ELECTROPHORETIC AND CHROMATOGRAPHIC STUDY OF SOME CHEMICAL TRANSAMINATION REACTIONS INVOLVING VITAMIN B_6

P. FASELLA, H. LIS*, N. SILIPRANDI AND C. BAGLIONI
Institute of Biological Chemistry, University of Rome (Italy)

The possibility of nonenzymic transamination between PL** and amino acids and PM and α-keto acids has been demonstrated by METZLER AND SNELL[1] and confirmed by CENNAMO[2]. The proposed mechanism of the reaction involves the formation of Schiff-base chelates between PL and amino acids and PM and keto acids[3]. Spectrophotometric evidence has been reported for the formation of Schiff-base chelates from PL and amino acids and PM and keto acids in the presence of metal ions[4]; and of non-chelated Schiff-bases between PLP, glycine and serine in the absence of metal ions[5]. An insoluble crystalline potassium salt of a Schiff-base, involving PL and alanine (pyridoxyliden-D-alanine), has been obtained from alcoholic solutions by HEYL *et al.*[6]. BADDILEY[7] has succeeded in isolating an insoluble compound, which has been identified as a Schiff-base chelate between PL and tyrosine.

An insoluble PL-glycine-aluminium chelate has been obtained by METZLER *et al.*[8] upon heating a solution of PL and glycine in the presence of Al^{+++}.

None of these results, however, directly proves the participation of these compounds in the transamination reactions.

It seemed interesting to reinvestigate these reactions by means of chromatographic and electrophoretic methods recently developed[9]. By these procedures, it became possible to identify, isolate and characterize the intermediate reaction products of transamination reactions between PL or PLP and amino acids, and between PM or PMP and keto acids, in the presence of metal ions.

EXPERIMENTAL

PL hydrochloride and PM dihydrochloride were commercial samples from Hoffmann La Roche. Pyridoxal phosphate Ca salt and pyridoxamine phosphate were generously supplied by Merck and Co. (Rahway N.J., U.S.A.). Sodium pyruvate and DL-alanine were commercial samples from Hoffmann La Roche.

Assay methods

Alanine was detected by spot test according to FEIGL[10] and identified by paper chromatography according to CONSDEN AND GORDON[11].

Pyruvate was identified and determined by paper chromatography according to CAVALLINI *et al.*[12], and according to METZLER AND SNELL[1]. For the determination of small amounts of keto acids, the method was slightly modified as follows: the extraction with toluene was repeated twice. The water phase was acidified and extracted three times with 5 ml of ether. The ethereal solution

* Fellow of the Swedish Institute for Culture Exchange. Present address: Biokemiska Institutionen, Upsala Universitet (Sweden).

** Abbreviations, PL = pyridoxal; PLP = pyridoxal phosphate; PM = pyridoxamine; PMP = pyridoxamine phosphate.

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was concentrated in vacuum and the residuum was dissolved in 4 ml NaOH 1.25 *N* for the determination of the absorption spectrum in the Beckman D.U. spectrophotometer.

Vitamin B_6 derivatives were determined:

(a) *by paper electrophoresis* according to SILIPRANDI *et al.*[9]. Acetate buffer, pH 5.1, 0.05 *M*, was used for the analysis of non-phosphorylated compounds and triethylamine-acetate buffer pH 5.9, 0.05 *M*, was used for the analysis of the phosphorylated compounds. All determinations were carried out at 0°C.

(b) *by cellulose column electrophoresis*. The apparatus design and the technical details were according to FLODIN AND PORATH[13] and SILIPRANDI *et al.*[9]. The following minor modifications were, however, introduced: a volatile triethylamine-acetate buffer 0.05 *M* was used, at pH 5.1 for the non-phosphorylated compounds, and at pH 5.9 for the phosphorylated ones. Acetate buffer, 0.05 *M*, pH 5.1, and phosphate buffer, 0.05 *M*, pH 5.9, were used when the eluates from the column were to be tested for the amino groups.

Before application of the current, the mixture was displaced 3.5 cm down the column by allowing a sufficient amount of the buffer to flow through the column. For the separation of the non-phosphorylated compounds the top of the column was connected to the anode and the bottom to the cathode. For the separation of the phosphorylated derivatives, the connection of the column to the electrodes was inverted. A potential of 300 V, giving a current of about 20 mA, was applied for 50 hours. On completion of the run, the column was eluted with the same buffer at the rate of 8 ml/hour and the effluent collected in 4 ml fractions. The entire procedure was carried out at 0° C. The optical densities of the fractions were measured in the Beckman Model D.U. spectrophotometer at 325 mμ, and the fluorescence in the Klett fluorimeter, using a lamp filter 5970, a "thiamine" photocell filter, and a quinine sulphate solution (0.5 mμ/ml in sulphuric acid 0.1 *N*) as reference. The fractions were then concentrated at 0°C under high vacuum.

(c) *by paper chromatography*, using Munktell paper O.B. and a mixture of *n*-propanol, formic acid 10% (4:1) as solvent. This procedure allows a better separation of the non-phosphorylated derivatives than does paper electrophoresis.

(d) *spectrophotometrically* according to METZLER AND SNELL[1].

Copper was detected by the *o*-tolidine spot test, according to FEIGL[14].

TRANSAMINATION BETWEEN PYRIDOXAL AND ALANINE AND BETWEEN PYRIDOXAMINE AND PYRUVATE

Reactions were carried out at 100°C; solutions were 0.01 *M* in reactants, 0.001 *M* in alum and 0.05 *M* in buffer acetate pH 5. Analyses were made at zero time and at 3 to 6 intervals ranging from 5 to 60 minutes. Two reaction mixtures were used: (1) PL, alanine and alum; (2) PM, pyruvate and alum.

In both cases transamination occurs, as evidenced by the progressive decrease of the reactants and by the appearance of the corresponding reaction products. After 60 minutes equilibrium is reached, as formerly demonstrated by METZLER AND SNELL[1], and the chromatograms obtained from the two mixtures become identical; examination of the chromatograms, in U.V. light, for vitamin B_6 derivatives revealed the presence of four fluorescent spots (see Fig. 1) corresponding respectively to PL, PM and to two unknown compounds, C_I and C_{II}.

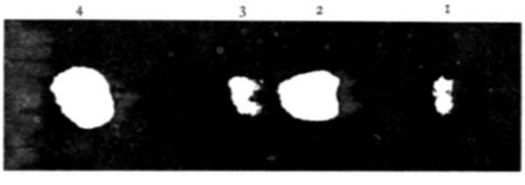

Fig. 1. Chromatographic separation of a reaction mixture containing PL 0.01 *M*, alanine 0.01 *M*, alum 0.001 *M*, heated at 100°C for 30 minutes in acetate buffer 0.05 *M*, pH 5.1. 1 = C_{II}; 2 = PM; 3 = C_I; 4 = PL.

By submitting the same reaction mixtures to paper electrophoresis four fluorescent spots, displaced towards the cathode were also obtained. The spots corresponding to C_I and C_{II} occupy an intermediate position between PL and PM; C_{II} is not very sharply separated from PM.

In U.V. light the spot corresponding to C_I exhibits a very intense light-blue fluorescence, and the spot corresponding to C_{II} a violet fluorescence closely resembling

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that of PM. Both C_I and C_{II} give negative reactions for free amino (ninhydrin[11]) and aldehydic (ammoniacal silver nitrate[15]) groups.

Chemical nature of C_I and C_{II}

To investigate the composition of the two compounds, samples of the reaction mixtures at equilibrium were analyzed by: (a) two-dimensional chromatography and (b) two-dimensional electrophoresis.

For two-dimensional chromatography 0.05 ml of a reaction mixture at equilibrium were separated chromatographically along one side of a square paper. After separation of the four compounds (PL, PM, C_I and C_{II}), the paper was dried at about 50 °C for 30 minutes, in an attempt to split the intermediate compounds, and developed in the other direction using the same solvent. A schematic representation of the results so obtained is given in Fig. 2.

Fig. 2 clearly shows that C_{II} gives rise to three spots corresponding respectively to PL, PM and C_I, while C_I gives rise to PL.

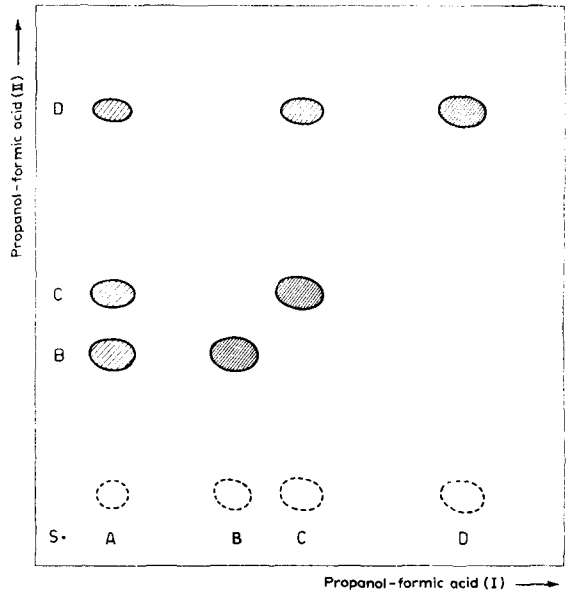

Fig. 2. Diagram of the chromatographic resolution of the breakdown products of the intermediate compounds upon examination in U.V. light. Dashed lines: position of the spots after the first chromatographic separation. Continuous lines: position of the spots after the second chromatographic separation. Intensity of the fluorescence: feeble (coarse-hatching); strong (fine-hatching). I = first run; II = second run.

For two-dimensional electrophoresis 0.05 ml of a reaction mixture at equilibrium was spotted at the corner of a square paper and separated electrophoretically along one side of the paper in a volatile triethylamine-acetate buffer pH 5.1, 0.05 *M*. After separation of the components the paper was dried at 50°C for 60 minutes to volatilize the buffer and to split the intermediate compounds, and developed electrophoretically in the other direction using an acetate buffer pH 5.1, 0.05 *M*. Examination of the electrogram in U.V. light revealed that C_{II} gives rise to PL, PM and C_I, and that C_I gives rise to PL, thus confirming the results obtained by two-dimensional chromatography. After spraying the papers with ninhydin, moreover, a violet spot appeared among the breakdown products both of C_I and of C_{II}, which on the basis of its electrophoretic behaviour, could be identified as alanine.

To obtain more convenient amounts of the intermediate compounds 2 ml of the above indicated reaction mixture were submitted to electrophoresis on a cellulose column. The separation obtained is reproduced in Fig. 3. The identity of the peaks was checked by paper chromatography. As can be seen in the figure, C_{II} cannot be separated from PM under these conditions. The solution of C_I eluted from the column was

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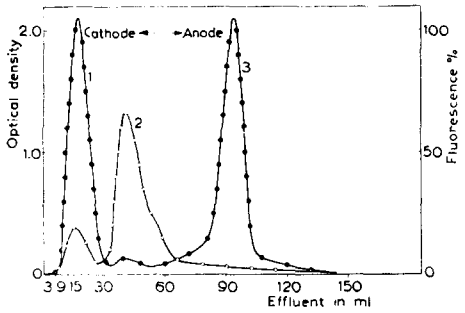

Fig. 3. Separation by cellulose powder column electrophoresis of a reaction mixture containing 0.01 *M* PL, 0.01 *M* alanine and 0.001 *M* alum in acetate buffer pH 5, 0.05 *M*, heated at 100°C for 30 minutes. •—•—• = optical density at 325 mμ; o—o—o = fluorescence (% of the fluorescence of a quinine sulphate solution 0.50 μg/ml in 0.1 *N* sulphuric acid). 1 = PM, and C_{II}; 2 = C_I; 3 = PL.

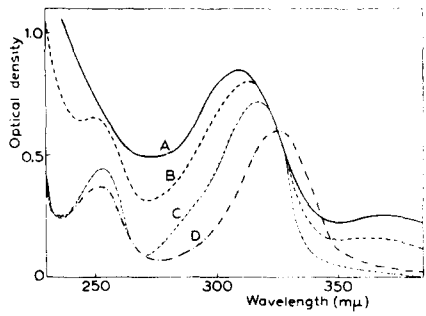

Fig. 4. Absorption spectra in acetate buffer 0.05 *M*, pH 5 of C_I (A); C_I after partial hydrolysis (B); PL (C); PM (D).

heated at 100°C for 30 minutes to split C_I. The analysis of the solution after heating revealed the presence of PL and alanine. These results confirm those obtained for C_I by two-dimensional paper chromatography and electrophoresis.

Absorption spectra of C_I and C_{II}

More evidence about the character of the intermediate compounds could be obtained by the study of their U.V. absorption spectra. Fig. 4 reproduces the spectrum of a solution of C_I obtained by cellulose column electrophoresis. The spectra of PL and PM at the same pH (pH = 5) and of the same solution of C_I, after it had been heated at 100°C for 30 minutes in order to split the compound, are given for comparison.

Fig. 5 shows the absorption spectra of C_I at different pH values.

The spectrum of C_I displays at all pH values a peak at 375–380 mμ that is unlike any of the absorption bands of PL or PM and is probably due to the formation of a new bond.

In the attempt to establish the absorption spectrum of C_{II}, and because of the above-mentioned difficulty in separating this compound by column electrophoresis, the spots corresponding to C_{II} were collected from several chromatograms and eluted with 4 ml acetate buffer pH 5, 0.05 *M*; the whole procedure was done at 2°C. The spectrum of the elution fluid was then determined; correction was made for the absorption due to the paper by using, as reference, the fluid collected by washing identical strips from the same paper. The spectrum of C_{II}, eluted from the paper, is not sufficiently stable to be determined. Only after a few hours at room temperature does the eluted solution acquire a stable spectrum, which closely resembles that of C_I after heating. This fact can be explained by the great instability of C_{II} and its tendency to give rise to PL, PM and C_I, which has already been ascertained by two-dimensional chromatography.

A Beckman D.U. spectrophotometer was used in these experiments.

Order of formation of C_I and C_{II}

Chromatographic analyses of the reaction mixtures in the course of transamination provide useful information concerning the formation of C_I and C_{II}.

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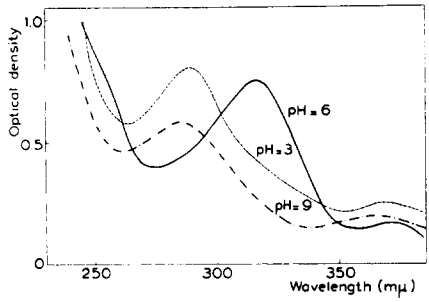

Fig. 5. Absorption spectra of C_I in aqueous buffered solution at pH 3 (·····), pH 6 (———) and pH 9 (·—·—·).

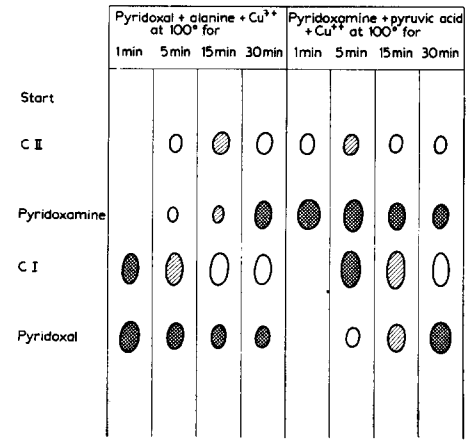

Fig. 6. Diagram of the chromatographic analysis of the transamination reaction involving the nonphosphorylated forms of vitamin B_6 at different times. Intensity of fluorescence: feeble (no hatching); intermediate (diagonal hatching); strong (cross-hatching).

Copper (0.001 *M* copper sulphate) was chosen as chelating agent, because the fluorescence of C_{II} is much stronger when this metal is used instead of aluminium. It was thus possible to follow the formation of C_{II} more accurately. The results are given in Fig. 6.

As is seen in the figure, the order of formation of the reaction products in the reaction between PL and alanine, is as follows (1) C_I, (2) C_{II}, (3) PM; and in the reaction between PM and pyruvate: (1) C_{II}, (2) C_I, (3) PL.

Kinetics of the reaction

An insight into the kinetics of the transamination reaction could be gained under the following conditions: reactions were carried out at 100°C, pH 5; the solutions were 0.01 *M* in the reactants and 0.001 *M* in alum. Analyses were made at zero time and at 6–8 intervals ranging from 30 seconds to 1 hour. PL and total vitamin B_6 were determined according to METZLER AND SNELL[1], and C_I fluorimetrically. Both the reaction between PL and alanine, and that between PM and pyruvate have been investigated, the latter more extensively because PL production could be measured more accurately than PL loss. The results are given in Fig. 7.

Fig. 7 shows that the increment of the PL concentration is roughly proportional to the concentration of C_I. This relationship can be more easily appreciated by comparing the curve of the concentration of C_I *vs.* time with that of $\frac{d[PL]}{dt}$.

Continuous variation

The method of continuous variation[4,16] has been applied to the study of the composition of C_I. By the assumption of the formation of a Schiff-base between PL and alanine and between PM and pyruvate, the three-component system has been simplified to a two component system (Schiff-base and aluminium). Considering the above-mentioned high intensity of the fluorescence of C_I compared to that of PL, PM and C_{II}, fluorescence was chosen as the most suitable property of the resulting complex (C_I) for the continuous variation study.

To determine the number of the moles of Schiff-base coordinated to one aluminium

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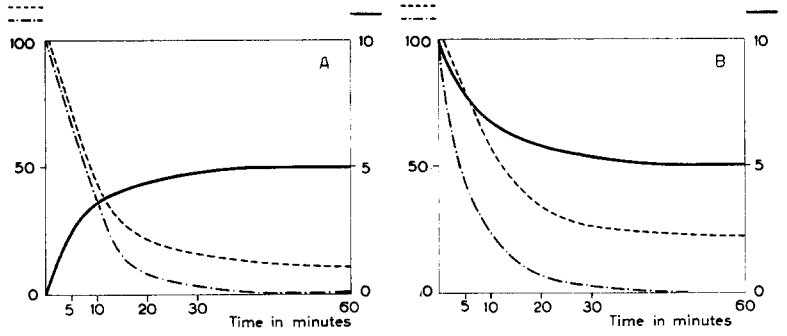

Fig. 7. C_I and PL concentrations *vs.* time during transamination reactions at 100°C between: (A) 0.01 *M* PM and 0.01 *M* pyruvate, (B) 0.01 *M* PL and 0.01 *M* alanine. Both solutions were 0.001 *M* in alum and at pH 5.0 in acetate buffer 0.05 *M*. ——— PL concentrations (millimolar). – – – – C_I concentrations in arbitrary "Fluorescence" units (the solutions were diluted 1:500 in cold acetate buffer 0.05 *M*, pH 5; their fluorescence, determined in the Klett fluorimeter and corrected for the fluorescence of PM and PL is expressed as % of the fluorescence of a standard quinine sulphate solution (0.5 μg/ml in 0.1 *N* sulphuric acid); the fluorescence due to C_{II} was disregarded). ·—·—· $\frac{d[PL]}{dt}$.

ion, equimolar solutions of PL-alanine and of alum, or of PM-pyruvate and of alum, were mixed in varying proportions to a constant final volume, so that the total molarity of the solutions was constant. Fluorescence readings were made after all solutions had been heated at 100°C for 10 minutes; the fluorescence values found for each solution were corrected for the fluorescence of the reactants, and expressed as % of a standard quinine sulphate solution; these values (Y) were plotted against the concentration of Schiff-base expressed as per cent of the total concentration of the solution (see Fig. 8).

For longer periods of heating, the fluorescence decreases, but the general shape of the curve remains constant. The composition at which the value of Y reaches a maximum bears a simple relation to the number of molecules co-ordinated to one aluminium ion, and is independent of the equilibrium constant[16]. The Y values pass through a maximum at a point corresponding to a solution that contains two moles of PL-alanine, or of PM-pyruvate, to one of aluminium (see Fig. 8).

TRANSAMINATION BETWEEN PYRIDOXAL PHOSPHATE AND ALANINE, AND BETWEEN PYRIDOXAMINE PHOSPHATE AND PYRUVATE

The transamination conditions described by METZLER AND SNELL[1] were slightly modified by increasing the concentration of copper ions. Two reactions were studied:

(a) 0.004 *M* PLP + 0.004 *M* alanine, in the presence of 0.02 *M* pyruvate.

(b) 0.004 *M* PMP + 0.024 *M* pyruvate.

Both solutions were 0.0005 *M* in copper sulphate and 0.1 *M* in phosphate buffer, pH 7.0.

The reactions were carried out at 100°C under nitrogen to prevent excessive air oxidation of PMP to PLP.

Analyses were made at zero time and at 3 to 6 intervals ranging from 5 to 60 minutes. The progressive decrease of the reactants and the appearance of the corre-

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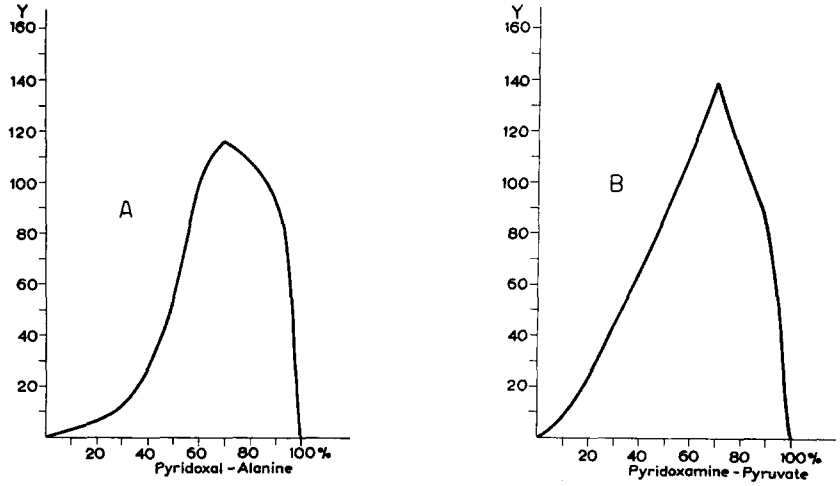

Fig. 8. Continuous variation of aluminium with PL-alanine (A) and PM-pyruvate (B). Total concentration of all solutions was 0.01 *M*. For the fluorescence determinations all solutions were diluted 1:200. Y = corrected fluorescence values expressed as % of the fluorescence of a standard quinine sulphate solution (0.5 μg/ml 0.1 *N* sulphuric acid).

sponding reaction products indicate that transamination takes place in both reactions. After 90 minutes equilibrium is reached.

Examination of the electropherograms for the vitamin B_6 derivatives in U.V. light revealed the presence of two fluorescent spots beside those corresponding to PLP and PMP. Both spots migrate towards the anode and occupy an intermediate position between PLP and PMP (Fig. 9).

One spot (C_IP) is of a pale green colour, and gives a violet fluorescence in U.V. light. The other spot ($C_{II}P$) is visible only in U.V. light by its green-blue fluorescence.

The intermediate spots were not observed with reaction mixtures containing only two ofthe three reactants involved.

With lower concentrations of copper sulphate, such as those used by METZLER AND SNELL[1], the intermediate products were observed only when the reaction mixture was incubated at low temperature (20°C).

Fig. 9. Electrophoretic separation of 0.05 ml of a reaction mixture containing 0.004 *M* PMP, 0.004 *M* alanine, 0.02 *M* pyruvate and 0.0005 *M* copper sulphate in phosphate buffer pH 7, heated at 100°C for 15 minutes in the presence of oxygen. 1 = PLP; 2 = $C_{II}P$; 3 = C_IP; 4 = PMP.

Chemical nature of C_IP and $C_{II}P$

For the chemical characterization of C_IP and $C_{II}P$ on the paper, we have proceeded as previously indicated for the non-phosphorylated compounds. It should be observed, however, that the papers used for the detection of free amino groups had been run in a phosphate buffer. Moreover, the papers were sprayed with ferric chloride and salicylsulphonic acid, according to WADE AND MORGAN[17], to detect the phosphoric radicals.

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Both C_IP and $C_{II}P$ gave a positive reaction for the phosphoric group and a negative one for the amino and aldehydic groups.

To investigate the composition of C_IP and $C_{II}P$, samples of the reaction mixture were spotted at the corner of a square paper (30 × 30 cm) and developed electrophoretically using a volatile triethylamine buffer. After separation, the paper was dried at about 60°C for two hours.

The paper was then developed electrophoretically in the other direction, using a phosphate buffer 0.05 *M* pH 5,9. C_IP is thus resolved into PMP, $C_{II}P$ and PLP; and $C_{II}P$ into PLP and $C_{II}P$. The findings are schematically reproduced in Fig. 10.

PMP and C_IP cannot be sharply separated by cellulose column electrophoresis; nevertheless, it has been possible to obtain a pure solution of C_IP by heating the reaction mixture at 100°C in the presence of oxygen for 30 minutes. Under these conditions PMP is quantitatively oxidized to PLP. The solution of PLP, $C_{II}P$ and C_IP is shown in Fig. 11.

The identity of the peaks was checked by paper electrophoresis.

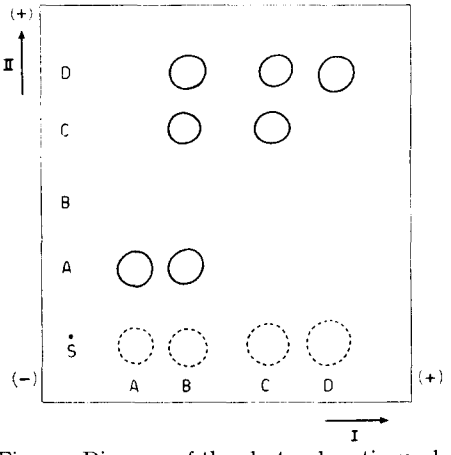

Fig. 10. Diagram of the electrophoretic resolution of the breakdown products of the phosphorylated intermediate compounds. S = start; A = PMP; B = C_IP; C = $C_{II}P$; D = PLP. Dashed lines: position of the spots after the first electrophoretic separation. Continuous lines: position of the spots after the second electrophoretic separation. I = first run; II = second run.

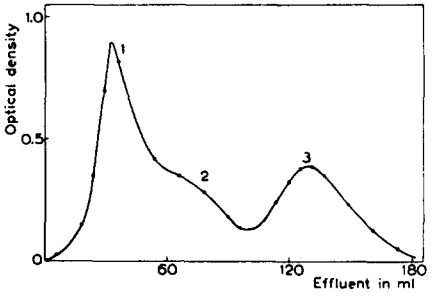

Fig. 11. Separation of C_IP from the reaction mixture (see text) by cellulose powder column electrophoresis: 1 = PLP; 2 = $C_{II}P$; 3 = C_IP. Optical density at 325 mμ.

The solution of pure C_IP was heated at 100°C for 60 minutes, in order to split the compound, and analysed. The analysis revealed the presence of PLP, alanine, pyruvate and copper.

PMP, being readily oxidized to PLP when heated at 100°C in the presence of copper ions, was not detected among the breakdown products.

Absorption spectra of C_IP

The spectrum of a pure solution of C_IP, obtained by cellullose column electrophoresis, at pH 5.9 is reproduced in Fig. 12. The spectra of PLP, PMP at the same pH

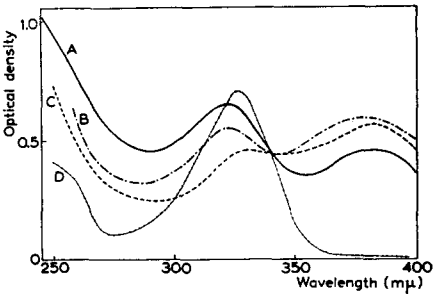

Fig. 12. Absorption spectra at pH 6 of C_IP (A), C_IP after partial hydrolysis (B), PLP (C) and PMP (D).

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and of the solution of C_IP after heating at 100° C for 30 minutes are given for comparison.

It has not been possible to obtain $C_{II}P$ in sufficient amounts for the determination of the spectrum.

Order of formation of C_IP and $C_{II}P$

The electrophoretic picture of the reaction mixtures during transamination is reproduced in Fig. 13.

In the reaction between PLP and alanine, the reaction products are formed in the following order: (1) C_IP, (2) $C_{II}P$, (3) PMP.

In the reaction between PMP and pyruvate $C_{II}P$ is formed first, followed successively by C_IP and alanine. Small amounts of PLP are produced very early owing to the partial oxydation of PMP in the presence of copper ions; the formation of alanine has therefore been taken as a criterion that transamination has taken place.

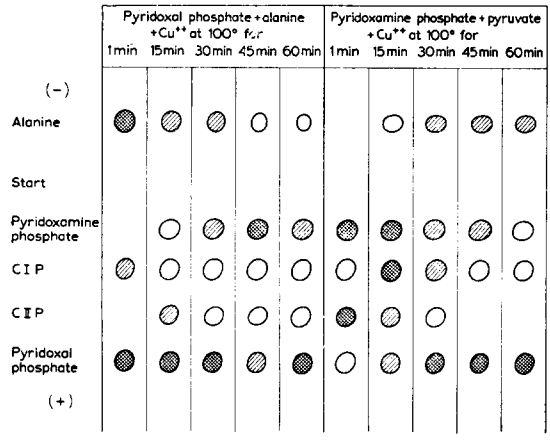

Fig. 13. Diagram of the electrophoretic analysis of the transamination reactions involving the phosphorylated forms of vitamin B_6 at different times. Intensity of fluorescence: feeble (no hatching); intermediate (diagonal hatching); strong (cross-hatching).

Continuous variation

For the continuous variation study of the phosphorylated compounds we have proceeded as previously indicated for the non-phosphorylated forms. The simplified two-component systems resulted in a Schiff-base (PLP-alanine, or PMP-pyruvate) and alum. Aluminium was chosen instead of copper because the fluorescence of C_IP is much stronger when aluminium is used.

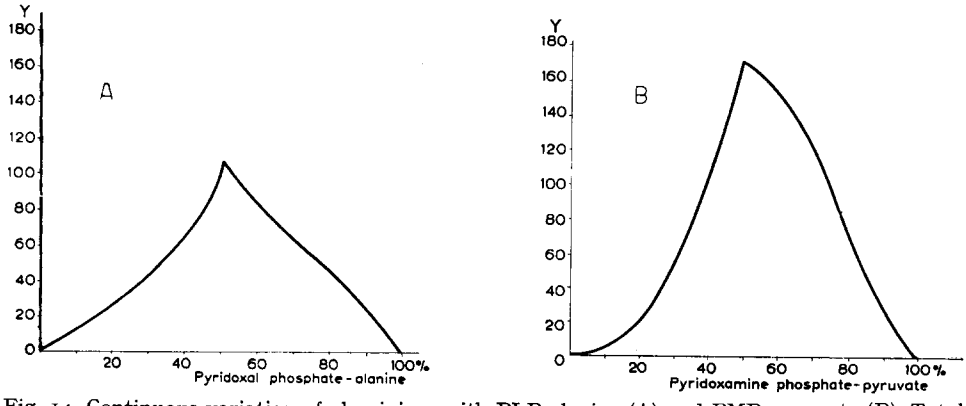

Fig. 14. Continuous variation of aluminium with PLP-alanine (A) and PMP-pyruvate (B). Total concentrations of all solutions were 0.004 *M*. For the fluorescence readings all solutions were diluted 1:60. Y = corrected fluorescence values expressed as % of the fluorescence of a standard quinine sulphate solution (0.5 μg/ml in 0.1 *N* sulphuric acid).

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The fluorescence readings were made after all solutions had been heated at 60°C for 10 minutes. The plot of the *Y* values against the composition of the solutions, obtained as previously described for the non-phosphorylated forms, are given in Fig. 14.

For longer periods of heating the fluorescence decreases but the general shape of the curve remains fairly constant. The *Y* values pass through a maximum at a point corresponding to a solution that contains one mole of PLP-alanine, or of PMP-pyruvate to one of aluminium.

DISCUSSION

The electrophoretic and chromatographic study of the transamination reactions involving vitamin B_6 has led to the isolation and characterization of two intermediate products for each of the reactions considered, and has provided convincing evidence for the participation of these compounds in the transamination mechanism.

The intermediate compounds have been isolated both chromatographically and electrophoretically. They are characterized by an intense fluorescence when irradiated with U.V. light, and by highly specific absorption spectra (C_I and C_IP).

The evidence for the nature of these compounds is the following:

(1) Their formation requires the presence of three reactants: PL, alanine and metal ions; or PM, pyruvate and metal ions.
(2) The compounds have no free aldehydic or amino groups.
(3) Upon heating they split into pyruvate or alanine and the corresponding vitamin B_6 derivatives. In the case of C_IP it has, moreover, been possible to demonstrate the presence of copper among the hydrolysis products. The hydrolysis is accompanied by a characteristic change of the absorption spectrum which indicates the formation of PL or PM.
(4) C_I and C_IP are formed first when the initial reaction mixture contains PL, or PLP, and alanine; C_{II} and $C_{II}P$ are formed first when the initial reaction mixture contains PM, or PMP, and pyruvate.
(5) From the continuous variation study it can be concluded that two molecules of Schiff-base are co-ordinated to one aluminium ion to form C_I. These data are consistent with those obtained by EICHHORN[4] for a pyridoxal-alanine-nickel complex and by BADDILEY[7] for a pyridoxal-tyroxine-copper complex. Figures obtained for the phosphorylated compounds (see Fig. 14) would indicate, on the other hand, that one Al^{+++} is coordinated with one Schiff-base molecule. These results might account for the electrophoretic behaviour of the phosphorylated and non-phosphorylated compounds.

It can therefore be concluded that C_I and C_IP derive from alanine and PL or PLP, and C_{II} and $C_{II}P$ from pyruvate and PM or PMP, respectively. The condensation involves a reaction between an amino and an aldehydic (or ketonic) grouping and the intervention of metal ions to form Schiff-base chelates.

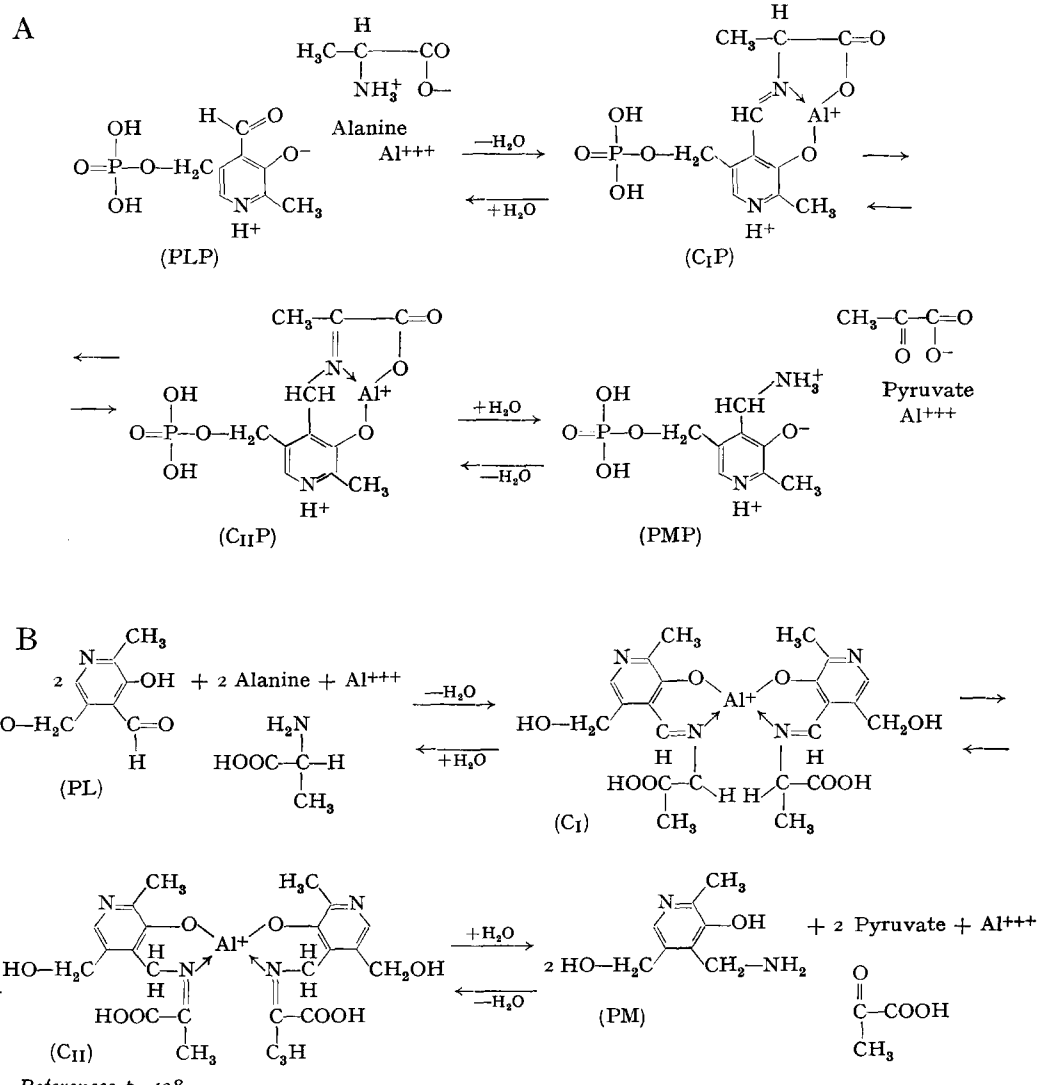
One metal ion is co-ordinated to two molecules of Schiff-base in the case of the non-phosphorylated compounds and to one molecule of Schiff-base in the case of the phosphorylated derivatives. To C_I and C_{II}, therefore, can be attributed the formulas proposed by BADDILEY[7] and to C_IP and $C_{II}P$ those proposed by METZLER *et al.*[3] (see reaction schemes A and B).

References p. 428.

Evidence for the participation of these compounds in transamination is the following:

(1) Upon heating, the intermediate compounds are converted one into the other and to the final reaction products.
(2) In the initial phase of the reaction the transamination rate is proportional to the concentration of C_I.
(3) The order of formation of the intermediate compounds in the transamination between PL or PLP and alanine is the reverse of the order of formation between PM or PMP and pyruvate.

On the basis of these experimental results the mechanism of the chemical transamination reactions can be represented as follows:



References p. 428.

Our findings therefore provide further experimental evidence for the reaction mechanism postulated by METZLER AND SNELL[1,8] for chemical transamination.

SUMMARY

Analytical investigation of chemical transamination reactions between α-keto or amino acids and phosphorylated and non-phosphorylated derivatives of vitamin B_6, has been carried out by means of chromatography and electrophoresis on filter paper. It has thus been possible to give evidence that in each reaction two strongly fluorescent intermediates are formed.

Two of these compounds have been isolated in appreciable amounts by means of electrophoresis on a cellulose column.

The spectra and chemical properties of the intermediate compounds indicate that they are Schiff-base metal chelates between pyridoxal or pyridoxal phosphate and amino acids and pyridoxamine or pyridoxamine phosphate and α-keto acids.

Evidence has, moreover, been obtained for the participation of these compounds in the transamination mechanism.

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Short Communications

Studies of bacterial resistance to 6-azauracil and its riboside*

It was previously reported that 6-azauracil (*as*-triazine-3,5-dione, AzU) is an effective inhibitor of the growth of a number of species of microorganisms and experimental neoplasms in mice[1,2,3,4]. However, in the microbial studies, resistant populations ultimately evolved in most experiments. The nature of this resistance has been investigated in *Streptococcus faecalis* (A.T.C.C. 8043) and less completely in *Escherichia coli* B and *Lactobacillus leichmannii* (A.T.C.C. 7830). When *S. faecalis* was grown in the presence of 6-azauracil-2-^{14}C** essentially no free AzU or nucleic acid-bound

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** This compound, *as*-triazine-3,5-dione-3-^{14}C, was prepared by Dr. P. K. CHANG in this department by a method to be submitted for publication.