PYRUVAMIDE-PROMOTED DEAMINATION AND DECARBOXYLATION OF AMINES AND AMINO ACIDS; A SYSTEM MODELLING HISTIDINE DECARBOXYLASE

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1. Introduction

Pyruvamide residues and related functions appear to be involved in a variety of enzyme-catalyzed reactions [1,2]. Histidine decarboxylase (histidine carboxy-lyase, EC 4.1.1.22) from *Lactobacillus* 30a, for example, has been convincingly demonstrated by Snell and co-workers [1] to contain N-terminal pyruvylphenylalanine residues which function as prosthetic groups for the decarboxylation of histidine to histamine. The mechanism advanced [1a] as being consistent with the very substantial experimental information is shown in fig. 1. The analogy between this mechanism and that widely accepted for the function of pyridoxal in related reactions is immediately evident, the pyruvate keto group substituting for the pyridoxal aldehyde in the imine-forming step and the carboxamide moiety serving as electron sink in lieu of the pyridine nitrogen atom in the tautomerisation which is the essential intramolecular redox reaction. Since pyridoxal itself deaminates α-amino acids [3], albeit under somewhat heroic conditions, one speculates that simple pyruvamides might well promote deamination, and perhaps decarboxylation, of similar substrates. As with pyridoxal [4], a study of such reactions might well yield useful progress towards an understanding of enzymic-catalysis.

We now report that pyruvamide itself does indeed promote both deamination and decarboxylation, and under quite mild conditions.

2. Materials and methods

Pyruvamide (mp. 124–125, ref. [5] 124–125) was prepared by permanganate oxidation of lactamide in acetone containing a little acetic acid. Details of this and other procedures for preparing pyruvamides will be published elsewhere. Amines and amino acids (Aldrich Chemical Co. Inc.) were used as received. Benzaldehyde, invariably recognisably by smell, was extracted into ether from acidified reaction mixtures, authenticated by mass spectrometry (parent ion m/e 106, base peak m – H 105, m – CHO 77 a.m.u.) and roughly quantitated as its 2,4-dinitrophenylhydrazone. Acetophenone was similarly authenticated (parent ion m.e 120, base peak m – CH_3 115, m – $COCH_3$ 77 a.m.u.) and quantitated. Alaninamide and recovered amines were detected and roughly quantitated with ninhydrin (0.5% in *n*-butanol) after separation by paper electrophoresis (10 V · cm^{-1}, 2 hr) in molar pyridine–formic acid buffer pH 4.5. Recovered phenylglycine was precipitated at pH 6.5, dried and weighed. Consumption of pyruvamide was monitored by n.m.r. (methyl singlet at 2.56 δ) and mass spectrum (parent ion m/e 87, base peak doublet at 44 and 43 a.m.u.). Imines (Schiff's bases, II *b,d,e*) were authenticated by NMR, ir and, in certain cases, mass spectrum and elemental analysis (Galbraith Laboratories, Inc.). Instruments: Varian EM 600 mass spectrometer, Beckman Acculab 1 infrared, Varian A-60 NMR

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3. Results and discussion

Pyruvamide and N-substituted pyruvamides deaminate benzylamine to benzaldehyde very readily. The unmistakable almond odor of the aldehyde is immediately observed when mixtures in ethanol, tetrahydrofuran, water or other solvents, kept for a while or briefly warmed, are acidified. For example, ethanol or tetrahydrofuran solutions of equimolar amounts of pyruvamide itself and benzylamine, kept 1–2 hr or overnight, respectively (or briefly boiled), evaporated to dryness at room temperature and the residues taken up in 5% HCl and extracted with ether, afforded oils whose mass spectra clearly corresponded to slightly impure benzaldehyde and which gave the appropriate 2,4-dinitrophenylhydrazone in about 25% overall yield. Electrophoresis of the aqueous layers revealed alaninamide and substantial amounts of recovered benzylamine as major ninhydrin-positive products. α-Methylbenzylamine afforded acetophenone and alaninamide equally well. In sharp contrast, no detectable amount of *n*-butyraldehyde resulted from *n*-butylamine even under much more vigorous conditions. Reactions 4 and 3 of fig. 1 followed by reactions 6 and 7 of fig. 2 provide an obviously plausible pathway for alaninamide and aldehyde or ketone production.

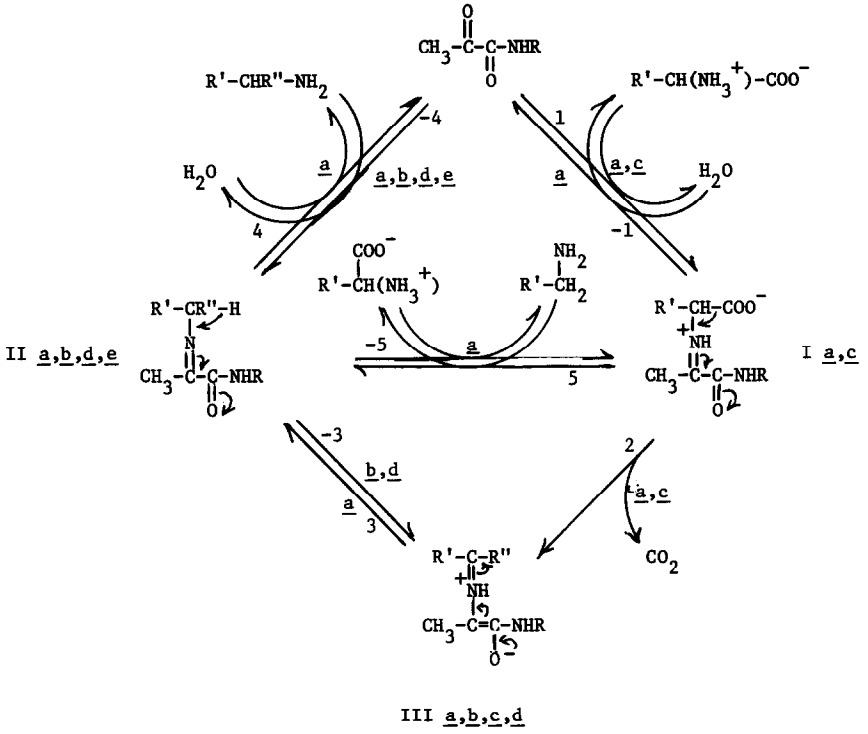


Fig. 1. Mechanism of action of histidine decarboxylase (modified from Recsei and Snell [1a] and related model reactions: *a* (enzymic), R = protein, R′ = $C_3H_3N_2$-CH_2-, R″ = H; *b* (benzylamine) and *c* (phenylglycine), R = R″ = H–, R′ = C_6H_5–; *d* (α-methylbenzylamine), R = H, R′ = C_6H_5–, R″ = CH_3–; *e* (*n*-butylamine), R = R″ = H, R′ = *n*-C_4H_9–.

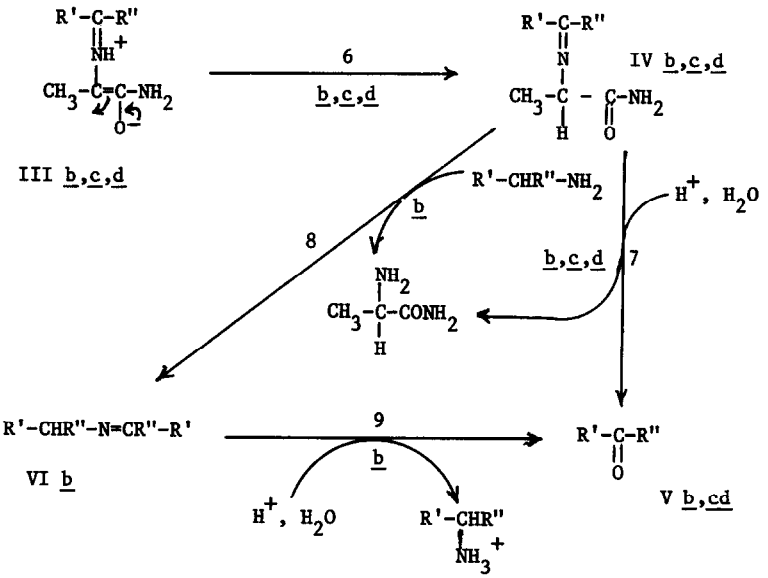
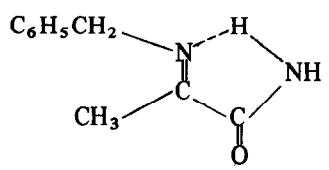


Fig. 2. Fate of imine enolate III in non-enzymic model systems; *b–d* as in fig. 1.

Chang and Snell [1e] have shown that reaction 3 is essentially irreversible in the enzymic system. In view of the absence of substituents which would promote protolysis from the amine methylene group of histamine this finding is not at all surprising, and the inability of pyruvamide to deaminate *n*-butylamine in the non-enzymic system is entirely analogous to it. However, and as might well be expected, the phenyl group of benzylamine or α-methylbenzylamine promotes protolysis (or, equivalently, stabilises the transition state between II and III) sufficiently for reaction 3 to proceed. The conjugated aldimine IV, hydrolysable by acid to the observed products, would reasonably be a favoured component of the resulting II–III–IV equilibrium.

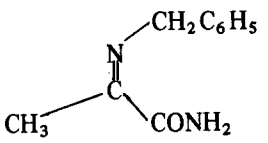
The α-amino acid phenylglycine is smoothly decarboxylated by pyruvamide. Carbon dioxide (15% yield) was obtained upon acidification of a solution of pyruvamide and tetraethylammonium glycinate in a little methanol kept at 30°C overnight. Benzaldehyde (smell, mass spectrum, 5% yield of 2,4-dinitrophenylhydrazone), alaninamide and unchanged phenylglycine (75% recovery) were found in the acidified mixture. Thus, CO_2 and benzaldehyde production account for at least 60% and 20%, respectively, of the amino acid consumed. No trace of phenylglyoxylate or other base-soluble dinitrophenylhydrazone was detected in the crude dinitrophenylhydrazine precipitate. Since phenylglycine was chosen, in the light of the benzylamine results, to offer the optimum opportunity for protolysis (step 3, leading to phenylglyoxylate) to compete with decarboxylation (step 2, leading to benzaldehyde), the non-production of phenylglyoxylate suggests a strong intrinsic preference for the latter pathway. This contrasts sharply with the behaviour of pyridoxal.

The proposed pathway is supported by the isolation and characterization of the ketimines II *b,d* and *e*. The imine II b, a cis (E, 40%) – trans (Z, 60%) mixture (NMR) precipitated in up to 80% yield within 1–2 min at 25°C from mixtures of pyruvamide and benzylamine in ethanol, chloroform, water or (best) tetrahydrofuran.

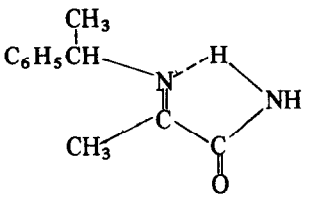


cis(E)

IIb



trans(Z)



cis(E)

IId

The α-methylbenzylimine IId is more soluble and does not precipitate. The pure cis(E) isomer was obtained in fair yield (33%) when residues (88% crude) from evaporation of solutions in ethanol (kept overnight) or benzene (refluxed overnight) were recrystallized from pentane–ether mixtures. An oil, plausibly the *n*-butyl imine IIe, was obtained in excellent yield upon evaporation of solutions resulting from the exothermic reaction of *n*-butylamine with pyruvamide in benzene.

Cis IId had mp. 72.5°, NMR ($CDCl_3$) 7.44 (6H, C_6H_5 and intramolecularly hydrogen-bonded NH), 6.1 (broad singlet, 1H, NH), 4.82 (quartet, J=7 hz, 1H, CH), 2.18 (singlet, 3H, $-N=C-CH_3$) and 1.49 δ (doublet, J=7 hz, 3H, $CH-CH_3$) and elemental analysis C, 69.70, H, 7.51 ($C_{11}H_{14}N_2O$ requires C, 69.95, H, 7.41%). Cis IIb, recovered in low yield from hot ethanol, had mp. 82°C, NMR ($CDCl_3$) 7.31 (∿5H, C_6H_5), 4.63 (singlet, 2H, CH_2) and 2.16 δ (singlet, 3H, CH_3); low solubility precluded clear recognition of amide proton signals. Slightly impure trans IIb (exceedingly sensitive to standing or warming even in aprotic solvents or in the solid state), recrystallized by chilling barely-warm solutions of the mixture in tetrahydrofuran, had mp. ∿95°C, NMR ($CDCl_3$) 7.31 (∿5H, C_6H_5), 3.86 (singlet 2H, CH_2) and 2.41 δ (singlet 3H, CH_3). Butylimine IIe had mass spectrum m/e 142 (5%, parent ion), 98 (35%, m – $CONH_2$), 56 (30%) and 42 (base peak) a.m.u., infrared 3450, 3310 (NH), 2960, 2930, 2870 (CH), 1690, 1640 and 1570 (C=N,C=O) cm^{-1}.

These imines are notably more easily formed and, in the case of the cis isomers at least, more stable than is usual for wholly aliphatic ketimines. This may be attributed to enhancement of the electrophilicity of the keto group by the adjacent carboxamide moiety and to intramolecular hydrogen bonding of the imine nitrogen to the carboxamide N–H. The NMR spectra of IId and the IIb isomers are entirely consistent with the assigned stereochemistries. Kinetic control and low solubility undoubtedly account for the predominance of the less stable trans IIb in the initial product.

The isolation and relative stability of these imines leads us to anticipate that intermediates of this sort can be prepared generally, so that it may prove possible to dissect the multi-step transamination and decarboxylation pathways into their constituent steps in product and kinetic studies. Some preliminary results in this direction are as follows: None of the purified imines gave aldehyde or ketone upon acidification. However, after modest maltreatment (warming in ethanol or water or boiling in benzene or tetrahydrofuran) or prolonged standing (some weeks) in the solid state, imines IIb and IId did do so. Thus, isomerization II → IV does not compete with hydrolysis in acid but proceeds best in neutral or weakly basic solution in protic solvents. The NMR spectrum of the oil produced upon heating the benzyl imine mixture IIb in ethanol indicated it to be benzylidenebenzylamine VIb. This trans-Schiffisation product of IVb and IIb undoubtedly is the immediate precursor of the benzaldehyde produced upon acidification. Reaction 8, akin to 5 in the enzymic system, seems the most likely route for its production. Ill-characterized pyruvamide polymers and pyruvamide–alaninamide copolymers account for the pyruvamide consumed. Corroborating this pathway, the NMR spectrum of a 2:1 (moles) mixture of benzylamine and pyruvamide, heated in ethanol and then evaporated, was clearly that of a mixture of alaninamide and benzylidenebenzylamine.

In summary, benzylamine and α-methylbenzylamine are readily deaminated to benzaldehyde and acetophenone by pyruvamide. n-Butylamine is not.

Phenylglycine is decarboxylated and deaminated. The reactions of the enzymic pathway, fig. 1, and of the non-enzymic branch, fig. 2, which occur in this model system are: *n*-butylamine: Reaction 4 only. Benzylamine and α-methylbenzylamine: Reactions 4, 3, 6, 8 (or 7 and 9) and 9. Phenylglycine: Reactions 1, 2, 6, and 7 (or 8, 9).

Acknowledgements

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