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CONDITIONS FOR THE FORMATION OF COMPLEXES BETWEEN PYRIDOXAL PHOSPHATE AND SOME IMIDAZOLE DERIVATIVES

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Abstract—Imidazole derivatives form cyclic compounds with pyridoxal phosphate (PLP). The conditions of the cyclization reaction depend on both the pH of the media and the concentration of the substrate. Imidazole and imidazolelactic acid do not react with PLP.

Histamine, histidine and its methyl derivatives each form a Schiff's base which is transformed during incubation into a complex compound with a maximum absorption spectrum at 330 mμ—probably imidazoletetrahydropyridine.

The optimal pH for the reaction with histamine is 7·0–9·0; optimal concentration is 5×10^{-2} M, but the complex is still formed with 10^{-5} M of histamine. The optimal pH for reaction of PLP with histidine and its methyl derivatives is 7·2; optimal concentration 10^{-1} M; the complex is still formed in 10^{-4} M of histidine.

Methyl derivatives of histidine (L-1-methyl and L-3-methylhistidine) form a Schiff's base and cyclic compound. The mechanisms of the cyclization reaction and the importance of the phenomenon in the regulation of the level of the amines in the tissues are discussed.

PYRIDOXAL, pyridoxal phosphate (PLP), or PLP-dependent enzymes under certain conditions may react in an irreversible way with amines and aminoacids. In such reactions Schiff's bases are transformed into cyclic compounds. Various complex combinations may occur, depending on the reacting aminoacid and pyridoxal or PLP. Pyridoxal or PLP may enter into the irreversible reaction with 1-amino-2-thiol compounds (cysteine, cystamine) to form thiazolidine derivatives.[1–4] A similar type of reaction may occur with 1-amino-2-imidazole compounds, to form a cyclic compound imidazoletetrahydropyridine.[1–3] As a result of PLP reaction with 3-hydroxyphenylethylamine, cyclic compounds are formed of the tetraisoquinoline type.[5]

The formation of complex cyclic compounds, which lead to the removal of the coenzyme, may be of importance in the processes of inhibition of enzymatic reactions involving PLP. Under certain conditions some amines and aminoacids may inhibit decarboxylation processes;[5–7] there may be a competition for kynureninase between kynurenine and aminoacids,[8] which may be inhibitors of the reaction catalyzed by this enzyme.[4] A complex cyclic compound may result from PLP reaction with histamine;[7] the reaction was shown to occur at histamine concentrations only slightly higher than those found in certain tissues under physiological conditions.

In the experiments now reported an attempt has been made to determine the optimal conditions (pH and substrate concentration) for cyclization of the Schiff's base and for the formation of complex combinations of PLP and some imidazole derivatives.

METHODS

Tests were performed on the course of the *in vitro* reaction of PLP with imidazole (Im) as well as with its derivatives: imidazolelactic acid (ImLA), histamine acid phosphate (Hi-P), histamine dihydrochloride (Hi-2HCl), DL-histidine (DL-His), L-histidine-dihydrochloride (L-His-2HCl), L-1-methylhistidine (L-1-MeHis) and L-3-methylhistidine (L-3-MeHis). The PLP concentration used was kept constant at 7×10^{-5} M. Substrate concentrations were varied as follows:

Substrate	10^{-1} M	5×10^{-2} M	10^{-2} M	10^{-3} M	10^{-4} M	10^{-5} M
Im	+	+	+			
ImLA	+	+	+			
Hi-P	+	+	+	+	+	+
Hi-2HCl	+	+	+	+	+	+
DL-His	+	+	+	+	+	
L-His-2HCl	+	+	+	+	+	
L-1-MeHis	+	+	+	+	+	
L-3-MeHis	+	+	+	+	+	

With each concentration a series of determinations was performed, in phosphate buffer within a pH range from 5 to 9 at pH intervals equal to 1. In some experiments other pH ranges were used in addition and these are specified in the text.

The course of the reaction was tested by plotting the absorption curve obtained for PLP at wavelengths from 250 to 500 mμ. 1 ml of PLP solution was mixed with 1 ml of the compound tested in suitable concentration and 1 ml of buffer of defined pH. The absorption spectrum was determined at 10 mμ intervals (zero time) then the reacting mixture was incubated at 37° in water bath.

Readings were then taken every 15 min using a Hilger–Watts Uvispek H 700 spectrophotometer for absorption measurements, and an LBS 3A (Poland) pH meter for pH determinations from pH 5·0 to 14·0 as well as an IL (U.S.A.) instrument with a scale expander for pH 6·8–8·0.

RESULTS

1. *General character of the reaction*

PLP shows two absorption maxima: at 390 and 330 mμ; the latter being a weaker one. When a Schiff's base is formed (zero time) the absorption maximum shifts toward visible light. At high hydrogen ion concentrations (pH=5·0–6·0) the maximum was observed at 400 mμ, while it occurs at 410 mμ in alkaline solutions. The shift is accompanied by the appearance of a yellow color, characteristic for the formation of a Schiff's base.[9] During incubation of PLP-imidazole derivative mixtures the azometin peak gradually disappeared and at the same time a new absorption maximum appeared at 325–330 mμ, probably due to gradual cyclization of the Schiff's base and the formation of a complex compound.

2. *PLP reaction with Im and ImLA*

No shifts of the absorption spectrum were observed and neither a Schiff's base nor a complex compound were formed, during the reactions of Im or ImLA with PLP.

3. *PLP reaction with histamine*

The reaction was additionally tested at pH=7·2 and 7·5. The results of the reaction between Hi-2HCl with PLP are illustrated in Fig. 1 (Hi-P reaction with PLP was identical).

With both Hi-2HCl and Hi-P, the absorption spectrum of the reacting mixture shifted to 400–410 mμ, i.e. characteristic for Schiff's base. During incubation the

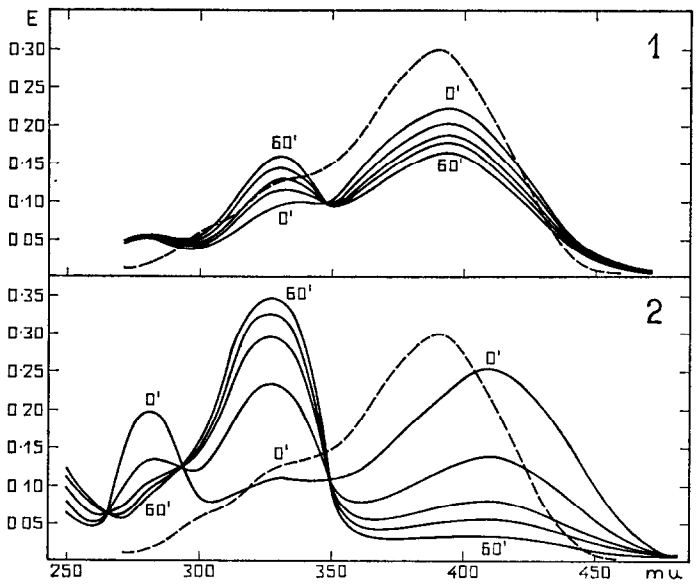

FIG. 1. Reaction of histamine-2HCl (5×10^{-2} M) with pyridoxal phosphate (PLP) (7×10^{-5} M) 0, 15, 30, 45 and 60 time of incubation in min. (1) Phosphate buffer pH 6·0 (2) Phosphate buffer pH 8·0. Dotted line—the absorption spectrum of PLP.

azometin peak gradually decreased and a new absorption maximum appeared at 330 mμ.

It may be inferred that during incubation azometin is transformed into a new product; within 1 hr the Schiff's base is completely transformed into the new compound. The absorption spectrum remained unchanged when tested 24 hr after incubation was stopped. The course of the reaction depends on pH. Figure 2a presents the results for Hi-2HCl.

The reaction was slowest at pH 5·0; optimal at pH 7·2 and was not modified by further alkalization. It should be noted, however, that the pH of the reacting mixture was acidified by both the compounds tested. At optimal substrate concentration (5×10^{-2} M) pH was lowered by 0·2, thus suggesting that the rate of complex formation is highest at neutral pH of the medium.

The kinetics of azometin transformation into the new compound depends on the pH of the medium. In acid medium the increase in extinction with unit time (tested every 15 min) was linear.

In neutral or alkaline medium the formation of cyclic compound was most rapid during the first 15 min (Fig. 3a).

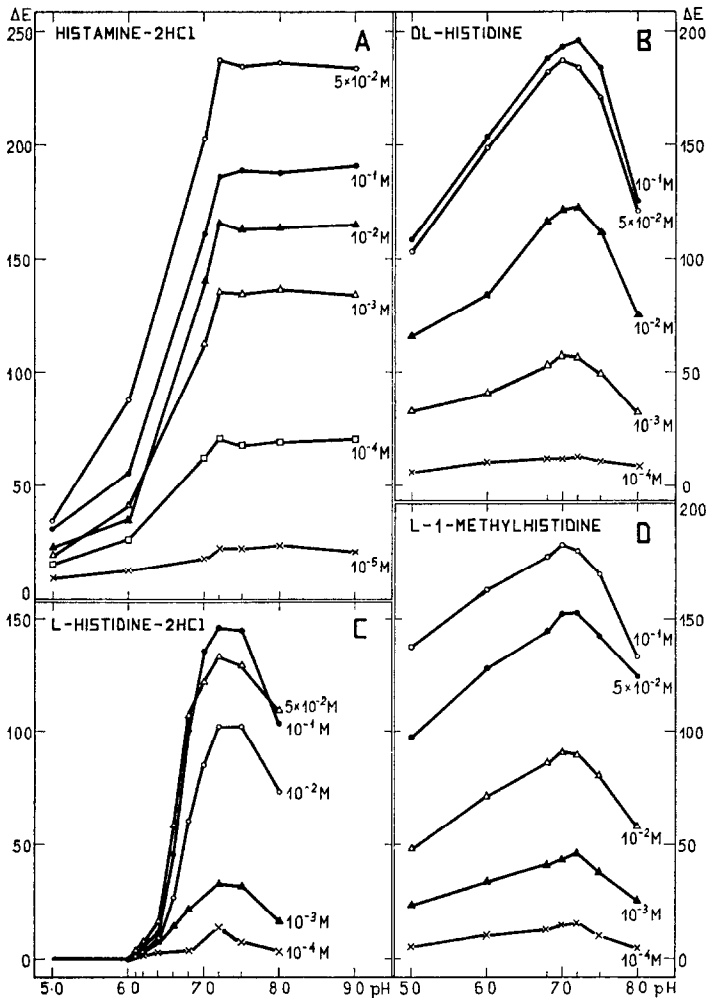

FIG. 2. pH-dependence of cyclization reaction between pyridoxal phosphate (7×10^{-5} M) and (a) Histamine-2HCl (b) DL-histidine (c) L-histidine-2HCl (d) L-1-methylhistidine (in various concentrations).

The course of the reaction depended on the substrate concentration (Fig. 2a). The concentration of 5×10^{-2} M was optimal. Concentration of 10^{-5} M was the lowest at which azometin was still found to be transformed into a complex compound.

4. *PLP reaction with histidine*

The reaction was tested at pH 6·8, 7·2 and 7·5 with DL-His and at pH=6·2, 6·4 and 6·6 with L-His-2HCl. With both substrates absorption maximum typical for that of a Schiff's base was observed, as well as transformation of azometin into the cyclic compound. However, there were marked differences between the two histidine compounds tested.

In the reaction with DL-His the complex compound was formed over the whole pH

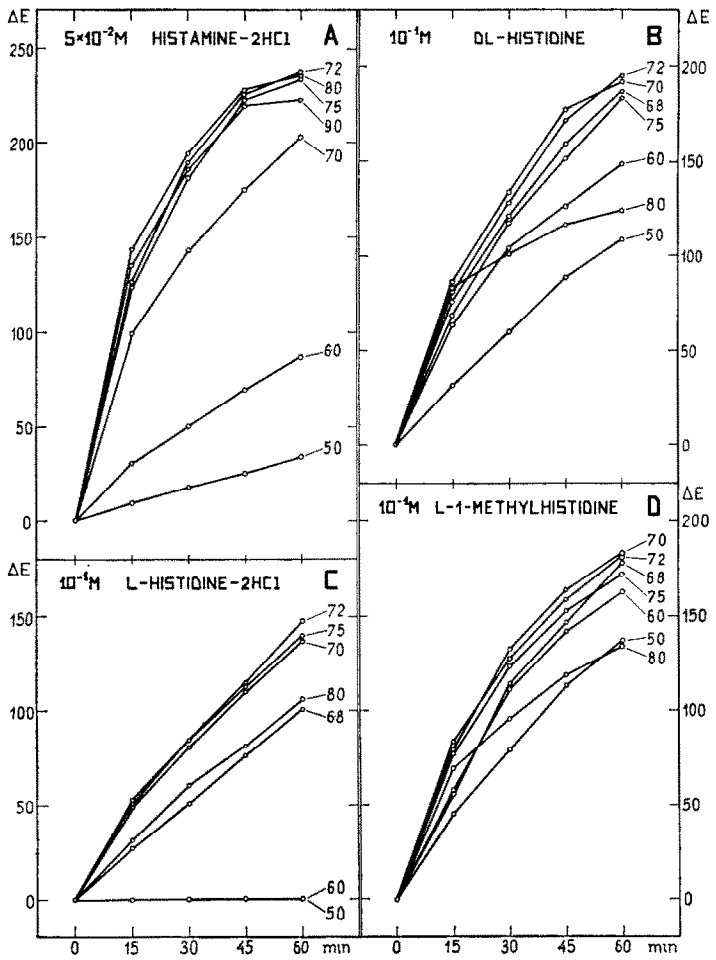

FIG. 3. The rate of cyclization reaction during incubation of pyridoxal phosphate (7×10^{-5} M) with: (a) Histamine-2HCl (5×10^{-2} M) (b) DL-histidine (10^{-1} M) (c) L-histidine-2HCl (10^{-1} M) (d) L-1-methylhistidine (10^{-1}M); pH 5–9.

range tested (5·0–8·0), but changes in pH were clearly accompanied by changes in the dynamics of the reaction (Fig. 2b). Three rates can be distinguished, depending on pH; the reaction ran rather quickly at pH 5·0–6·0, but its rate was highest at pH 7·0–7·4 and further alkalization of the medium resulted in a decrease in the reaction rate. As the DL-His itself only altered the pH of the mixture by 0·05, the optimum pH for the reaction was found to be only slightly alkaline at pH 7·2. The reaction was most rapid within the first 15 min of incubation, especially at pH 8·0 when it is almost complete (Fig. 3b), then its rate decreased, though to an extent lower than in the case of histamine. DL-His concentration of 10^{-1} M was optimal, although complex compound was still formed at 10^{-4} M concentration.

The reaction with His-2HCl was found not to occur at pH 5·0–6·0 (Fig. 4). The formation of the Schiff's base and its transformation into the new compound was observed at pH higher than 6·2; the reaction was optimal at pH 7·2–7·5 (Fig. 2c) and

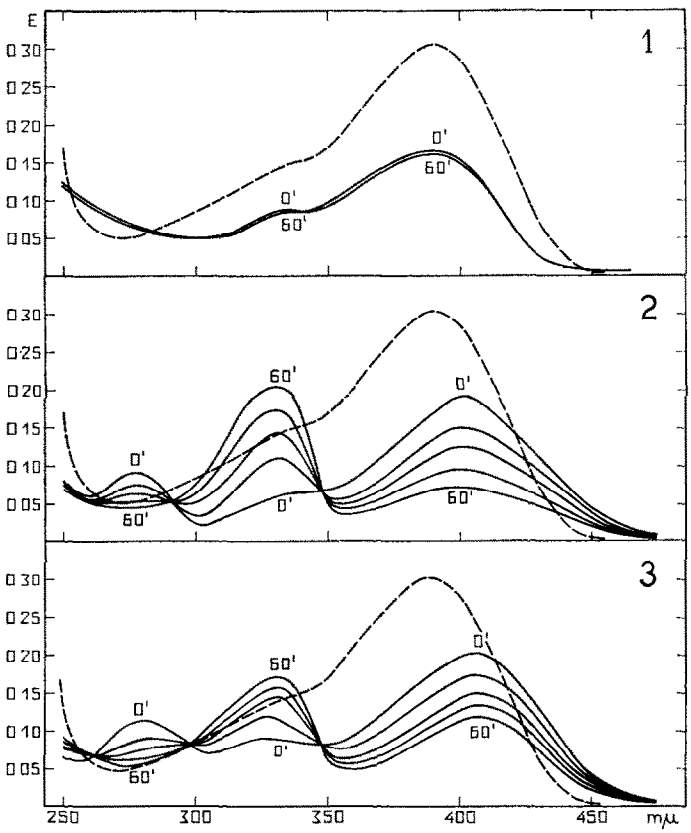

FIG. 4. Reaction of L-histidine-2HCl (10^{-1} M) with pyridoxal phosphate (PLP) (7×10^{-5} M). 0, 15, 30, 45 and 60 time of incubation in min. (1) Phosphate buffer pH 5·0 (2) Phosphate buffer pH 7·2 (3) Phosphate buffer pH 8·0. Dotted line—the absorption spectrum of PLP.

at more alkaline pH cyclization of the Schiff's base was lower. His-2HCl itself acidified the mixture by about 0·5, thus optimum pH of the reaction PLP–His-2HCl mixture is equal to 6·7–7·0. Cyclization of azometin was most rapid within the first 15 min of incubation (Fig. 3c). The concentration of His-2HCl for complex compound formation was optimal at 10^{-1} M, although the reaction was still found to occur at 10^{-4} M.

5. *PLP reaction with methyl histidine derivatives*

It was suggested[7] that a proton at position 1 of the imidazole ring is involved in the process of cyclization of the Schiff's base. If this hypothesis is true, the process should not occur when hydrogen is substituted by another group, for example the methyl one. L-1-MeHis and L-3-MeHis were tried. The results of reaction between L-1-MeHis with PLP being presented in Fig. 5.

Methyl derivatives appeared to react with PLP like DL-His. Figure 2d presents the course of the reaction of L-3-MeHis depending on pH (L-3-MeHis reaction was identical). Optimal pH was 7·0–7·2 for both methyl derivatives and optimal concentration 10^{-1} M. Both the compounds produced a slight alkaline shift of the mixture (by

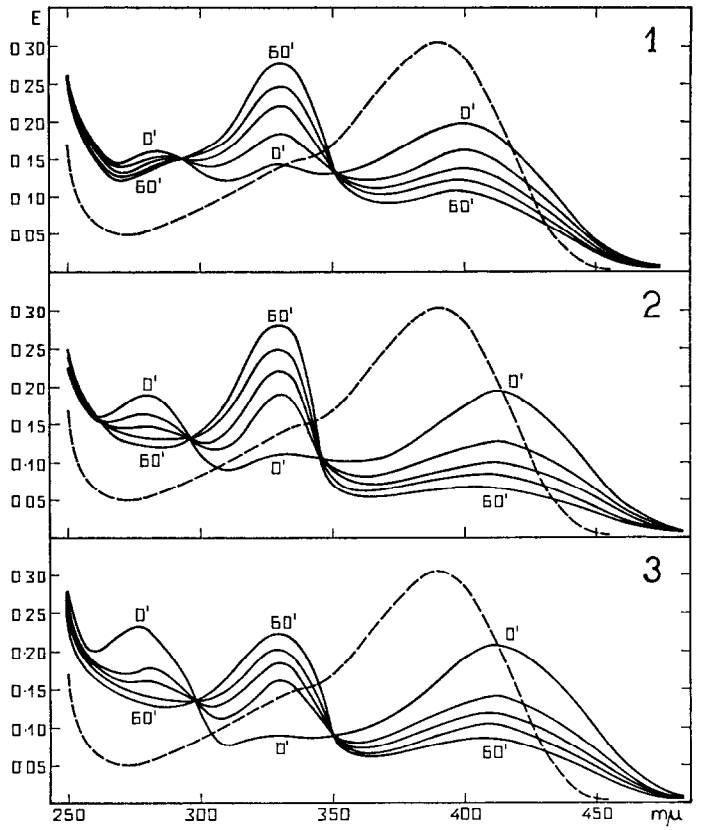

FIG. 5. Reaction of L-1-methylhistidine (10^{-1} M) with pyridoxal phosphate (PLP) (7×10^{-5} M). 0, 15, 30, 45 and 60 time of incubation in min. (1) Phosphate buffer pH 5·0 (2) Phosphate buffer pH 7·0 (3) Phosphate buffer pH 8·0. Dotted line—the absorption spectrum of PLP.

about 0·05). The reaction rate was highest within the first 15 min of incubation. It was striking that at that time and at pH 8·0 the Schiff's base almost totally transformed into the cyclic compound (Fig. 3d).

DISCUSSION

A Schiff's base is formed by the reaction of an aldehyde with an amino group. In the results described above, Im and ImLA failed to react with PLP because of the lack of an amino group.

In the reaction of PLP with imidazole derivatives containing the amino group, a Schiff's base was formed immediately after mixing the compounds. During incubation this base was transformed into a new compound with an absorption maximum 330 mμ; believed to be an imidazoletetrahydropyridine. It was isolated in crystalline form from alcoholic solutions.[3] It is stable, since the extinction was unaltered after 24 hr. It cannot be excluded, of course, that dissociation may occur under different experimental conditions.

The formation of a cyclic compound is closely associated with the pH of the medium. This is in turn related to the properties of the imidazole ring—its secondary

'pyrrol" and tertiary "pyridine" nitrogen atoms,[10] amphoteric features,[11] and the character of the side chain.

In the case of Hi the reactions ran slowly at low pH. The reaction was optimal at pH 7·0 and further rise in pH was without effect on the dynamics of the reaction. It may be related with an ionization of histamine. In pH 7·5 histamine monocation (Hi^+) concentration is 96·6%, while in pH 8·5 the concentration is 95·1%.[12] The cyclization reaction was optimal at a Hi (Hi-P and Hi-2HCl) concentration of 5 × 10^{-2} M, and not at highest concentration of 10^{-1} M. A similar finding was also reported in the reaction of PLP with glycine.[9] It seems possible that the bond at position 5 cannot be increased in length when there are too many molecules in the medium and this may be the reason why the complex is formed at a lower rate. A Hi concentration of 10^{-5} M was the lowest one at which a cyclic compound was still formed and this concentration is only slightly higher than found in many body tissues.[13–16]

The effect of changes in pH on the formation of the cyclic compound was most obvious in the reaction of PLP with Hi. Like all aminoacids, His has the ability to transfer a proton from a carboxyl to an amino group and thus form a dipolar ion. The fact that PLP reacts at a lower rate with His than with Hi might thus be associated with the presence of the carboxyl group on the alfa C of His and with the fact that this group, located near H atom, makes the latter more acid in character. The effect of pH was more distinct with the PLP–His–2HCl reaction than with the PLP–DL-His reaction. At acid pH (5·0–6·0) the reaction with His-2HCl did not occur while the reaction with DL-His was still observed. This was probably due to the higher acidity of His-2HCl, resulting from both the presence of the COOH group and the neighboring H atom and the presence of two HCl molecules per one His molecule. The acidity of the solution was thus higher (by 0·5 compared with the buffer) and the dynamics of the cyclic compound formation might be made lower in this way. In alkaline solution an excess of OH^- ions is accompanied by the presence of aminoacid $RCOO^-$ ions. The presence of this carboxyl group, which forms negative ions at such a pH, probably results in a lower rate of azometin cyclization.

The formation of a complex compound in the PLP reaction with methyl derivatives of His and similarity of this reaction with PLP–DL-His reaction, clearly indicate that the azometin rings is closed without participation of the proton at the N atom of the imidazole ring. It might be supposed that the double bond in the imidazole ring (Fig. 6) does not equally belong to both carbon atoms (C_4 and C_5), but it exerts a certain inductive effect on the atom at position 5. In the C—H bond the H atom is less electronegative than the C atom, which results in the appearance of a partial charge on these atoms. Some minus charge δ^- may appear in C_5, which may lead to polarization and a loose C—H linkage (H^+ proton is weakly bond with C_5). Since the cyclization

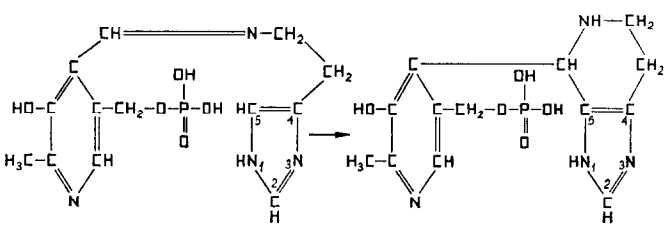

FIG. 6. Formation of a cyclic product between histamine and pyridoxal phosphate.

reaction runs at the highest rate in a weakly alkaline medium, an excess of OH^- ions may make this linkage still more loose. The nonpolarized electron pair on the N atom of the azometin bond may exert an attractive force on the H^+ proton of this loose linkage, thus making it possible for the transfer of H^+ to N atom. The process is associated with the disappearance of the CH=N double bond and with the formation of a transient electron deficiency on the C atom of the formyl group of PLP. Attractive forces may then appear between the two differently charged carbon atoms: $C^{\delta+}$ of the PLP formyl group and the $C^{\delta-}$ at position 5 of the imidazole ring: the new ring can be closed and the cyclic compound can be formed.

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