Coenzyme Model Studies. Part 3.[1] Transimination and Reduction using NADH Models

Serjinder Singh,* Iqbal Singh, Ravjitinder K. Sahota, and Sarita Nagrath
Department of Chemistry, Guru Nanak Dev University, Amritsar-143005, India

N-Arylidenealkylamines (**1**) undergo transimination with aniline derivatives in glacial acetic acid. In the presence of 3,5-bis(ethoxycarbonyl)-2,6-dimethyl-1,4-dihydropyridine (Hantzsch ester) transimination is followed by reduction of the transimination products (**3**) but not of (**1**). Transimination also takes place in acetonitrile on treatment of the amines (**1**) with anilinium trifluoroacetate. These phenomena are analogous to transiminations at the catalytic sites of pyridoxal-dependent enzymes and a similar transimination is followed by reduction in the mechanism of glutamate dehydrogenase.

A variety of enzymes which catalyse the transformations and reactions involving imines as intermediates are known to possess a primary ε-amino group of a lysine residue at the active site. The role of this primary amino group is initially to bind a carbonyl-containing molecule at the catalytic site by forming a carbon–nitrogen double bond. The bound molecule may be an α-keto acid substrate as in glutamate dehydrogenase[2] (E.C.1.1.4) or even a coenzyme as in the case of pyridoxal phosphate dependent enzymes (E.C.2.6.1). This not only facilitates binding of the carbonyl compound at the catalytic site but also converts the carbonyl groups into more easily polarizable imine bonds through *N*-protonation for attack by other nucleophiles. These nucleophiles are generally ammonia or primary amines but could also be carbanions as in the case of aldolases. Attack by ammonia or a primary amine on the imine group thus formed results in the displacement of the lysine amino residue and the formation of a new imine which undergoes reduction in glutamate dehydrogenase and other important transformations in pyridoxal-dependent enzymes. This replacement of one amino component in an imine by another is generally known as transimination.[3]

Results and Discussion

We report here the results of an investigation[4] consisting of model experiments aimed at understanding the role and mechanism of biological transiminations. Whereas a more basic amine is known[5] to replace less basic amines in neutral solvents *in vitro*, the reverse is the case *in vivo*. Furthermore, an acidic group at the catalytic site is essential for transimination in enzymes. In the model experiments we chose primary aliphatic amines to represent the ε-amino group of the lysine residue and aromatic aldehydes as the model for pyridoxal and α-keto acids. Various anilines were chosen as the less basic amino components for transimination and glacial acetic acid as the

Table. Transimination of imines (**1**) with anilines and reduction with an NADH model (**2**)

Substrate imines	Ar	R	Aniline p-$XC_6H_4NH_2$	Methods[a]	Product	Yield (%)	M.p. (°C)[b]
(**1a**)	Ph	Bu	H	A	(**3a**)	90	48 (54)[c]
(**1a**)	Ph	Bu	H	B	(**3a**)	50	48 (54)[c]
(**1a**)	Ph	Bu	H	C	(**4a**)	60	107 (108)[d]
(**1a**)	Ph	Bu	OMe	B	(**3b**)	52	70 (72)[e]
(**1a**)	Ph	Bu	OMe	C	(**4b**)	55	62 (64)[f]
(**1b**)	Ph	C_6H_{11}	H	A	(**3a**)	94	48 (54)[c]
(**1b**)	Ph	C_6H_{11}	H	C	(**4a**)	92	107 (108)[d]
(**1b**)	Ph	C_6H_{11}	OMe	A	(**3b**)	85	70 (72)[e]
(**1b**)	Ph	C_6H_{11}	OMe	C	(**4a**)	80.5	107 (108)[d]
(**1b**)	Ph	C_6H_{11}	NO_2	A	(**3c**)	82.0	116 (118)[g]
(**1b**)	Ph	C_6H_{11}	NO_2	C	(**4c**)	82.0	146 (147)[h]
(**1c**)	m-$O_2NC_6H_4$	C_6H_{11}	H	A	(**3d**)	90.0	65 (66)[i]
(**1c**)	m-$O_2NC_6H_4$	C_6H_{11}	H	C	(**4d**)	87.0	82 (85)[j]
(**1c**)	m-$O_2NC_6H_4$	C_6H_{11}	OMe	A	(**3c**)	76.0	82 (83)[k]
(**1c**)	m-$O_2NC_6H_4$	C_6H_{11}	OMe	C	(**4e**)	81.3	60 (62)[k]
(**1d**)	p-$MeOC_6H_4$	C_6H_{11}	H	A	(**3f**)	91.0	63 (64)[l]
(**1d**)	p-$MeOC_6H_4$	C_6H_{11}	H	C	(**4f**)	88.0	46 (49)[m]
(**1d**)	p-$MeOC_6H_4$	C_6H_{11}	OMe	A	(**3g**)	82.0	145 (146[n]
(**1d**)	p-$MeOC_6H_4$	C_6H_{11}	OMe	C	(**4g**)	78.0	94 (95)[o]
(**1c**)	Ph	$PhCH_2CH_2$	H	A	(**3a**)	70.0	48 (54)[c]
(**1c**)	Ph	$PhCH_2CH_2$	H	C	(**4a**)	66.0	107 (108)[d]

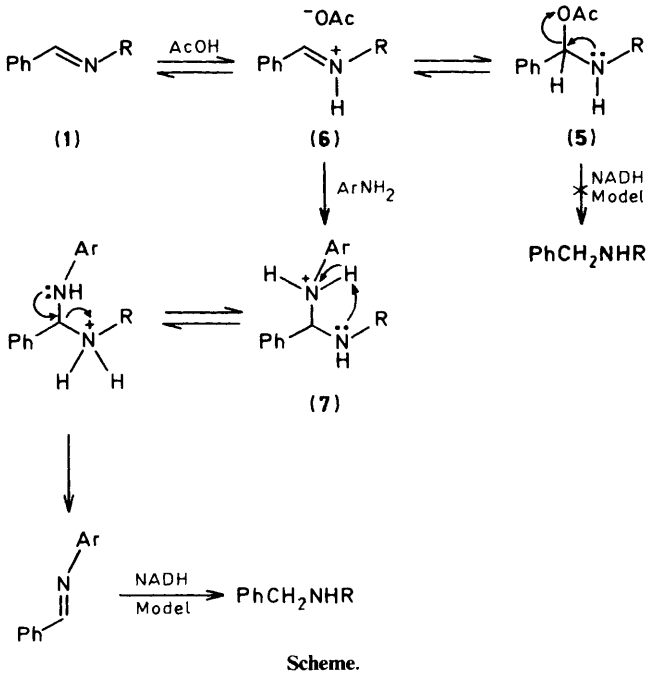
[a] See the Experimental section. [b] Known compounds, literature values in parentheses. [c] L. A. Biglow and H. Eatough, *Org. Synth.*, Coll. Vol. 1, 80, 82. [d] J. H. Billman and A. C. Diesing, *J. Org. Chem.*, 1957, **22**, 1068. [e] A. F. M. Iqbal, *J. Org. Chem.*, 1972, 2791. [f] L. Zeckmeister and J. Truka, *Chem. Ber.*, 1930, **63**, 2883. [g] E. Votocek, *Chem. Ber.*, 1915, **48**, 1002. [h] F. Kehrmann and M. Tichvinsky, *Justus Liebigs Ann. Chem.*, 1896, **290**, 293. [i] P. Gramaticakis and H. Texier, *Bull. Soc. Chim. Fr.*, 1971, 1323. [j] J. H. Billman and A. C. Diesing, *J. Org. Chem.*, 1957, **22**, 1068. [k] Y. Ogata and A. Kawasaki, *J. Chem. Soc., Perkin Trans. 1*, 1972, 792. [l] H. Schiff, *Justus Liebigs Ann. Chem.*, 1869, **150**, 195. [m] O. J. Sterngart, *Justus Liebigs Ann. Chem.*, 1887, **241**, 337. [n] M. Masuil and H. Ohmari, *J. Chem. Soc., Perkin Trans. 2*, 1972, 1882. [o] M. Julia and J. Igolen, *Bull. Soc. Chim. Fr.*, 1962, 1056.

solvent, it was felt, would play the role similar to that of an acidic group at the catalytic site in enzymes. Thus, when *N*-benzylidene derivatives (**1**) of cyclohexylamine, butylamine, or phenethylamine were taken up in glacial acetic acid and an equimolar amount of aniline added, a fairly good yield (Table) of *N*-benzylideneaniline (**3a**) was isolated after one hour at room temperature confirming the acetic acid-catalysed displacement of more basic aliphatic primary amines (pK_a *ca.* 10) by the less basic aniline (pK_a 4.76) analogous to the process *in vivo*. The primary amino group of amino acids which displaces the amine group of lysine in transaminases is similar but less basic. In glutamate dehydrogenase, a similar transimination with ammonia is followed by reduction of the imine thus formed with NADPH to give glutamate. We sought to mimic this sequence *in vitro*. When *N*-benzylidene(cyclohexyl)amine in glacial acetic acid was treated with equimolar amounts of aniline and 3,5-bis(ethoxycarbonyl)-1,4-dihydro-2,6-dimethylpyridine (**2**), *N*-benzylaniline (**4a**) was isolated in 92% yield after 6 h at room temperature. The dihydropyridine acted as a model for NADPH, and the formation of *N*-benzylaniline indicated that the reaction conditions were appropriate not only for transimination but also for the biomimetic reduction of the imine thus formed. Similar results were obtained with other substituted anilines.

In transaminases, in the absence of amino acid substrates, the coenzyme pyridoxal phosphate binds at the catalytic site by forming a Schiff base with an ε-amino residue of lysine. Transimination of this Schiff base with an amino acid is possible only if the amino group of the zwitterionic amino acid deprotonates itself. We believe that the imine nitrogen is sufficiently basic to deprotonate the amino acids and such a proton transfer from the amino acid to the imine not only makes the amino acid nitrogen nucleophilic but also polarizes the imine as its iminium salt prompting the former to attack it. The *gem*-diamino intermediate thus formed should further result in the elimination of the lysine amino group and formation of the new imine. To verify the chemical feasibility of this idea we took *N*-benzylidene(butyl)amine (**1a**) in acetonitrile and treated it with an equimolar amount of anilinium trifluoroacetate. A 50% yield of *N*-benzylideneaniline was isolated on diluting the reaction mixture with ice-cold water. No change occurred when aniline in acetonitrile was used in place of anilinium trifluoroacetate.

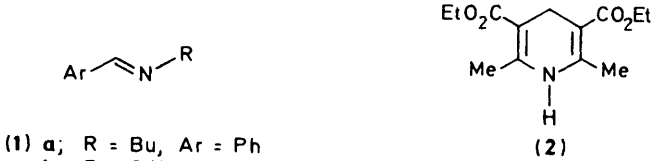
The two amino components involved in the enzymatic transimination usually differ in basicity to a much smaller extent (ΔpK_a *ca.* 0.5) than those in our model experiments mentioned above. Therefore, the above experiments were repeated employing amines with a smaller difference in pK_a. Instead of a more basic aliphatic and less basic aromatic amine both the amines employed were anilines differing slightly in basicity. Thus, when *N*-benzylidene-*p*-anisidine (**3b**) in acetic acid was treated with equimolar amounts of aniline and the dihydropyridine (**2**), *N*-benzylaniline was isolated in 82% yield indicating that the less basic aniline had displaced the slightly more basic *p*-anisidine. Other less basic anilines behaved similarly. This ability of less basic anilines to displace more basic ones in arylideneanilines was exploited in a competitive experiment for the formation of the latter. Thus, when benzaldehyde was treated with equimolar amounts of aniline and *p*-methoxyaniline in the presence of Hantzsch ester in glacial acetic acid, *N*-benzylaniline (**4a**) and *N*-benzyl-*p*-methoxyaniline (**4b**) were obtained in 66 and 33% yield respectively. This reflected the proportion in which *N*-benzylidene aniline and *N*-benzylidene-*p*-methoxyaniline must have been formed. This is with the knowledge[6] that the rate of formation of such imines is much slower than the rate of reduction with NADH models. The imines being formed equilibrate in acetic acid with free amines such that the less basic aniline tends to displace the more basic *p*-methoxyaniline as the concentration of free aniline falls. Some *p*-methoxybenzylideneaniline also becomes available for reduction by the NADH model.

The above observations can be explained by keeping in view the fact that acetic acid has a pK_a value higher than that of most anilines and much lower than those of aliphatic amines. The case of the corresponding *N*-benzylidene compounds is similar although the pK_a is further lower by *ca.* 2 upon condensation with benzaldehyde. Thus, *N*-benzylidene aliphatic amines are easily protonated in acetic acid and the iminium species (**6**) thus formed should be susceptible to attack by either acetate anion or unprotonated anilines (Scheme).



Scheme.

The attack of acetate results apparently in the formation of *o*-acetyl carbinolamine (**5**) which is unlikely to be reduced by the NADH model (**2**), but is in equilibrium with (**6**). The attack of aniline should give the *gem*-diamino intermediate (**7**). This intermediate has the aniline nitrogen protonated and the aliphatic amino nitrogen as yet unprotonated. It is at this stage that the difference in the basicity of the two amines matters. Rapid proton transfer should take place from the less basic



(**1**) **a**; R = Bu, Ar = Ph
b; R = C_6H_{11}, Ar = Ph

(**2**)

(**3**)

(**4**)

a; Ar = Ar' = Ph
b; Ar = Ph, Ar' = *p*-$MeOC_6H_4$
c; Ar = Ph, Ar' = *p*-$NO_2C_6H_4$
d; Ar = *m*-$NO_2C_6H_4$, Ar' = Ph
e; Ar = *m*-$NO_2C_6H_4$, Ar' = *p*-$MeOC_6H_4$
f; Ar = *p*-$MeOC_6H_4$, Ar' = Ph
g; Ar = Ar' = *p*-$MeOC_6H_4$

anilinium nitrogen to the aliphatic nitrogen and the new monoprotonated *gem*-diamino intermediate should undergo irreversible elimination to give *N*-benzylideneaniline. The latter is easily reduced to *N*-benzylaniline with the NADH model. It must be pointed out that the *N*-benzylidene aliphatic amines above are not reduced by NADH models in acetic acid although their trifluoroacetate salts in neutral solvents are reduced [7] thus indicating the intermediacy of the non-reducible *o*-acetylcarbinolamine (**5**). The role of *gem*-diamino intermediates such as (**7**) in enzymatic transiminations where one nitrogen is more basic than the other has been described in the literature.[3] It is mainly kinetic studies which have been carried out to infer the involvement of such intermediates. Our model experiments further confirm this and demonstrate the *in vitro* chemical feasibility of this crucial process in enzymes involving imines as intermediates.

Experimental

Column chromatography was carried out using silica gel (60—200 mesh) and benzene or a benzene–ethyl acetate mixture (90:10) as the eluant. T.l.c. was performed on silica gel (Merck GF_{254}). Glacial acetic acid of analytical grade (B.D.H.) was used as supplied. Anilinium trifluoroacetate was prepared by treating aniline with an excess of trifluoroacetic acid and recrystallizing the residue after evaporation, from ethanol. Imines derived from aromatic amines and aldehydes were prepared by condensation in methanol whereas those derived from aliphatic amines were prepared by literature procedures.[8]

General Procedure for Transimination.—(*a*) *In glacial acetic acid* (*Method A*). The imines (**1**) (1 mmol) were dissolved in the minimum quantity of glacial acetic acid and aniline (1 mmol) was added. The mixture was left at room temperature for 24 h and then poured onto crushed ice. The solid that separated was filtered off and recrystallized from ethanol to give the imines (**3**) which were characterized by comparison (t.l.c., n.m.r.) with authentic samples.

(*b*) *In acetonitrile* (*Method B*). The imine (**1**) (1 mmol) was taken up in dry acetonitrile (10 ml) and anilinium trifluoroacetate (1 mmol) was added. The reaction mixture was left at room temperature for 6 h and the poured onto crushed ice. The solid imines (**3**) that separated were filtered off, recrystallized, and characterized by comparison (t.l.c., n.m.r., i.r.) with authentic samples.

General Procedure for Transimination and Reduction (*Method C*).—*N*-Arylidenealkylamines (**1**) or *N*-arylidene-4-methoxyaniline (**3b**) (1 mmol) were taken up in glacial acetic acid (20 ml) and aniline, or an equimolar mixture of aniline and *p*-methoxyaniline (1 mmol) was added. To this reaction mixture was added compound (**2**) (1 mmol) and the reaction mixture was left at room temperature overnight. The solution was neutralized with aqueous sodium hydrogen carbonate and the aqueous mixture was extracted with benzene. The extract was dried (anhydrous Na_2SO_4) and evaporated to give the amines (**4**) which were separated by chromatography on silica gel. The products were characterized by comparison (t.l.c., n.m.r. i.r.) as such or as the *N*-acyl derivatives with authentic samples.

Acknowledgements

Financial assistance from the U.G.C. India and the C.S.I.R. India is gratefully acknowledged.

References

1 Part 2, S. Singh, S. Gill, and S. Nagrath, *J. Chem. Soc., Perkin Trans. 1*, 1986, 1273.
2 E. L. Smith, B. H. Austen, K. M. Blumenthal, and J. F. Nyc, '*The Enzymes*,' ed. P. D. Boyer, vol. XI, Academic Press. New York, 1975, p. 357.
3 W. P. Jencks, 'Catalysis in Chemistry and Enzymology,' McGraw Hill, 1969, p. 505.
4 Preliminary communication, S. Singh and I. Singh, *J. Chem. Soc., Chem. Commun.*, 1982, 1000.
5 B. A. Porai-Koshits and A. L. Remizov, *Sbvenik Statei Oshchei Khim*, 1953, **2**, 1570 (*Chem. Abstr.*, 1955, **49**, 5367*i*).
6 S. Singh, B. S. Lark, and A. S. Bhui, unpublished results.
7 S. Singh, V. K. Sharma, S. Gill, and R. K. Sahota, *J. Chem. Soc., Perkin Trans. 1*, 1985, 437.
8 Z. Eckstein and A. Lakariewicz, *Bull. Acad. Pol. Sci., Ser. Sci. Chim.*, 1959, **7**, 789.

Received 21*st November* 1985; *Paper* 5/2050