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BIOMIMETIC MODELS OF LACTATE DEHYDROGENASE

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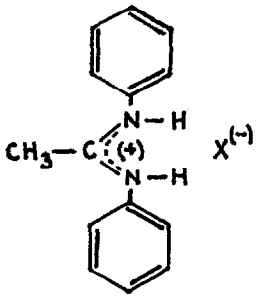
A biomimetic system that models the enzyme lactate dehydrogenase was designed and its capability to reduce α-oxoacids in absence of divalent metal ion was investigated.

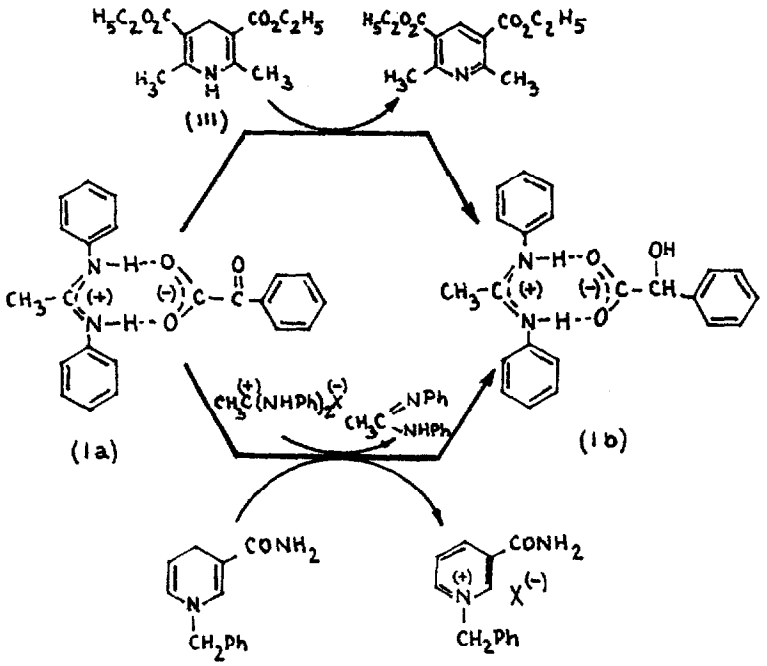
Enzymatic reductions depend greatly on the function of the apoenzyme which exhibits the catalytic properties. Within the large molecule of an enzyme only a few parts seem to be indispensable for the catalytic activity. If a few catalytic groups are arranged appropriately with respect to the substrate by proper molecular design, the excellent reactions exerted by the enzyme can be realized artificially. Among many kinds of enzymic reactions it is relatively easy to simulate the ones in which coenzyme acts as reagent - the active part of the coenzyme can be combined with a small part of the apoenzyme in one model. As a simulation of oxidoreductases reaction reductions by model compounds of NAD(P)H coenzymes have been investigated frequently[1]. These reductions were mostly directed towards the alcohol dehydrogenase models supported by divalent metal which activates the carbonyl oxygen and corresponds to Zn(II) ion in native alcohol dehydrogenase. Lactate dehydrogenase (LDH) is known[2] to apply another feature to maintain its substrate, i.e. a pair of hydrogen bonds between the guanidinium of the arginine in active centre and substrate carboxylic group. In previous communications on the LDH models either a divalent metal was used[3,4] to activate the substrate and/or Hantzsch dihydropyridines served[5,6] as coenzyme model. Yields obtained were low.1-Benzyl-3-aminocarbonyl-1,4-dihydroquinoline was also used for the reduction[7] of phenylglyoxylic acid. No attention has been paid so far to the importance of arginine for substrate fixation and activation.

Preparation[8] of compounds I is described elsewhere[9] the synthesis of N,N'-diphenylacetamidinium carboxylates (I) which provide the important amidinium - carboxylate interaction mediated by hydrogen bonds[10]. They are



Ia X = $O_2C-CO-C_6H_5$
Ib X = $O_2C-CH(OH)-C_6H_5$
Ic X = $O_2C-CO-CH_3$
Id X = $O_2C-CH(OH)-CH_3$
Ie X = $O_2C-COOH$
If X = O_2C-CF_3
Ig X = $O_2C-C_6H_5$
Ih X = Cl
Ii X = ClO_4

soluble in aprotic media. Phenylglyoxylic and pyruvic acids were chosen as substrates (models Ia and Ib, respectively) and dihydropyridines II and III served as NADH models (Scheme 1). The reductions were carried out in acetonitrile solutions at room temperature in dark and the mixture was



(Scheme 1)

deaerated by a stream of argon. The course of the reaction was followed as decrease of the dihydropyridine absorption at λ_{max} 354 nm and was compared with an otherwise identical reaction mixture lacking substrate. The reaction mixture was then treated with diazomethane, to yield methyl esters either of the non reacted substrates or products, the mandelic or lactic acid, respectively. The yield of the reaction was then established as substrate and product methyl esters ratio by HPLC. In several cases (see Table I) also NMR, UV and GLC was applied.

Table I
Model reductions performed in dry acetonitrile

Substrate	NADH model	Proton donor	Concentration mol/l substr.	Concentration mol/l others[b]	Time[a] hrs.	Volume ml	Detection	Yield %
Ia	II	Ih	0.01	0.01	3	20	HPLC	9.1
*[c]	II	Ih	0.01	0.01	5	15	HPLC	0.0
Ia	II	Ii	0.01	0.01	25	12	HPLC	1.3
Ia	II	Ie	0.01	0.01	25	12	HPLC	22.5
Ia	II	If	0.006	0.006	50	15	HPLC	47.2
Ia	II	Ig	0.01	0.01	50	15	HPLC	35.3
Ia	II	-	0.02	0.02	23	5	HPLC	9.8
Ia	III	-	0.01	0.01	26	5	HPLC	10.4
Ia	III	-	0.01	0.02	26	5	HPLC	18.3
Ia	III	-	0.01	0.04	26	5	HPLC	82.3
Ic	II	-	0.05	0.05	25	10	UV	0.0
Ic	II	*[d]	0.025	0.025	1.5	18	UV	0.0
Ic	II	*[e]	0.0002	0.0002	28	3	UV	0.0
Ic	II	Ih	0.01	0.01	25	27	NMR	10.0
Ic	II	*[f]	0.01	0.01	21	21	GLC	22.0

[a]Time for completion of the reduction followed by absorption peak at 354 nm; [b]equimolar concentration of NADH model and proton donor; [c]ethyl phenylglyoxylate; [d]4-imidazolylcarboxylic acid; [e]imidazole hydrochloride; [f]2-benzimidazolacetic acid.

Another problem which had to be solved was the necessity for a proton donor, which function is presumably fulfilled in the native system by amino acid His-195. When dihydronicotinamide (II) was used as a NADH model the compounds I were found as the best proton source although other compounds were examined (see Table I). Hantzsch dihydropyridine (III) affords the desired proton itself on aromatization.

From the Table it is seen that very good yields were achieved in reductions of substrate Ia by coenzyme model II and Ia by III, respectively. In contrast to previously described reductions no divalent metal was necessary. This phenomenon may be attributed to substrate activation by the arginine model, ethyl phenylglyoxylate is not reduced under these conditions to an observable extent. Our present efforts are directed towards preparation of a LDH model bearing all components in a single molecule.

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8. N,N'-diphenylacetamidine was prepared from acetanilide by the action of phosphorus oxychloride in anhydrous benzene. Salts of N,N'-diphenylacetamidine were prepared either by the reaction of equimolar amounts of base and acid (compounds Ia, Ic, Ie and Ih) or by the reaction of Ih with sodium salt of the respective acid (compounds Ib, Id, If, Ig and Ii) in methanol-water solution. The crude amidinium salt was crystallized from methanol.
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