibacterium ammoniagenes[b,c]	*pro-4S*	*Si*	*Si*	*anti*	II
Yeast[b,c]	*pro-4S*	*Si*	*Si*	*anti*	II
Rat[b,c]	*pro-4R*	*Re*	*Si*	*syn*	IV
Chicken[b,c]	*pro-4R*	*Re*	*Si*	*syn*	IV
E. coli (chain elongation)	*pro-4R*	*Re*	*Re*	*anti*	III
E. coli[b]	*pro-4S*	*Si*	*Re*	*syn*	I
S. collinus (ChcA)[b]	*pro-4S*	*Si*	*Si*	*anti*	II
S. collinus (CCR)[b,d]	*pro-4S*	*Re*	*Re*	*anti*	V

[a] Only enoyl thioester reductases where all three cryptic stereochemical details have been elucidated are included. Roman numerals are used to correlate the observed stereochemical outcomes with those depicted in Scheme 4. Most of the enzymes are enoyl ACP reductases involved in *de novo* fatty acid synthesis. The exceptions are the crotonyl CoA reductase (Ccr) and 1-cyclohexenylcarbonyl CoA reductase (ChcA) of *S. collinus* and the *E. coli* enoyl thioester reductase involved in fatty acid elongation. [b] Denotes enzymes whose predicted amino acid sequence is available. [c] Denotes two groups of enzymes that have significant amino acid sequence similarity *within* the group. The remaining enzymes have no significant amino acid sequence similarity with each other. [d] Findings from this work.

eospecificity has diverged. It has been noted that the tremendous diversity observed in both the predicted amino acid sequence and putative evolutionary origin of enoyl thioester reductases presents clear evidence contrary to the latter of these proposals.[14] Evidence consistent with the former of these proposals has been provided by examination of the stereospecificity and amino acid sequence of the enoyl thioester reductases listed in Table 1.

Enoyl thioester (ACP) reductases are involved in fatty acid biosynthesis. The *Escherichia coli* enoyl ACP reductase involved in *de novo* synthesis exhibits a different stereospecificity to the eukaryotic enoyl ACP reductases (Table 1). Consistent with this observation, the predicted amino acid sequence data of *fabI* (the gene that putatively encodes this enzyme) shows similarity to some alcohol dehydrogenases, but not with other enoyl ACP reductase.[16] In contrast, the enoyl ACP reductase sites in the chicken and rat fatty acid synthases (FASs) exhibit 83% identity (overall these two synthases exhibit 67% identity), and catalyse reactions with the same stereospecificity (Table 1). The yeast and *Brevibacterium ammoniagenes* have significant sequence homology (30 and 46% identity along the entire sequence for FAS1 and FAS2, respectively)[17] and the respective enoyl ACP reductases catalyse reactions with identical stereospecificities (Table 1).[18] Finally, it has been noted that the yeast–*B. ammoniagenes* and the chicken–rat FAS enoyl ACP reductases which catalyse reactions with different stereochemical outcomes have no clear amino acid sequence similarity.[14] It has even been suggested that the yeast and chicken FAS either diverged from a common evolutionary pathway at a very early time, or were obtained *via* a different evolutionary pathway.[19]

The crotonyl CoA reductase (CCR) of *S. collinus* has a different stereospecificity to other enoyl thioester reductases where the overall stereospecificity is known.[14] Analysis of the amino acid sequence of this CCR with these enoyl thioester reductases, where the sequence is known, reveals no obvious similarity. In fact CCR is not related to enoyl thioester reductases at all, but rather to alcohol dehydrogenase members of the quinone oxidoreductase superfamily.

Finally, the stereospecificity of the 1-cyclohexenylcarbonyl CoA reductase (ChcA) of *S. collinus* matches that observed for the yeast–*B. ammoniagenes* FAS enoyl ACP reductase. Significantly, the amino acid sequence of ChcA shows no sequence similarity with other enoyl thioester reductases but rather with members of the short-chain alcohol dehydrogenase superfamily.[20]

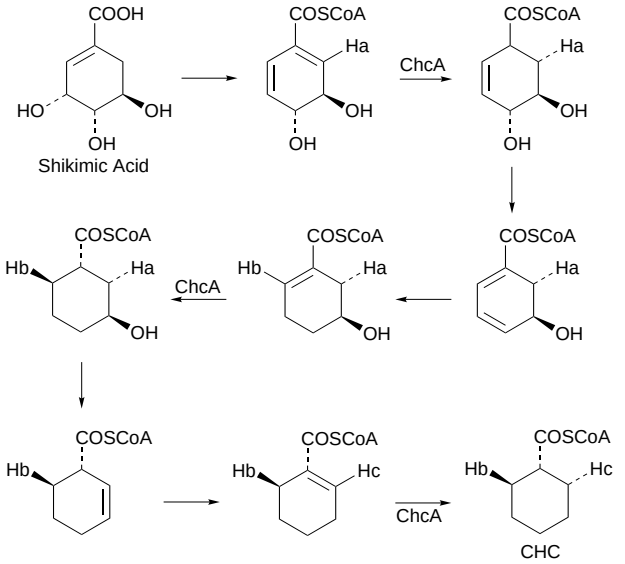
These observations for CCR, ChcA and the FAS enoyl ACP reductases are all consistent with the suggestion that an observation of diversity in stereospecificity indicates unrelated enzymes while identical stereospecificity may indicate related enzymes. The comparison of the yeast FAS enoyl ACP reductase and ChcA, however, demonstrates that the enzymes need not be related.

This historical model for enoyl thioester reductase stereospecificity has been presented only very recently, and is based on the availability of both a complete stereochemical analysis of each enzyme-catalysed reaction and the predicted amino acid sequence.[14] However, the principles of this model have been used for more than a decade to make predictions regarding relationships between enoyl thioester reductases based simply on a comparison of just one or two stereochemical details. Subsequent analyses have always proved consistent with these initial predictions.

For instance, collected findings with fungal systems have shown that within an organism the enoyl thioester reductase reactions that occur in both fatty acid and polyketide biosynthesis proceed with opposite stereospecificities from the perspective of solvent hydrogen addition at the α-carbon. The data have often been used to argue that different enzymes catalyse the reductions in these processes.[21] Furthermore, it has been argued that these enzymes would have to be unrelated. All available data supports this hypothesis.[14] The one exception to the findings with fungal polyketide and fatty acid biosynthesis has been the long standing studies of the biosynthesis of averufin and fatty acids in *Aspergillus parasiticus*. In this case the first three enoyl thioester reduction steps in both processes proceed with solvent hydrogen addition to the same face of the growing polyketide and fatty acid. This observation has been used to argue that the first three addition reactions in the polyketide biosynthetic process cannot be catalysed by a polyketide synthase (which has a different stereospecificity) but rather by a fatty acid synthase (*i.e* conserved stereospecificitiy *may* indicate that a reaction is carried out by a related enzyme).[21] This prediction has recently been substantiated by genetic analysis of the averufin polyketide synthase (PKS) gene cluster which reveals a FAS-like gene essential to the biosynthetic process.[22]

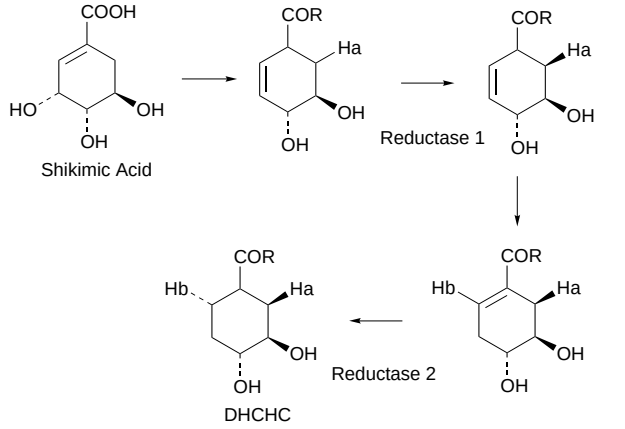
In *S. collinus* it has been shown that a pathway from shikimic acid to cyclohexanecarboxylic acid (CHC) proceeds with three separate enoyl thioester reductions, each proceeding in an *anti* fashion with addition of solvent hydrogen to the *Si* face of the α-carbon (this *in vivo* analysis did not allow the nucleotide specificity to be determined) (Scheme 5).[23] The enzymes which catalyse these reactions, therefore, may be similar. In fact the possibility that one enzyme is responsible for catalysing all three reductions was raised.[23] Recent enzymatic and genetic analysis of ChcA has proved consistent with this prediction.[20]

In *S. hygroscopicus* it has been shown that a pathway from shikimic acid to dihydroxycyclohexanecarboxylic acid (DHCHC) proceeds with two separate enoyl thioester reduc-



Scheme 5 Conversion of shikimic acid to cyclohexanecarboxylic acid (CHC) in *S. collinus*. The three α,β-enoyl thioester reduction steps have been shown to be catalysed *in vitro* by 1-cyclohexenylcarbonyl CoA reductase (ChcA).

tions (Scheme 6).[24] The stereospecificity of both of these reductions differs from that observed in the CHC pathway. Thus it has been predicted that the enoyl thioester reductases involved in DHCHC biosynthesis are unrelated to the ChcA protein. All data obtained to date are in full agreement with this proposal. In addition it has been noted that the stereochemical outcome of the two enoyl thioester reductions in the DHCHC pathways differs, suggesting that they must be carried out by different enzymes.[24] In fact it has been suggested that the first reduction may not even be carried out with the substrate activated as a thioester.[14]



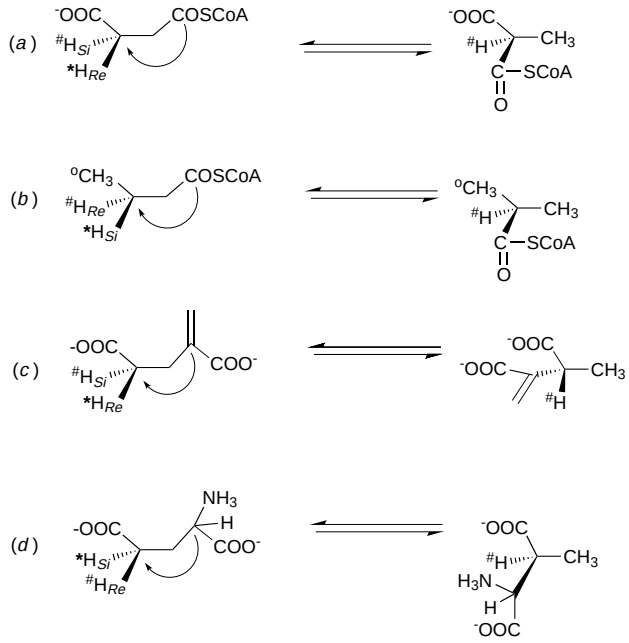
Scheme 6 Conversion of shikimic acid to dihydroxycyclohexanecarboxylic (DHCHC) acid in *S. hygroscopicus*

Taken together these observations are all consistent with the following conclusions; (a) enoyl thioester reductases that have different stereospecificities have unrelated amino acid sequences and (b) the stereospecificity of an enoyl thioester reductase is not a functional trait and reflects the evolutionary origin of the protein. Finally, there is no evidence that stereospecificity of enoyl thioester reductases can diverge.

5 Coenzyme B_{12}-dependent rearrangements

A number of coenzyme B_{12}-mediated rearrangements also involve the stereospecific removal of a heterotopic hydrogen.[25,26] For the purposes of this review we have only considered mutases involved in carbon skeleton rearrangements: methylmalonyl CoA mutase, isobutyryl CoA mutase, glutamate mutase and methyleneglutarate mutase.

Methylmalonyl CoA mutase catalyses the conversion of succinyl CoA to methylmalonyl CoA by removal of the *pro*-3*R* hydrogen of succinyl CoA (Scheme 7). A high degree of homology between the predicted amino acid sequences of the human, mouse and bacterial (*Propionibacterium shermanii* and *S. cinnamonensis*) methylmalonyl CoA mutases suggests that these enzymes have evolved from one common ancestor.[27] Isobutyryl CoA mutase catalyses the conversion of butyryl CoA to isobutyryl CoA with the abstraction of the *pro*-3*S* hydrogen (Scheme 7). As shown in Scheme 7 the absolute stereochemistry for this hydrogen abstraction is the same as that observed for methylmalonyl CoA mutase. As there is no obvious mechanistic rationale for the stereospecific selection of either the *pro*-3*S* or the *pro*-3*R* hydrogen in these reactions it is reasonable that such a choice is based on an historical contingency. Thus, methylmalonyl CoA mutases and isobutyryl CoA mutase may be related. Professor J. A. Robinson has determined that the isobutyryl CoA mutase and large subunit of methylmalonyl CoA mutases of *S. cinnamonensis* share 44% identity (personal communication).



Scheme 7 Stereochemical course for the coenzyme-B_{12}-dependent rearrangements catalysed by (*a*) methylmalonyl CoA mutase, (*b*) isobutyryl CoA mutase, (*c*) 2-methyleneglutarate mutase, and (*d*) glutamate mutase. *H represents the hydrogen which is abstracted in the rearrangements.

Glutamate mutase catalyzes the interconversion of glutamate and (2*S*,3*S*)-3-methylaspartate. This reaction proceeds with abstraction of the *pro*-4*S* hydrogen and thus differs from the reactions catalysed by either isobutyryl CoA mutase or methylmalonyl CoA mutase (Scheme 7).[26] Methyleneglutarate mutase catalyses the interconversion of 2-methyleneglutarate and (*R*)-3-methylitaconate (2-methylene-3-methylsuccinate). In this reaction the *pro*-4*R* hydrogen methyleneglutarate is removed (Scheme 7).[26] It has been suggested previously that the stereochemical difference in the proton abstraction for these two enzymes may have arisen due to small changes in the active site geometry with respect to the carboxylate binding functionalities.[26] In other words the stereospecificity of these coenzyme B_{12}-dependent rearrangements has diverged. In apparent agreement with this proposal is the amino acid similarity between the cobalamin-binding domains of the methyleneglutarate mutase and glutamate mutase.[28]

An alternative explanation for the diversity in stereospecificity for these two enzymes is that it reflects diversity in evolutionary origin. As such, glutamate mutase should be

unrelated to methyleneglutarate mutase, while the latter may be related to isobutyryl CoA and methylmalonyl CoA mutase. Such a proposal might seem to contradict the observed sequence similarity between the cobalamin-dependent domains of these enzymes. However, it has been noted that while these enzymes share a homologous region of about 110 residues in the cobalamin binding domain, there are no homologies outside of this region.[25] This domain is located in the C-terminus of 2-methyleneglutarate mutase and methylmalonyl CoA mutase and in a separate subunit of glutamate mutase. The larger catalytic subunit of this latter enzyme shows no significant similarity to any known protein (including methyleneglutarate mutase). These and additional observations have led to the proposal that the different mutases have not diverged from a common ancestor but rather have acquired their cobalamin-binding domain by a gene fusion event.[25]

Thus this aspect of the stereochemical diversity of mutases, like the dehydratases–hydratase and enoyl thioester reductases, may be driven by a historical contingency in which the stereospecificity does not readily diverge. Proponents of the functional model for stereospecificity in dehydrogenases have contended that stereospecificity can diverge during evolution.[2] It has been suggested that changes in the amino acid residues that interact with the nicotinamide ring may allow a 180° rotation around the glycosidic bond and thus alter the stereospecificity (Scheme 2). A double mutant of yeast alcohol dehydrogenase has been created to demonstrate that with the appropriate substitutions the enzyme specificity can be from *Re* specific to *Si* specific.[2] The corresponding mutant transferred the *pro*-4*R* hydrogen of NADH 850 000 times for every transfer of the *pro*-4*S* hydrogen (as compared to 7 000 0000 000 to 1 in the native protein). While there is a dramatic drop in the ratio of the transfers of the different heterotropic hydrogens, it is still clear that the mutant YADH retains significant stereospecificity. No clear evidence that dehydrogenase stereospecificity can change during evolution has been presented thus far. The stereospecificity of the coenzyme B_{12}-dependent mutases by comparison is very fragile. When methylmalonyl CoA is presented with succinyl CoA containing a heavy isotope at the *pro*-3*R* position (the labilisable position) the enzyme will actually alter the steric course and abstract the *pro*-3*S* hydrogen.[29] A similar situation has been reported for the stereospecificity of the glutamate mutase reaction.[30] Thus a simple isotopic substitution can alter the stereospecificities of both of these mutases. Despite this fragility it appears that all methylmalonyl CoA mutases and the related isobutyryl CoA mutases have retained the same stereospecificity during evolution. Similarly there is no evidence to indicate that either the respective stereospecificities of glutamate mutase and 2-methyleneglutarate have changed.

The abstracted hydrogen in a coenzyme B_{12}-dependent rearrangement is replaced by a migrating group (COSCoA in the case of methylmalonyl CoA mutase). While this review has been limited to discussion of stereospecificity to discrimination between heterotopic hydrogens, it is noted that the related isobutyryl CoA mutase and methylmalonyl CoA mutase both carry out this replacement with retention of configuration, while glutamate mutase and methyleneglutarate mutase do so with inversion of configuration (Scheme 7).[26] Thus, only the reactions catalysed by related mutases proceed with identical stereochemical courses. Recently, a number of laboratories have identified a gene *mea*A from both methylotrophs and streptomycetes that appears to encode a novel coenzyme B_{12}-dependent mutase. The *mea*A gene product shares global homology with both methylmalonyl CoA mutase and isobutyryl CoA mutase.[31] While the substrate for this enzyme remains undetermined it is reasonable to predict that the stereochemical course of the rearrangement will exhibit the same characteristics as these mutases.

6 Concluding comments

There are four interrelated observations that can be drawn from the analyses of the stereospecificities and amino acid sequences of the three classes of enzymes described herein.

(a) Enzymes within a specific class that are related at the amino acid level all catalyse their respective reactions with the same stereospecificity. This observation supports the hypothesis that once stereochemistry is set it is retained in the process of evolution.

(b) If the enzymes within a specific class are unrelated at the amino acid level it is possible that they may catalyse reactions with opposite stereochemistry. However, as there are a limited number of stereochemical courses available for enzymes (a total of eight for enoyl thioester reductases and only two for hydratase–dehydratases) there will be examples where unrelated enzymes share the same stereochemical course. In either case the stereospecificity is dictated by an historical contingency and not a mechanistic imperative.

(c) Enzymes that catalyse similar or analogous reactions with different stereochemistries have unrelated amino acid sequences. The observation that enzymes can converge to catalyse analogous reactions with opposite stereospecificities is incongruous with mechanistic imperatives and fully aligned with historical interpretations of the origins of stereospecificity.

(d) Although probability predicts that the same stereochemical course will be shared by unrelated enzymes [conclusion (b)], it appears that enzymes that catalyse analogous reactions with the same stereochemistries are often related. This observation holds true for each of the following: the dehydroquinate dehydratases, each of the coenzyme B_{12}-dependent mutases and the enoyl thioester reductases involved in fatty acid biosynthesis.

Historical contingencies have now been shown to be the origin of the stereospecificities of a number of enzyme reactions which discriminate between diastereotopic hydrogens. As discussed above the historical model for interpreting stereospecificity makes the following assumptions (i) that enzymes from all organisms catalysing the same reaction with the same stereospecificity are homologous, and (ii) that stereospecificity is conserved during evolution. Critics of this model note that in cases where enzymes catalyse the same reactions with opposite stereochemistry the following additions or changes must be made; (1) there are separate ancestral genes for these enzymes or (2) that stereospecificity can diverge for some enzymes and not others. It now appears that the former of these modifications is consistent with the data available to date.

It has been over fifteen years since the functional imperative was proposed for dehydrogenase stereospecificity. Most of these interpretations were carried out without any significant protein sequence information. The rapidly growing protein sequence database should now provide an opportunity to revisit this controversial issue especially in the light of recent developments demonstrating the role of historical contingencies for stereospecificity in enoyl thioester reductases (which also use a nucleotide cofactor) and hydratase–dehydratases (where there was a substantially stronger case for a mechanistic imperative).

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