RATES OF EXCHANGE OF ISOTOPIC HYDROGEN BETWEEN REDUCED SUBSTRATES OF COUPLED NAD-DEPENDANT DEHYDROGENASES

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Received October 29, 1968

Summary

The exchange of isotopic hydrogen between reduced substrates of coupled NAD-dependant dehydrogenases possessing the same and opposite steric specificities for NADH is considered. Kinetic analysis of the equilibrium state of the interacting systems shows that when dehydrogenases of unlike stereospecificity are paired, two exchanges between labeled NAD and NADH are required before the isotope can be transferred from one substrate to the other whereas no exchanges of this kind are required when dehydrogenases of like stereospecificity for NADH interact. The obligatory exchanges between NAD and NADH reduce the equilibrium velocity of isotope transfer between the substrates. Accordingly the exchanges of hydrogen isotopes between reduced substrates of coupled dehydrogenases possessing opposite stereospecificity for NADH will, in general, be found to be slower than between systems of the same stereospecificity.

In an earlier study we observed that, when L-lactate-2-^{3}H was infused into an isolated rat liver perfused with homologous whole blood, the liver malate rapidly came to isotopic equilibrium with the liver lactate but not with the liver glycerol-3-phosphate (G-3-P) (Hoberman, 1965). On the other hand, deuterium incorporation from glycerol-2-^{2}H into position 4 of the glucosyl residues of liver glycogen, a position corresponding to the aldehydic hydrogen of glyceraldehyde-3-phosphate, was more than four times greater than when L-lactate-2-^{2}H was the source of hydrogen for the reduction of 1,3-diphosphoglycerate (Hoberman and D'Adamo, 1960). More recently we have noted a lack of isotopic equilibration between liver G-3-P and malate when glycerol-2-^{3}H is

* This investigation was supported by Public Health Service Research Grant No. CA-03641-12 from the National Cancer Institute and Grant No. GB-7860 from the National Science Foundation.

infused into the isolated perfused liver*. Taken together these experimental observations imply that, when NAD-dependant dehydrogenase systems possessing like steric specificity for NADH are coupled, the rate of transfer of isotopic hydrogen from a reduced substrate of one of the dehydrogenases to the oxidized substrate of the other is more rapid than when systems of unlike stereospecificity for NADH are paired; the steric specificity of lactate and malate dehydrogenases is towards the 4A hydrogen of NADH whereas that of G-3-P and glyceraldehyde-3-phosphate dehydrogenases is towards the 4B (Levy and Vennesland,1957).

It is now widely accepted that the steady state ratios of lactate:pyruvate, malate; oxalacetate, and G-3-P: dihydroxyacetonephosphate (DHAP) in the liver reflect the equilibrium concentrations (Bücher and Klingenberg, 1958). Accordingly the kinetics of hydrogen transfer between coupled dehydrogenase systems is susceptible to analysis by applying principles outlined by Yagil and Hoberman (1969). We show that the equilibrium velocity of the overall reaction, $V_{X-Y,}$ is related to the equilibrium velocities of the separate reactions, $V_{X-H'}$ and $V_{Y-H'}$ as follows:

$$1/V_{X-Y} = 1/V_{X-H} + 1/V_{Y-H} \qquad (1)$$

where V_{X-H} is the rate of hydrogen exchange between substrate X(reduced) and NADH while V_{Y-H} is the corresponding rate of exchange between Y (reduced) and NADH. This relationship may be expected to hold when dehydrogenases with like stereospecificity for NADH are coupled. The oxidation-reduction reaction between the lactate and malate dehydrogenase systems can be depicted as follows:

$$\text{L-lactate-2-}{}^{3}\text{H} + \text{NAD} = \text{pyruvate} + \text{NADH-4A-}{}^{3}\text{H} + \text{H}^{+} \qquad V_{L-H}$$

$$\text{NADH-4A-}{}^{3}\text{H} + \text{H}^{+} + \text{oxalacetate} = \text{L-malate-2-}{}^{3}\text{H} + \text{NAD} \qquad V_{M-H}$$

And

$$1/V_{L-M} = 1/V_{L-H} + 1/V_{M-H} \qquad (2)$$

*Hoberman,H.D., Carnicero, H., and Moore, C., to be reported.

However, the situation is more complex when the exchange of tritium (or deuterium) involves coupling of systems of opposite steric specificity for NADH. This is illustrated by the interactions which occur when lactate and G-3-P dehydrogenases are coupled and the isotope is introduced into the system as L-lactate-2-^{3}H. The steps required to transfer tritium from lactate to G-3-P are:

L-lactate-2-^{3}H + NAD = pyruvate + NADH-4A-^{3}H + H^+ V_{L-H}

DHAP + NADH-4A-^{3}H + H^+ = G-3-P + NAD-4-^{3}H $V_{H-D(G-3-P)}$

lactate + NAD-4-^{3}H = pyruvate + NADH-4B-^{3}H $V_{H-D(L)}$

DHAP + NADH-4B-^{3}H + H^+ = G-3-P-2-^{3}H + NAD $V_{G-3-P-H}$

Note that NADH-4B-^{3}H is formed in a reaction with normal lactate; the probability of a bimolecular reaction between L-lactate-2-^{3}H and NAD-4-^{3}H is infinitisimal. The equilibrium velocity of the isotope exchange between L-lactate-2-^{3}H and G-3-P, $V_{L-G-3-P}$, is:

$$1/V_{L-G-3-P} = 1/V_{L-H} + 1/V_{H-D(G-3-P)} + 1/V_{H-D(L)} + 1/V_{G-3-P-H} \quad (3)$$

where the Vs are defined as follows: V_{L-H} is the equilibrium velocity of the isotope exchange between labeled lactate and NADH; $V_{H-D(G-3-P)}$ is the equilibrium velocity of the exchange between labeled NADH and NAD catalyzed by G-3-P dehydrogenase; $V_{H-D(L)}$ is the equilibrium velocity of the exchange between labeled NAD and NADH catalyzed by lactate dehydrogenase; and $V_{G-3-P-H}$ is the equilibrium velocity of the isotope exchange between labeled NADH and G-3-P catalyzed by G-3-P dehydrogenase.

Because of the compulsory binding order of NAD-dependant dehydrogenases so far studied, and well documented for lactate dehydrogenase by Silverstein and Boyer (1964), exchanges of NADH for NAD (and the reverse) are considerably slower than exchanges of hydrogen isotopes between substrate and coenzyme (Yagil and Hoberman, 1969). Accordingly exchanges of hydrogen isotopes between reduced substrates of coupled dehydrogenases possessing opposite stereospecificity for NADH will, in general, be found to be slower than

between systems of the same stereospecificity where no exchange of pyridine nucleotides is involved in the transfer of the isotope. We apply the condition, of course, that the enzyme activities of the systems being compared are the same.

The foregoing analysis explains the experimental observations which led to the present need of examining the relationship between rates of hydrogen transfer and the stereospecificity of coupled dehydrogenase systems. With the use of equation (3) $V_{L\text{-}G\text{-}3\text{-}P}$ may be calculated by evaluating $V_{L\text{-}H'}$ etc. assuming the conditions of concentrations of substrates, enzymes, and coenzymes existing in liver. The observed value of $V_{L\text{-}G\text{-}3\text{-}P}$ (Hoberman, 1965) and the calculated value agree. The retardation of the rate of exchange of tritium between lactate and G-3-P is considerable, i.e. the value of $V_{L\text{-}G\text{-}3\text{-}P'}$ observed and calculated, is about 1% of the rate of exchange between lactate and G-3-P if no exchanges between NAD and NADH were required. This is an effect on the rate which is much larger than any anticipated isotope effect on the reaction. Indeed it may be expected that, if the hydrogen isotope effect on the exchange of tritium between substrate (reduced) and NADH is about the same for lactate and G-3-P dehydrogenases, no isotope effect on the tritium exchange between lactate and G-3-P will be observed. This is because the predicted isotope effect approximates the isotope effect on an equilibrium constant and accordingly is small.

The present communication may serve to aid in the interpretation of data obtained from studies of the coupling of oxidations of substrates to reductive biosynthesis (Hoberman, 1958; Bloom, 1959; Loewenstein, 1961; Kemp and Rose 1964). The extent of utilization of isotopic hydrogen, present in an appropriately labeled substrate, in the reductions occurring in the course of synthesis of fatty acids, cholesterol, glycogen, etc. may be determined, at least in part, by the stereospecificities of the participating reactions.

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