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The stereoselectivity of alcohol dehydrogenases: A stereochemical imperative?[1]

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Summary. The stereoselectivity of NAD^+-dependent alcohol dehydrogenases (transfering either the pro-R or pro-S hydrogen of NADH) correlates with the thermodynamic stability of their substrates, and appears to reflect evolutionary pressure to adjust in the active site the conformation of NADH so as to match the cofactor's reducing power to the oxidizability of the substrate. A requirement that the free energies of protein-bound intermediates be matched suggests a new approach for understanding catalysis and evolution in enzymes.

Stereoselective transfer of hydrogen to and from nicotinamide cofactors is the best studied example of the power of enzymes to make stereochemical choices. Westheimer, Vennesland and coworkers[2] first demonstrated this selectivity in the early 1950's, showing that dehydrogenases acting on NADH occur in 2 stereochemically distinct classes. Members of 1 class catalyze the transfer of the pro-R (A) hydrogen; member of the 2nd class catalyze the transfer of the pro-S hydrogen. More recently, work on well over a 100 additional dehydrogenases has shown that roughly half of all dehydrogenases belong to the 1st class and half to the 2nd[3,4]. The large body of data for dehydrogenases has prompted many authors[4-7] to attempt to discern a pattern in the stereopreferences of these enzymes. Hoping to understand why a particular dehydrogenase belongs to a particular class, several investigators have formulated a variety of 'rules' regarding the stereochemical preferences of dehydrogenases[4-7]. However, most of the 'rules' have exceptions, and some appear decidedly ad hoc; none have a clear mechanistic basis. Thus, none appear to have been accepted as part of a general explanation for the stereopreferences of dehydrogenases. Still others have concluded that the data contain no pattern whatsoever, that no mechanistic explanation exists, and that the stereopreferences are 'random'[8]. Thus, despite a quarter century of speculation, the mechanistic basis for the different stereochemical behavior of dehydrogenases remains a mystery.

Stereochemical ambivalence in dehydrogenases is unusual, since stereochemical uniformity seems to be the rule for most classes of enzymes. These uniformities have been analyzed recently by Hanson and Rose[8], who argued that uniform stereochemical preferences displayed by the members of many classes of enzymes catalyzing similar reactions might reflect the existence of a 'mechanistic imperative' in these enzymes. For chemical reasons inherent in the nature of the reaction being catalyzed, a single mode of catalysis producing a certain stereochemical outcome is presumed to be more efficient than alternative modes that produce other stereochemical outcomes. Because an organism possessing highly efficient enzymes is more likely to survive than a competing organism possessing less efficient enzymes, Hanson and Rose[8] argued that evolutionary processes may have forced the selection of only those enzymes that are optimal catalysts, and thereby have selected only those enzymes making the optimal stereochemical choice.

The stereochemical similarities within a class of enzymes can be quite striking. For example, all enzymes using pyridoxal cofactors shuttle a proton by attacking C-4′ of the pyridoxal group from the si face. All amino acid decarboxylases proceed with retention of configuration. Of course, these uniformities can possibly be understood in 'historical' terms; if all of the members of these classes of enzymes have evolved from a common ancestral protein that catalyzed a primeval prototypical reaction, the stereochemical preferences of the ancestor may have been conserved. Nonetheless, Hanson and Rose[8] suggested a number of chemical explanations for why one stereochemical outcome might be catalytically superior to an alternative outcome. In the case of dehydrogenases, where non-uniformity is the rule, the arguments of Hanson and Rose force one to the conclusion either that no stereochemical imperative exists favoring one stereochemical course over the other, or that an imperative does exist, but the processes of evolution have not been able to exploit it[9]. The 1st conclusion is remarkable from the view of bio-organic chemistry, and suggest a need for further investigation to determine which stereochemical distinctions are important catalytically and which are not. The 2nd conclusion is remarkable from an evolutionary point of view, since it contradicts the emerging belief that the processes of evolution have produced enzymes that approach catalytic 'perfection'[10].

The data. We report here a hitherto unnoticed regularity which suggests that the stereochemical outcome in dehydrogenases acting on alcohols is neither random nor evolutionarily neutral, but reflects the evolution of these enzymes to produce an active site that is catalytically optimal. A plot (fig. 2) of stereochemical outcome (pro-R or Pro-S) versus equilibrium constant:

$(K_{eq} = [\text{ketone}][\text{NADH}][H^+]/[\text{alcohol}][NAD^+]$

for the overall reaction in dehydrogenases acting on simple alcohols shows this correlation most clearly. If the K_{eq} for a particular enzyme-catalyzed reaction is less than 10^{-12} M^{-1},

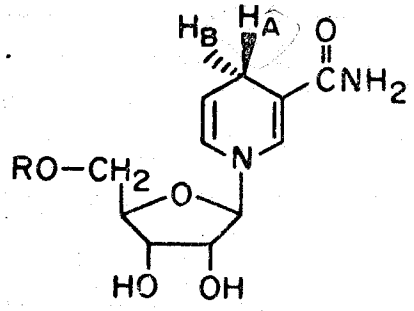

Figure 1. The pro-R (A) and pro-S (B) hydrogens of reduced nicotinamide adenine dinucleotide are diastereotopic.

the pro-R hydrogen is generally transferred; if the K_{eq} is greater than 10^{-12} M^{-1}, the pro-S hydrogen is transferred. We have interpreted these data as suggesting that more reactive carbonyls are reduced by the pro-R hydrogen, while less reactive carbonyls are reduced by the pro-S hydrogen of NADH.

The data summarized in figure 2 are experimental facts that stand independent of any hypothesis advanced to explain them. However, in view of such a striking correlation, we may attempt to provide an explanatory hypothesis, especially one that can be tested by experiment.

The postulates. We suggest that the stereochemical preferences of dehydrogenases reflect their evolution under selective pressure to become optimal catalysts. Specifically, we propose that dehydrogenases have evolved active sites that adjust the conformation of the nicotinamide cofactor so as to match its reduction potential to the ease with which the co-substrate is reduced. Thus, the stereochemical preferences observed in dehydrogenases can be understood on the basis of the following:

1. Enzymatic transfer of the pro-R hydrogen of NADH occurs when the nicotinamide ring is in the anti conformation, while transfer of the pro-S hydrogen occurs when the nicotinamide ring is in the syn conformation (fig. 3).
2. Anti-NADH is a weaker reducing agent than syn-NADH.
3. Enzymes are optimally efficient when the intermediate states along the reaction coordinate are of equal free energies.
4. Dehydrogenases have evolved to be optimally efficient catalysts.

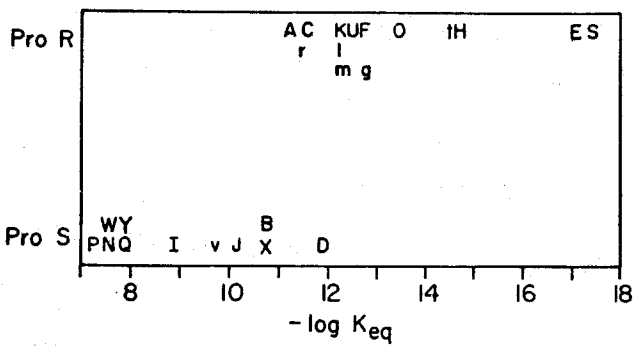

Figure 2. A plot of the stereochemical preference of dehydrogenases (pro-R or pro-S) versus the negative logarithm of the equilibrium constant for the reactions they catalyze. (K_{eq}=[H^+][NADH][ketone]/[NAD^+][alcohol]). A key is found in the table. I have attempted to include in the plot all dehydrogenases acting on simple, unconjugated ketones where both the stereochemical preference and equilibrium constant has been reported for the reduction of the biologically significant substrate (upper case letters). Occasionally, the K_{eq}-values, although not reported for the specific enzyme in question, have been obtained from measurements where the equilibrium was established on the physiological substrate using other enzymes or methods (lower case letters). A number of enzymes with defined stereoselectivity were omitted because of the difficulty of obtaining a K_{eq}-value that reflects the actual redox step. For example, the reaction catalyzed by isocitrate dehydrogenase (pro-R specific) is highly exergonic, since the overall reaction involves the loss of CO_2 in addition to a dehydrogenation. Similarly, the equilibrium constants obtained for the oxidation of poly-alcohols to sugars are invariably between the hemiacetal form of the sugar and the polyalcohol, again obscuring the equilibrium constant for the oxidation/reduction reaction. Also on occasion, an enzyme with defined stereochemistry was omitted if the physiological substrate is in question, or the substrate specificity was too broad to define reasonably the natural substrate. Alcohol dehydrogenase from horse liver is an example of this sort of enzyme.

Support for each of these hypotheses is enumerated below.

Evidence for the postulates. 1. Crystal structures of four dehydrogenases[13] have led several commentators[4,14] to suggest that dehydrogenases that are pro-R specific bind the NAD^+ cofactor in an anti conformation, while those that are pro-S specific bind the cofactor in the syn conformation. In view of the principles of stereoelectronic control[15], one might expect this rule to be general for all dehydrogenases. Transfer of the pro-R hydrogen from an anti conformation permits a desired 'cis' arrangement between the leaving hydride and the pair of electrons on nitrogen[16], distorted from planarity to optimize electron donation into the antibonding orbital of the C–O bond in the ribose ring[17]. Such a transfer would be from the sterically less hindered face of the dihydropyridine ring. Further, experimental work with NAD^+ analogs having methyl groups at the 2 or 6 positions[18], (which are thereby constrained to occupy the anti and syn conformations respectively) suggest that the arguments based on crystallography are valid in solution as well.

2. The 2nd hypothesis is difficult to confirm directly, but since the equilibrium constants between the syn and anti forms of both oxidized and reduced cofactors can be estimated from NMR-data on nicotinamide mononucleotides[19], this relationship can be obtained qualitatively from the thermodynamic diagram shown in figure 4. If for the reduced cofactor the anti conformation is lower in energy than the syn, while for the oxidized cofactor the anti conformation is higher in energy than the syn, then anti NADH will be a weaker reducing agent than syn NADH[20]. Unfortunately, the experimental data are not sufficient to permit extensive discussion of this point, but they are certainly suggestive. This 2nd postulate must await further experimental examination.

3. The 3rd assumption is supported by recent experimental data which show that for nearly a dozen enzymes (including a dehydrogenase), the 'internal' equilibrium constant (that between the enzyme's bound species) is close to unity, even when the 'external' equilibrium constant (between free species) favors reactants or products by 3 orders or magnitude or more[21]. While this 3rd postulate can be justi-

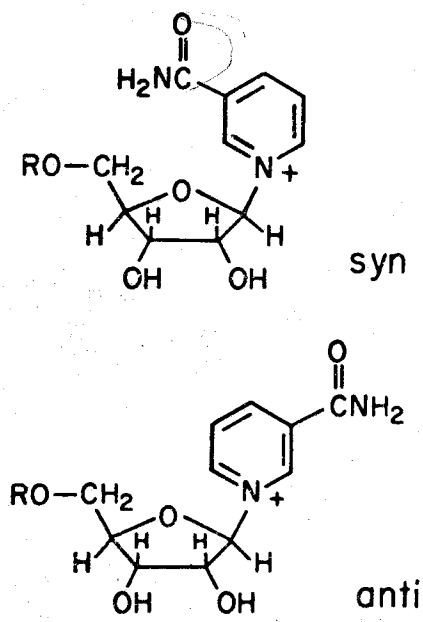

Figure 3. Syn and anti conformations of NAD^+.

Data for figure 2

Symbol	E.C.	Reference	Name	Stereochemistry
A	1.1.1.1	25	Alcohol dehydrogenase (Yeast)	A
B	1.1.1.3	26	Homoserine dehydrogenase	B
C	1.1.1.6	27	Glycerol dehydrogenase	A
D	1.1.1.8	28	Glycerol-3-phosphate dehydrogenase	B
E	1.1.1.26	29	Glyoxylate reductase	A
F	1.1.1.27	30	L-Lactate dehydrogenase	A
g	1.1.1.28	30	D-Lactate dehydrogenase	A
H	1.1.1.29	31	Glycerate dehydrogenase	A
I	1.1.1.30	32	3-Hydroxy butyrate dehydrogenase	B
J	1.1.1.35	33	3-Hydroxy acyl CoA dehydrogenase	B
K	1.1.1.37	34	Malate dehydrogenase	A
l	1.1.1.38	34	Malic enzyme	A
m	1.1.1.40	34	Malic enzyme (NADP)	A
N	1.1.1.51	35	β-Hydroxysteroid dehydrogenase	B
O	1.1.1.60	36	Tartronate semialdehyde reductase	A
P	1.1.1.62	37	Estradiol 17 β-dehydrogenase	B
Q	1.1.1.64	35	Testosterone β-dehydrogenase	B
r	1.1.1.72	27	Glycerol dehydrogenase (NADP)	A
S	1.1.1.79	29	Glyoxylate reductase (NADP)	A
t	1.1.1.81	31	Hydroxypyruvate reductase	A
U	1.1.1.82	34	Malate dehydrogenase (NADP)	A
v	1.1.1.91	38	Aryl alcohol dehydrogenase	B
W	1.1.1.100	39	3-Oxoacyl acyl carrier protein reductase	B
X	1.1.1.108	40	Carnitine dehydrogenase	B
Y	1.1.1.50	35	3-α-Hydroxysteroid dehydrogenase (P. test.)	B

fied on these empirical grounds, an argument by Albery and Knowles suggests that such an arrangement might be expected for an efficient catalyst, especially if it operates reversibly[22].

Together, these postulates permit the interpretation of the observed correlation in figure 2. Dehydrogenases select the weaker reducing agent (anti NADH, and hence the pro-R hydrogen) to reduce more reactive carbonyls, and the stronger reducing agent (syn NADH, and hence the pro-S hydrogen) to reduce less reactive carbonyls. This matching of reduction power to the ease with which the substrate is reduced must help match the free energies of the 2 ternary complexes (enzyme-NAD^+-reduced substrate, and enzyme-NADH-oxidized substrate), and thereby optimize the catalytic efficiency of the enzyme.

We emphasize that it is the free energies of the bound states that actually occur along the reaction coordinate in the enzyme catalyzed process that are matched, not those of the unbound species, which the enzyme cannot alter. Yet it is the equilibrium constants between unbound species, which are far easier to measure, that are plotted in figure 2. We presume that this external equilibrium constant correlates reasonably with the equilibrium constants for the bound states. Because the chemistry of the intermediates along the reaction coordinate is not well understood, it is not possible to define this correlation exactly. Where one expects the 'break' in figure 2 to occur depends entirely on the nature of the correlation[23].

Finally, consideration of the thermodynamics of these systems lends some support to our suggestion that the conformation of the cofactor might be chosen to match the reducing power of the cofactor to the reducibility of the substrate. The equilibrium constants for the reactions catalyzed by these dehydrogenases span a range of approximately 8 orders of magnitude, equivalent to a $\Delta\Delta F$ of about 11 kcal/mole. Based on the data for NMN^+ and NMNH, rotation around the glycosidic bond of the cofactor can be estimated to change the reducing strength of NADH by 1.3 kcal/mole, which is roughly 10% of the total range involved. Thus, while dehydrogenases clearly must have available a number of strategies in addition to the one suggested here for affecting the energies of bound species, the energetic consequence of rotating about the glycosidic bond is a significant part of the overall energetic requirements. If our proposed interpretation proves to be valid, dehydrogenases will reflect a most remarkable instance of convergent evolution, in which dozens of enzymes from organisms from the 3 kingdoms of life have all evolved to exploit a device that provides a catalytic advantage of only a factor of 10.

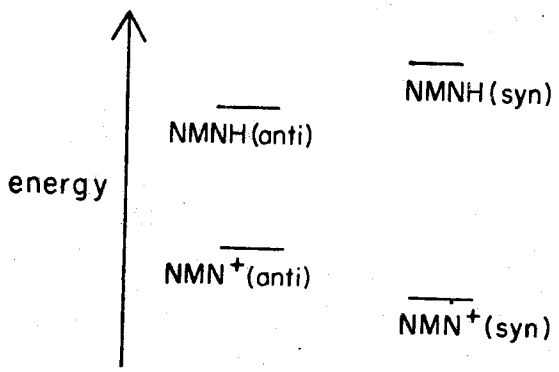

Figure 4. An energy diagram based on the preference of NMN^+ for the syn conformation and of NMNH for the anti conformation shows that anti NMNH is a weaker reducing agent than syn NMNH.

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