The Reaction of Dihydrolipoic Acid with Flavines. A Biochemical Model System

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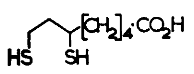
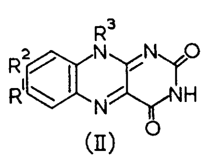
THE study of the mechanisms of chemical "model reactions" has greatly helped our understanding of enzymic mechanisms in recent years.[1] We now report our investigations of a reaction that may serve as a suitable model for the enzyme lipoyl dehydrogenase. This enzyme is a flavoprotein and it catalyses the transfer of two electrons from reduced nicotinamide–adenine dinucleotide to lipoic acid or the reverse of this reaction.[2]

There has been some doubt whether dihydrolipoic acid (I) reacts with riboflavine (II; R^1 = Me; R^2 = Me; R^3 = ribityl) or only interacts with it to form a charge-transfer complex.[3,4] We find that dihydrolipoic acid does react with flavine mononucleotide (II; R^1 = Me; R^2 = Me; R^3 = ribityl phosphate) and a number of its analogues under anaerobic conditions at room temperature. The products of the reaction are dihydroflavine and lipoic acid. The reaction is of first order in flavine concentration, and at a given pH, of first order in reduced lipoic acid concentration. The rate is strongly pH-dependent and is of first order in hydroxyl-ion concentration. This suggests that the reaction proceeds by a two-step mechanism, the first being the fast dissociation of one of the sulphydryl groups of lipoic acid into its anion followed by a second-order reaction between this anion and the flavine. The second step may involve the transfer of two electrons step by step leading to the formation of free radicals as intermediates (as suggested for the enzymic process[2]) or may involve a two-electron reduction step. On the following evidence we suggest the second (ionic) mechanism (see the Table).



(I)



(II)

Reactivities and half-wave potentials of substituted flavines.

substituent			$E_{\frac{1}{2}}$	k_2 for reaction with (I)	k_2 for reaction with NADH
R^1	R^2	R^3	(mV)	l. mole^{-1} sec.$^{-1}$	l. mole^{-1} sec.$^{-1}$
Me	Me	Ribityl phosphate	$-0{\cdot}473$	$5{\cdot}04 \times 10^{-2}$	$1{\cdot}86 \times 10^{-1}$
Me	Me	Ribityl	$-0{\cdot}460$	$1{\cdot}03 \times 10^{-1}$	$4{\cdot}12 \times 10^{-1}$
Me	Me	Me	$-0{\cdot}460$	$7{\cdot}99 \times 10^{-2}$	$4{\cdot}25 \times 10^{-1}$
Me	Me	$[CH_2]_2{\cdot}OH$	$-0{\cdot}450$	$6{\cdot}66 \times 10^{-2}$	$2{\cdot}67 \times 10^{-1}$
H	H	Me	$-0{\cdot}435$	$5{\cdot}78 \times 10^{-1}$	$8{\cdot}06 \times 10^{-1}$
Cl	OMe	$[CH_2]_2{\cdot}N(C_4H_8)$	$-0{\cdot}400$	$1{\cdot}94 \times 10^{-1}$	1·85
H	H	$[CH_2]_2{\cdot}NMe_2$	$-0{\cdot}385$	1·30	1·72
Me	H	$[CH_2]_2{\cdot}NEt_2$	$-0{\cdot}360$	1·63	3·92
Cl	Cl	Me	$-0{\cdot}333$	12·50	5·27
H	Cl	$[CH_2]_3{\cdot}NEt_2$	$-0{\cdot}315$	5·62	2·33
Cl	H	$[CH_2]_3{\cdot}NEt)_2$	$-0{\cdot}301$	33·40	4·03

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[4] V. Massey and N. W. Atherton, *J. Biol. Chem.*, 1962, **237**, 2965.

1. The rates of the reactions of a number of flavines substituted in the benzenoid ring or in the R^3-position are considerably dependent on the nature of the substituent, the reaction being speeded up by electron-withdrawing substituents and slowed down by electron-donating groups.

2. The polarographic half-wave potentials ($E_{\frac{1}{2}}$) of these flavines (determined under the same conditions as the reaction rates), which can be shown to measure the ease of reduction by a two-electron process from the shape of the current–voltage curves (*cf.* ref. 5), show a reasonable linear correlation (correlation coefficient 0·955) with the logarithm of their rates with dihydrolipoic acid. The slope of the line is 14·9 mv^{-1}. (Because of the differences in the sizes of the R^3-side chains the correlation cannot be expected to be more precise).

3. A similar rough correlation is observed between the rates of reactions of these flavines with reduced nicotinamide–adenine dinucleotide (NADH) and their half-wave potentials (correlation coefficient 0·914, slope 7·3 mv^{-1}). This reaction is known to involve a two-electron reduction in the form of a hydride-ion transfer.[6,7] (The poorer correlation here is mainly due to deviations from the line by the two compounds with the bulkiest side-chains. Without these the correlation coefficient is 0·965). The greater susceptibility of the reaction of dihydrolipoic acid than of the pyridine nucleotide system to substituent effects is an indication of the larger polarisability of the electrons in the sulphydryl groups than in the NADH system.

4. As for the reaction of NADH with flavine mononucleotide,[6,7] we found by electron spin resonance spectroscopy that the reaction of dihydrolipoic acid with the flavine ($R^1 = Cl$, $R^2 = H$, $R^3 = [CH_2]_3{\cdot}NEt_2$) produces a flavine free-radical. The concentration of this radical is at its maximum when the reaction is approximately half complete. This indicates that the radicals are not intermediates in the reaction but are produced as a result of a disproportionation equilibrium between the reduced and oxidised forms of the flavine.[7,8]

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