TRANSAMINATION BETWEEN ω-AMINO ACIDS, α,ω-DIAMINO ACIDS AND PYRIDOXAL PHOSPHATE: FORMATION OF ALDIMINES

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1. Introduction

In contrast with a considerable progress accomplished in the study of the mechanism of non-enzymic transamination of α-amino acids, little work is devoted to the aldimines (*N*-pyridoxylidene amino acids) formed between ω-amino acids or α,ω-diamino acids and pyridoxal phosphate (PLP) [1–3].

In the present paper we report data on the formation of aldimines of ω-amino acids, α,ω-diamino acids and α-amino acids (the latter as reference substrates), in order to elucidate the significance of the carbon chain-length and the position of the amino group upon the kinetics and equilibrium of formation, as well as the values of the molar absorption coefficients of the various aldimines.

2. Materials and methods

The following substrates were tested as NH_2-group donors: series of D,L-amino acids.* C-3, Ala, β-Ala, α,β-diamino propionic (A_2pr); C-4, α-amino butyric (Abu), γ-amino butyric(γ Abu) α,γ-diamino butyric (A_2bu); C-5, α-amino valeric(Nva), δ-aminovaleric (δPtn) Orn; C-6, α-amino caproïc(Nle), ε-amino caproïc(εAhx) Lys.

Amino acids ('Sigma' grade) were dissolved in phosphate buffer (pH 7.8) and used at final concentrations from 1×10^{-3} to 6×10^{-2} M.

*Symbols for less common amino acids are used according to recommendations of IUPAC–IUB Commission on Biochemical Nomenclature (1971) J. Biol. Chem. 1972, 247, p. 977.

PLP 'Merck' solution was prepared just before assay, with the same buffer and used at a final concentration of 1×10^{-4} M.

All solutions were prepared with water two-fold distilled in a quartz glass apparatus as to prevent the occurrence of metal ions, catalysing non-enzymic transamination [4].

Incubation temperature during assays for the determination of the apparent equilibrium constants (K_a) and of the molar absorption coefficient (ε) of the aldimines was 20 ± 0.5°C, and for the determination of reaction rate constants was 5 ± 0.05°C.

The spectra of aldimines were recorded using a 'SAFAS Spectralux 1800' spectrophotometer on the range 220–550 nm [1,5,6] with a 10 nm light path.

Reaction mixtures for a total volume of 3 ml/cell: PLP solution 0.075 ml, amino acid solution 0.6 ml, buffer (0.1 M) 2.325 ml.

Final concentration of reagents in the cell: PLP 10^{-4} M, amino acids 2×10^{-4} to 6×10^{-2},

Apparent equilibrium constant (K_a) and dissociation constant ($K_d = 1/K_a$) were determined according to the formula:

$$K_a = \frac{1}{K_d} = \frac{[\text{aldimine}]}{[\text{amino acid}]\ [\text{PLP}]}$$

and computed, using double reciprocal method of Lineweaver-Burk, plotting on abscissae the values of $1/[S]$ and on ordinate the values of $1/[E]$ [6].

K_a was also computed by the formula [7]:

$$\frac{1}{\epsilon - \epsilon_x} = \frac{1}{K_a(\epsilon_i - \epsilon_x)} \times \frac{1}{[S]} + \frac{1}{(\epsilon_i - \epsilon_x)}$$

where ϵ, ϵ_i, ϵ_x, represent the molar absorption coefficients of the reagent mixture before the reaction (time = 0) of the aldimine and of the PLP respectively. [S] is the substrate concentration.

Under conditions used, the course of aldimine formation followed pseudo first-order kinetics. Thus, the rate constant was calculated by

$$k = \frac{2.303}{t} \times \log \frac{a}{a - x},$$

where a in any parameter determining the initial concentration of PLP, x the fraction of converted to aldimine PLP at any time t of the reaction.

3. Results

3.1. *The kinetics of aldimine formation*

The kinetics of aldimine formation was studied with all the amino acids of the C-3–C-6 series.

Figure 1 illustrates the process of aldimine formation to equilibrium, determined by the $A_{275\,nm}$ of the reaction mixture for the valeric (A) and caproïc (B) series; the process is similar in the case of amino acids of the other series.

Table 1
Rate constants of aldimine formation

Amino acid Series	Rate constants (k) ($min^{-1} \times 10^3$)		
Propionic	Ala	β-Ala	A_2pr
	298	452	6300[a]
Butyric	Abu	γ-Abu	A_2bu
	416	432	6400[a]
Valeric	NVa	δ-Ptn	Orn
	502	271	3550
Caproïc	NLe	ε-Ahx	Lys
	149	253	782

[a]Reaction exhibits successive stages with varying k – reported values concern only the initial stage of the reaction

Incubation medium for a total volume of 3 ml: phosphate buffer M/10 at pH 7.8, each amino acid 6×10^{-2} M, PLP 10^{-4}M. Temperature 5 ± 0.02°C.

Rate constants were computed from the absorption curve of each aldimine. Mean values are reported in table 1.

Reported values exhibit significant differences in the rate constants of aldimine formation for α-and ω-amino acids of similar chain-length, as well as for amino acids bearing NH_2-group at the same position (α or ω) but differing by the carbon chain-length.

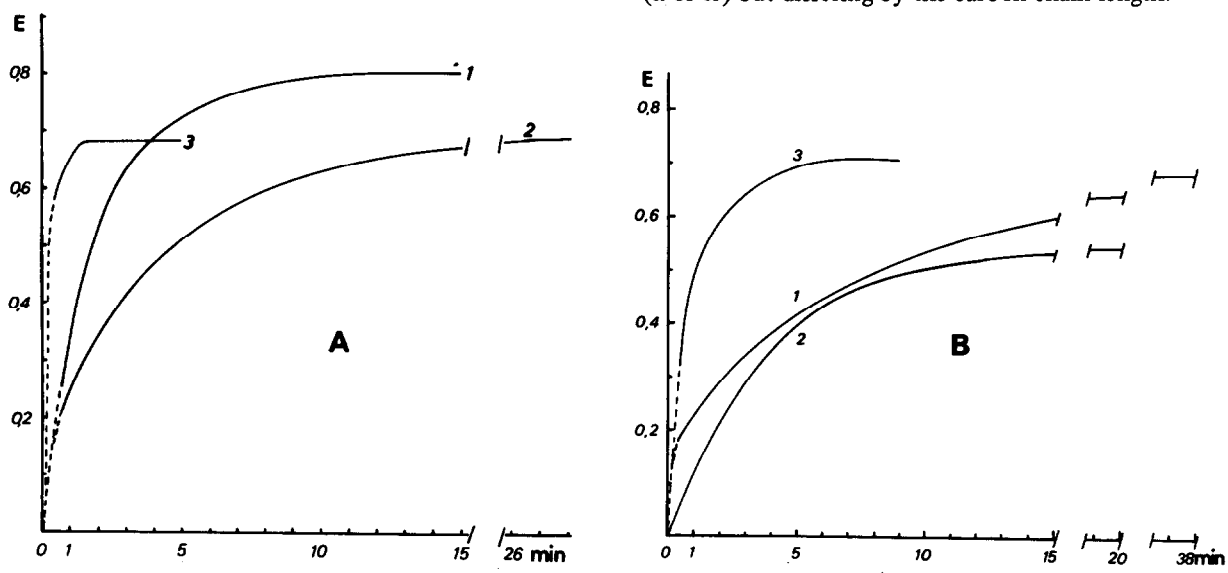


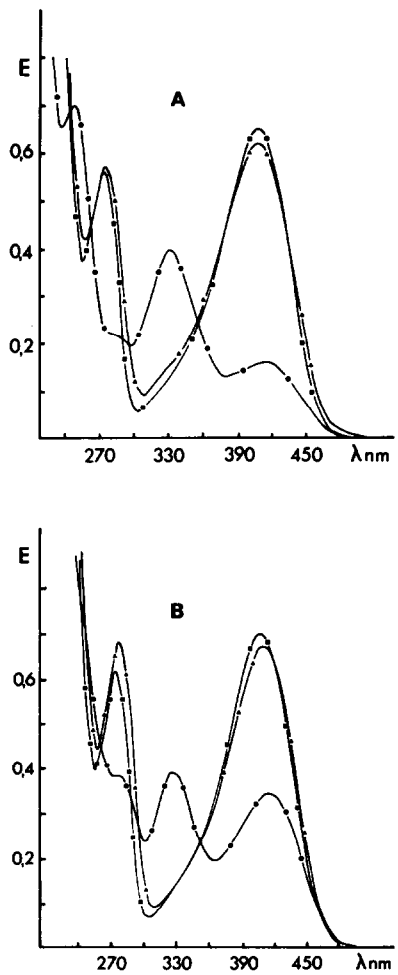
Fig.1. Kinetics of aldimine synthesis for amino acids of the valeric (A) and caproïc (B) series. (1) α-Amino (2) ω-amino (3) α,ω-diamino. The incubation medium contained for a total volume of 3 ml: phosphate buffer M/10 at pH 7.8, each amino acid at 6×10^{-2} M; PLP at 10^{-4} M. Temperature 5 ± 0.02°C.

3.2. *The effect of the number and position of NH_2-group on the absorption spectra of aldimines*

The absorption spectra of some of the formed aldimines are illustrated in fig.2.

Absorption spectra of aldimines of α-amino acids previously reported [6] and those of ω-amino acids reported here, show two characteristic bands at λ 275 nm, λ 410–415 nm respectively and a shoulder at λ 325 nm.

Spectra of diamino acids show marked differences depending on the carbon chain-length of the amino acid. Thus, the absorption bands of the Orn and Lys aldimines are almost similar to those of the mono-amino acids (α- or ω-), whereas the aldimine of A_2pr shows three characteristic bands with decreasing absorbance, at λ 245, λ 332, and λ 275 nm respectively. The aldimine of A_2bu shows a spectrum, intermediary with respect to those of A_2pr and Orn. It exhibits a marked decrease of the λ 245 nm band which is reduced to a shoulder. Bands at λ 275 nm, λ 410 nm exhibit a lower absorbance and one at λ 325 nm a higher absorbance value, with respect to the Orn aldimine.



3.3. *Molar absorption coefficients and equilibrium constants of aldimines*

Values of ϵ and K_a of various aldimines were determined using the absorption band at λ 275 nm, in the course of the reaction between a given concentration of PLP (10^{-4} M) and increasing in the 10^{-3}–6×10^{-2} M concentration range of each amino acid.

The results of the double reciprocal plot and computed values are reported in fig.3 and table 2.

4. Discussion

High values of the rate constants of aldimine formation from diamino acids may be, at least in part, ascribed to the level of their pK_{a2}, varying from 6.73–9.18 [8] a level significantly lower than that of the corresponding monoamino acids; the latter vary in the limits of 10.24–10.80 [8].

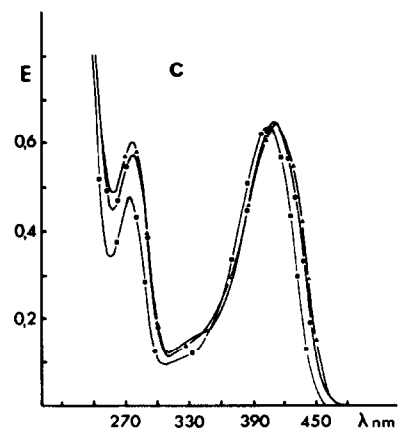
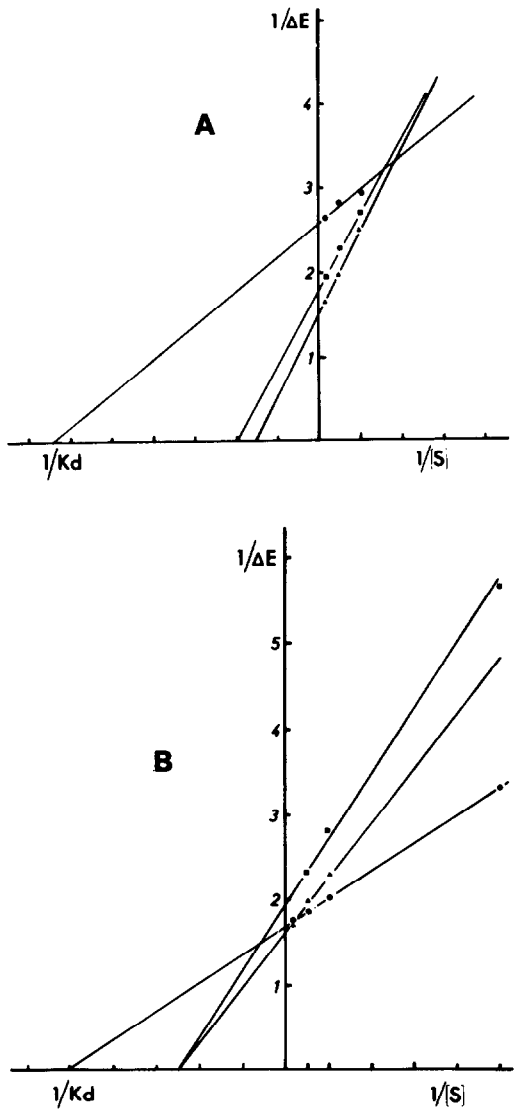


Fig.2. Absorption spectra of aldimines. Amino acid series: A-propionic, B-butyric, C-valeric. (▲) α-amino (■) ω-amino (●) α,ω-diamino. Incubation medium: same components and concentrations as in fig.1 Temperature 20 ± 0.5°C.



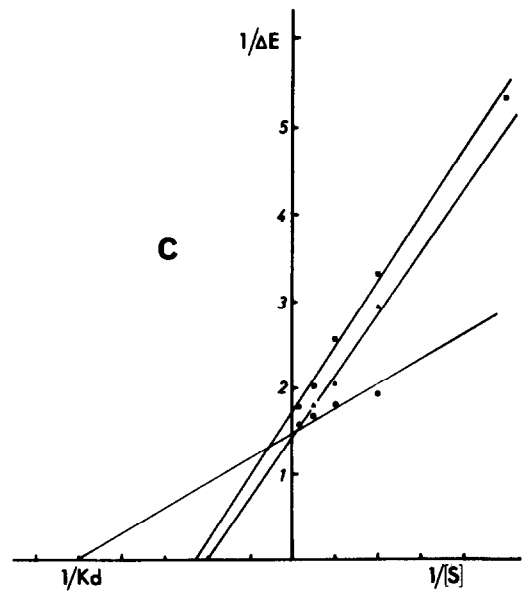


Fig.3. Double reciprocal plot of data on formation of aldimines. Amino acid series: A-butyric, B-valeric, C-caproïc. (▲) α-amino (■) ω- amino (●) α,ω-diamino. The incubation medium contained for a total volume of 3 ml: phosphate buffer M/10 at pH 7.8, each amino acid at concentration varying from 2×10^{-3}–6×10^{-2} M PLP 10^{-4} M.

In the incubation medium at pH 7.8 the diamino acids generate higher concentrations of bipolar ions, accepted as the reactive species, thus allowing the nucleophilic attack upon the carbonyl carbon (C-4′) of free PLP by the NH_2-group of the respective amino acids.

Plots of $\log(a-x)$ versus the time-course of incubation show a linear relationship, which points out that

Table 2
Molar absorption coefficients and equilibrium constants of aldimines

Amino acid series	ϵ			k_a		
	α	ω	α,ω	α	ω	α,ω
Propionic	5750	5600	3900	180	325	–
Butyric	6200	5700	3900	150	200	640
Valeric	6300	5800	5900	250	250	500
Caproïc	7600	5800	6800	200	225	500

The incubation medium contained for a total volume of 3 ml: phosphate buffer M/10 at pH 7.8, each amino acid at concentration varying from 10^{-3}–6×10^{-2} M, PLP 10^{-4} M.

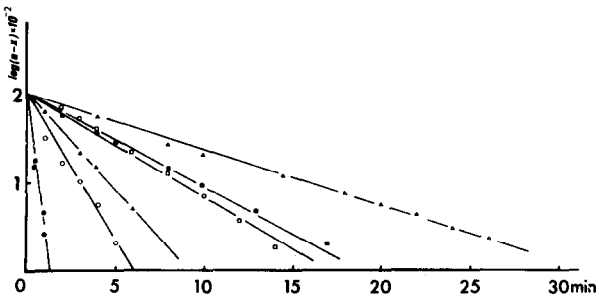


Fig.4. Plot of log(a−x) × 10^{-2} versus incubation time (in minutes). Conditions as in fig.1. The initial concentration of PLP is taken as 100. (▲) NVa (■) δPtn (●) Orn (△) Nle (□) ϵAhx (○) Lys.

aldimine formation follows pseudo first-order kinetics (fig.4).

The ratio of the $A_{275\ nm}$ to $A_{410\ nm}$ of the various aldimines allow some presumption relative to the amino group involved in the formation of diamino acid aldimines.

As reported in table 3, α-amino acid aldimines show a A_{275}/A_{410} ratio somewhat higher than that of the ω-amino acid aldimines.

Thus, under conditions used, aldimines from diamino acids seem to be formed preferentially at the α-NH_2 group. The hypothesis is supported by the A_{275}/A_{410} ratio of ϵ-Boc–Lys and α-Boc–Lys which show values similar to those of α-amino caproate and ϵ-amino caproate respectively. Similar interpretation was given by the analysis of NMR spectra of A_2pr aldimine [9].

The absorption band of the A_2pr aldimine at λ 330–332 nm (ϵ = 3900) and that of the A_2bu aldimine at λ 325 (ϵ = 3700) may be interpreted as the result of a cyclisation between ω-NH_2 of the amino acid and C-4′ of PLP, formerly involved in the azomethine bond, with the α-NH_2 of the same amino acid.

The hypothesis is proposed by analogy to the absorption spectra of Schiff bases formed between thiol compounds (cysteine, penicillamine) and PLP [10–12] and agree with the interpretation of NMR spectra of A_2bu and Orn aldimines by Abbott and Martell [9].

On the contrary, in the case of A_2pr aldimine the latter authors suppose that ring closure proceeds very slowly, whereas carbinolamine formation is much more extensive.

Values of K_a computed by the two methods used agree well and are close to values previously reported on some of the α-amino acids [6] γ-Abu [2] and ϵ-Ahx [3].

Table 3
Ratio A_{275}/A_{410} of aldimines

Amino acid series	A_{275} × 10^3	A_{410} × 10^3	A_{275}/A_{410}
Butyric: 6 × 10^{-2} M			
Abu	552	600	0.922
γ-Abu	522	682	0.768
A_2bu	380	410	0.927
Valeric: 6 × 10^{-2} M			
Nva	540	615	0.878
δPtn	480	630	0.762
Orn	565	640	0.883
Caproïc: 2 × 10^{-2} M			
Nle	581	621	0.936
ϵ-Ahx	520	650	0.790
Lys	600	660	0.910
ϵ-Boc–Lys	480	510	0.940
α-Boc–Lys	480	640	0.740

Data from experiments illustrated in fig.3. Each amino acid at a concentration of 6 × 10^{-2} M, PLP at 10^{-4} M.

Furthermore, K_a values suggest that reaction between all amino acids and PLP has obviously a tendency to shift to the right.

K_d (= $1/K_a$) values correspond to the concentration of amino acid at which half of the initial PLP is converted to aldimine. K_d of diamino acid aldimines show half-values of those of corresponding mono, α- or ω-amino acids, data which agree well with the hypothesis that each mole of diamino acid may combine with two moles of PLP.

The values determined for molar absorption coefficients of various aldimines, will be applied in the course of further investigations.

The most striking fact concerning the latter results is the marked difference between the molar absorption coefficients of the aldimines of different series of amino acids, depending upon the position and the number of NH_2 groups in the amino acids and the length of their carbon-chain.

Acknowledgement

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References

[1] Matsuo, O. (1957) J. Amer. Chem. Soc. 79, 2011–2015.
[2] Olivo, F., Rossi, C. S. and Siliprandi, N. (1962) in: Chemical and Biological Aspects of Pyridoxal Catalysis, Sympos. Rome, pp. 92–101, Pergamon Press, Oxford, 1963.
[3] Schonbeck, N. D., Stalski, M. and Shafer, J. (1975) J. Biol. Chem. 250, 5343–5351.
[4] Metzler, D. E. and Snell, E. E. (1952) J. Amer. Chem. Soc. 74, 979–983.
[5] Blakley, R. L. (1955) Biochem. J. 61, 315–323.
[6] Lucas, N., King, H. K. and Brown, S. (1962) Biochem. J. 84, 118–124.
[7] Ketelaar, J. A., Van de Stolpe, C., Goudsmit, A. and Dzcubas, W. (1952) Rec. Trav. Chim. Pays-Bas 71, 1104–1114.
[8] Peterson, E. A. and Sober, H. A. (1954) J. Amer. Chem. Soc. 76, 169–175.
[9] Abbott, E. H. and Martell, A. E. (1971) J. Amer. Chem. Soc. 93, 5852–5856.
[10] Bergel, F. and Harrap, K. R. (1961) J. Biochem. (London) 4051–4056.
[11] Mackay, D. (1962) Arch. Biochem. Biophys. 99, 93–100.
[12] Mackay, D. (1963) Biochim. Biophys. Acta 73, 445–453.