Vanadium Catalysis in the Nonenzymatic Transamination of δ-Aminolevulinic Acid

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Vanadium, δ-Aminolevulinic Acid, 4.5-Dioxovaleric Acid, Transamination, Pyridoxal Phosphate, Schiff Base Complexes

By nonenzymatic model reactions, the influence of vanadium (VO^{2+}, VO_3^-) and other metal ions (Al^{3+}, Fe^{2+}, Fe^{3+}, Zn^{2+}, Ni^{2+}, Cr^{3+}) on the transamination of 4,5-dioxovaleric acid (DOVA) with L-alanine (Ala) to δ-aminolevulinic acid (δ-ALA) and pyruvic acid (PA) with pyridoxal phosphate (PLP) is investigated. The examinations of the partial reactions show that VO^{2+}, VO_3^- and Fe^{2+} effectively enhance both conversions, while Ni^{2+} only promotes the conversion of DOVA to δ-ALA with pyridoxamine phosphate (PMP). Besides Cr^{3+}, which inhibits the transaminations, all other metals tried slightly enhance both reactions. The coupled transamination DOVA/Ala to δ-ALA/PA with PLP as a catalyst is performed at 40 °C, the extent of the reaction being judged by the amount of δ-ALA formed. Among all metal ions tested, VO^{2+} and VO_3^- were found to be the most effective ones. Fe^{2+}, Al^{3+} and Fe^{3+} also slightly enhance the reaction, but all other metals do not influence or inhibit the transamination. In presence of VO^{2+} or VO^{3-} ions, the optimum pH of the reaction shifts from pH 4 to pH 7.4. The positive influence of vanadium on δ-ALA transamination is discussed in connection with known structures of analogous Schiff base complexes with metal ions.

Introduction

Investigations have firmly established the essential role of the trace metal vanadium [1 – 3].For green plants, the element was found to be important for chlorophyll biosynthesis [2 – 4] and photosynthesis [5], while in animals, vanadium is assumed to be implicated in the metabolism of the erythrocytes [6]. We were anxious to define more precisely the essential function of vanadium in the important metabolic pathway of green plants, leading to the chlorophylls. Here, we first succeeded in tracing the positive influence of the metal to the formation of protoporphyrin-IX [2], the common intermediate for chlorophylls and also for heme pigments. We then discovered that the green alga *Chlorella pyrenoidosa* strongly increases its formation of δ-aminolevulinic acid (δ-ALA), when treated with vanadate in presence of levulinic acid [7]. Further studies with precursors of δ-ALA biosynthesis in the same organism led us to postulate a positive catalysis of vanadium in the transamination pathway to δ-ALA [8], where DOVA is transaminated by the enzyme δ-ALA transaminase with

L-alanine as a NH_2-group donor in presence of pyridoxal phosphate (PLP) [9].

The conversion of DOVA is indicated by the following equations:

$$Ala + PLP \rightleftharpoons PA + PMP \qquad (1)$$

$$PMP + DOVA \rightleftharpoons \delta\text{-}ALA + PLP \qquad (2)$$

$$Ala + DOVA \overset{PLP}{\rightleftharpoons} \delta\text{-}ALA + PA . \qquad (1+2)$$

The reactions follow the known scheme for transaminations, catalyzed by PLP, via a Schiff base intermediate, which is hydrolyzed after its tautomeric rearrangement [10]. This type of reaction is known to be influenced by metal ions which form complexes with the Schiff base [11], but vanadium has not been tested before. The function of the metal is now studied in detail in a non-enzymatic model system for δ-ALA transamination.

At first, we had to investigate, if vanadium forms Schiff base complexes with PLP and the substrates (Ala, DOVA), because the formation of such complexes is a precondition for the exspectation of a possible V-catalysis in the transamination. The reactions were performed with vanadyl salts (as $VOSO_4$) and also with vanadate (as $NaVO_3$), since stable complexes of both compounds with Schiff bases such as salicyaldehyde ethylenediimine are well established [12].

Experimental

Benzaldehyde, levulinic acid, PLP, PMP, Ala, and the metal salts were purchased from Merck, Darmstadt. DOVA was synthesized from levulinic acid and benzaldehyde via 5-benzylidene-levulinic acid [13], the latter being ozonized and catalytically hydrogenated according to Kissel and Heilmeyer [14].

Electron spectra (UV, vis) and photometrical measurements were performed in a Zeiss PMQ II-spectrophotometer, equipped with a Perkin-Elmer M 56-recorder. The transamination reactions were carried out at $40 \pm 0.2\ °C$. To avoid oxidation, the reactions with VO-salts were always performed under nitrogen. δ-ALA was photometrically estimated according to Mauzerall and Granick [15] after condensation with ethyl acetoacetate and a colour reaction with Ehrlich's reagent. The reaction was found to be disturbed by vanadium ions which were separated from the δ-ALA as follows: The transamination reactions were stopped by cooling the samples in an ice-bath. 1 ml of $FeCl_3$-solution (1 mg $FeCl_3 \cdot 6\ H_2O$/ml, pH 2.5) was added to 2 ml of sample solution. After 20 min, the precipitated $Fe(OH)_3$, containing more than 90% of the initial vanadium by co-precipitation [16], was centifuged (1000 g, 5 min). δ-ALA was then analyzed in the supernatant.

The following metal salts were employed for the transamination studies: $VOSO_4 \cdot 5\ H_2O$, $NaVO_3$, $NiSO_4 \cdot 6\ H_2O$, $Cr(NO_3)_3 \cdot 9\ H_2O$, $ZnSO_4 \cdot 7\ H_2O$, $MgSO_4 \cdot 7\ H_2O$, $(NH_4)_2Fe(SO_4)_2 \cdot 6\ H_2O$, $FeCl_3 \cdot 6\ H_2O$, $Al_2(SO_4)_3 \cdot 18\ H_2O$.

Results and Discussion

To follow the first partial reaction, leading to δ-ALA, PLP (0.1 mM) was allowed to react with a high excess of Ala (100 mM) at 40 °C for 90 min in buffered solution (0.2 M K-phosphate, pH 7.3) in absence and in presence of VO^{2+} and VO_3^- ions (1 mM each). It was observed that the yellow colour of the V-free solution deepened, indicating the formation of a Schiff base whose absorption maximum was found to be at 402 nm (Fig. 1). The V-containing samples, however, were more and more discoloured, the corresponding spectra showing high peaks in the region of PMP-absorption (325 nm), while the PLP-absorption at 390 nm had nearly vanished (Fig. 1). To detect any formation of a
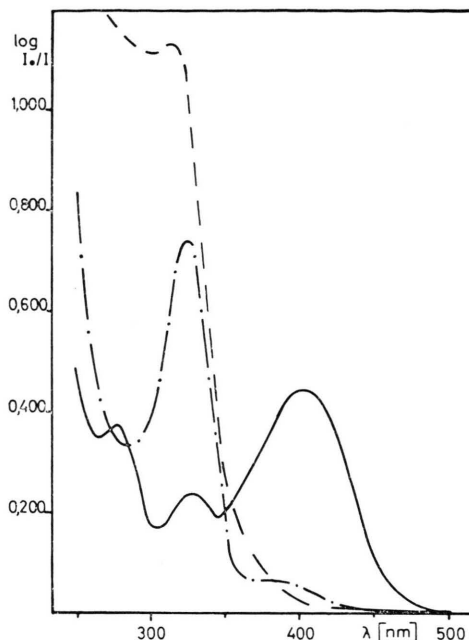
Fig. 1. UV and visible absorption spectra of PLP (0.1 mM) with Ala (100 mM) with and without VO^{2+} and VO_3^- ions (1 mM each) after 90 min at 40 °C (pH 7.3). ——— without metal, $- \cdot - \cdot$ VO^{2+}, $- - -$ VO_3^-.

Schiff base complex with VO^{2+}-ions, which should absorb in the visible range as it is known from the comparable system alanine/pyridoxal/copper ($\lambda_{max} = 650$ nm) [17], the concentration of PLP was raised from 0.1 to 1 mM. In this case, the colour of the solution turned to orange, caused by a new absorption band between 550 and 620 nm ($\lambda_{max} = 585$ nm). With VO_3^--ions, however, no characteristic absorption in the visible range could be detected.

The change of the absorption ratio 390/325 nm with time indicates that some transamination occurs between Ala and PLP under the conditions mentioned above, the reaction being remarkably influenced by the metal ions. In aqueous solution (pH 7.3), PLP has absorption maxima at $\lambda = 390$ nm ($\varepsilon = 5500$) and $\lambda = 325$ nm ($\varepsilon = 2400$), while PMP has only one maximum at 325 nm ($\varepsilon = 8400$) [18]. Since Ala and the metal salts show no absorption in this range, the amount of both, PLP and PMP, produced by the transamination, is thereby easy to calculate from their extinctions at 390 and 325 nm [19]. We measuerd therefore the time variation of the extinctions at 390 and 325 nm during the reaction of Ala with PLP in buffered solution (0.2 M K-phosphate, pH 7.3) at

40 °C. Equally treated assays were performed in presence of vanadyl and vanadate ions (1 mM each). Alanine (10 mM) was offered in a twofold excess with respect to PLP (5 mM), and the extinctions were measured after suitable times. In these samples, no interfering absorptions above 300 nm could be observed, thus indicating that the amount of any formed Schiff base or its metal complex was negligible. The kinetics of the transaminations are presented in Fig. 2.

Fig. 2 shows that in absence of the metal ions, only a slight amount of PMP is formed after some hours. In presence of both vanadium compounds, however, transamination rapidly occurs, reaching maximal yields after 150 min with a 10fold increase compared to the controls.

Besides vanadium, some other metals were tested for their catalytical influence upon Ala-transamination under the same conditions mentioned above. The amount of PMP, formed by transamination after 90 min at 40 °C is listed in Table I.

Table I indicates that VO^{2+} and VO_3^--ions were the most effective to catalyze the conversion of Ala to pyruvate. Fe^{2+}, Fe^{3+} and Al^{3+} also catalyze the reaction to a certain extent, while Zn^{2+}, Cr^{3+}, Mg^{2+}, and Ni^{2+} inhibit the transamination by forming stable complexes either with alanine (Cr^{3+}) [20] or with the Schiff base intermediate (Ni^{2+}) [19].

In the second partial reaction, leading to δ-ALA, the transamination of DOVA takes place, while PMP is converted back to PLP. This reaction was

investigated as before with and without VO^{2+}- and VO_3^--ions. The following observations were made: The electron spectra of solutions with high excess of DOVA (100 mM) with respect to PMP (1 mM) showed no characteristic shift of the PMP-absorption at 325 nm which would point to the formation of a Schiff base, but after some minutes, the PLP-absorption at 390 nm arises, thereby indicating that some transamination occurs. Only in presence of VO^{2+}-ions, a flat shoulder at 460 nm shows that a slight amount of a Schiff base complex had been formed. As known from previous studies, the new absorption should arise from the corresponding VO-Schiff base complex with PLP and δ-ALA which has its maximal absorbance at $\lambda = 456$ nm [21].

To follow the transamination with time, we measured the amount of δ-ALA formed *via* its colour reaction with ethyl acetoacetate and Ehrlich's reagent [15]. Among all reactants present in the transamination mixture, the vanadium compounds were the only ones to disturb the reaction with Ehrlich's reagent. Therefore, vanadium was co-precipitated with Fe(III)-hydroxide [16] after having stopped the reaction by cooling. Residual traces of V and Fe did not further interfere with δ-ALA analysis. DOVA (10 mM) and PMP (5 mM) were allowed to react at 40 °C (pH 7.3) in absence and in presence of VO^{2+} and VO_3^- ions (1 mM each). The kinetics of the DOVA transaminations are presented in Fig. 3. Fig. 3 shows that the vanadium ions have no accelerating effect upon δ-ALA formation, but after 300 min, where in all cases the saturation is attained, the metal ions cause a higher yield of δ-ALA, VO^{2+} being more effective than VO_3^-. The influence of the other metals (see above) upon DOVA transamination was judged after a constant reaction time (120 min) at 40 °C. The amount of the δ-ALA formed is listed in Table II. It was observed that Ni^{2+} and Fe^{2+} efficiently catalyze the conversion of DOVA to δ-ALA, while in presence of the other metal ions tried the δ-ALA formation was comparable to that of the metal-free controls.

The investigations, mentioned above, gave an insight into the two transamination systems involved in δ-ALA formation and their susceptibility to metal ions. It was therefore of great interest to examine non-enzymatically the coupled reaction of the two systems, as it occurs *in vivo* in presence of the enzyme δ-ALA transaminase. In contrast to the partial

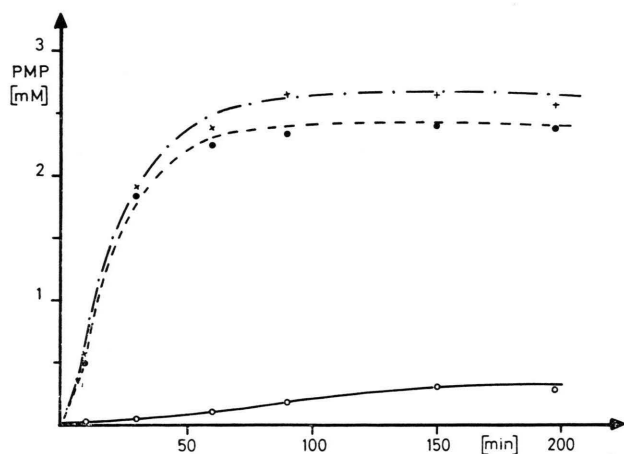

Fig. 2. Kinetics of PMP-formation by transamination from PLP (5 mM) and Ala (10 mM) in absence and in presence of VO^{2+} and VO_3^- ions (1 mM each) at 40 °C (pH 7.3). ——— without metal, — · — · — VO^{2+}, – – – VO_3^-.

Tab. I. Formation of PMP by transamination * from PLP and Ala with and without metal ions.

metal ion	none	VO^{2+}	VO_3^-	Fe^{2+}	Fe^{3+}	Al^{3+}	Zn^{2+}	Cr^{3+}	Mg^{2+}	Ni^{2+}
PMP [mM]	0.29	2.64	2.39	1.43	1.05	0.97	0.17	0.15	0.12	0.02

* Conditions: 5 mM PLP, 10 mM Ala, 1 mM metal ions, pH 7.3, 90 min at 40 °C.

Tab. II. Formation of δ-ALA by transamination * from DOVA and PMP with and without metal ions.

metal ion	none	VO^{2+}	VO_3^-	Ni^{2+}	Fe^{2+}	Zn^{2+}	Al^{3+}	Mg^{2+}	Cr^{3+}	Fe^{3+}
δ-ALA [mM]	0.250	0.270	0.242	0.427	0.416	0.302	0.297	0.258	0.236	0.224

* Conditions: 5 mM PMP, 10 mM DOVA, 1 mM metal ions, pH 7.3, 120 min at 40 °C.
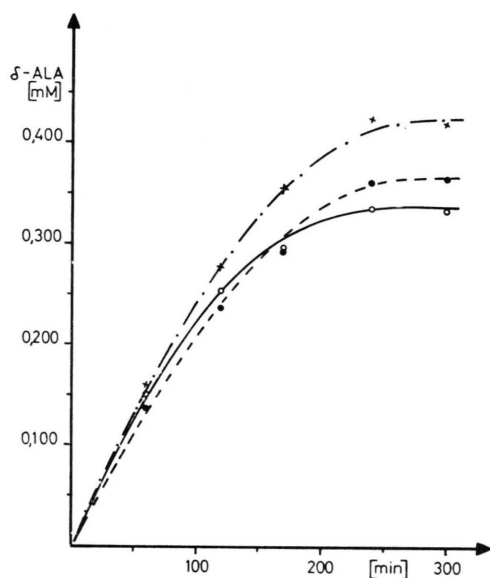
Fig. 3. Kinetics of δ-ALA formation by transamination from DOVA (10 mM) and PMP (5 mM) in absence and in presence of VO^{2+} and VO_3^- ions (1 mM each) at 40 °C (pH 7.3). ——— without metal, — · — · — VO^{2+}, — — — VO_3^-.

Fig. 4. Kinetics of δ-ALA formation by transamination from DOVA (10 mM), Ala (10 mM) and PLP (5 mM) in absence and in presence of VO^{2+} and VO_3^- ions (1 mM each) at 40 °C (pH 7.3). ——— without metal, — · — · — VO^{2+}, — — — VO_3^-.

reactions above, PLP has only a catalytic function, whereby PMP is formed as an intermediate. To follow the transamination with time, the δ-ALA formed was estimated colorimetrically as above. Most favourable conditions for measurable amounts of δ-ALA were found with 10 mM each of DOVA and Ala, 5 mM of PLP and 1 mM of the metal compounds. The pH of the medium (pH 7.3) and the reaction temperature (40 °C) were maintained constant as mentioned above.

In absence of metal ions, a slow reaction occurs, yielding maximal δ-ALA after 200 min. But vanadium, offered as $NaVO_3$ or $VOSO_4$, strongly in-

fluences the reaction by accelerating the initial rates and also by yielding far more δ-ALA (Fig. 4). In connection with these findings, the other metal ions, as tested above, were also investigated for their influence on δ-ALA transamination. The yield of δ-ALA, formed after a constant reaction time (180 min) at 40 °C was determined. The results are listed in Table III.

Table III shows that, besides vanadium, also iron and aluminium exert a positive influence on δ-ALA formation. The other metal ions have no effect (Zn, Mg) or even inhibit the conversion (Cr, Ni).

To estimate the optimal pH for the coupled δ-ALA transamination in absence and in presence of vanadium ions, δ-ALA was detected after the transamination at 40 °C (100 min) in solutions of various pH (Fig. 5), buffered with K-phosphate

Tab. III. Formation of δ-ALA by transamination * from DOVA, Ala and PLP with and without metal ions.

metal ion	none	VO_3^-	VO^{2+}	Fe^{2+}	Al^{3+}	Fe^{3+}	Zn^{2+}	Mg^{2+}	Cr^{3+}	Ni^{2+}
δ-ALA [mM]	0.030	0.197	0.170	0.111	0.076	0.047	0.027	0.027	0.026	0.020

* Conditions: 5 mM PLP, 10 mM DOVA and Ala, 1 mM metal ions, pH 7.3, 180 min at 40 °C.
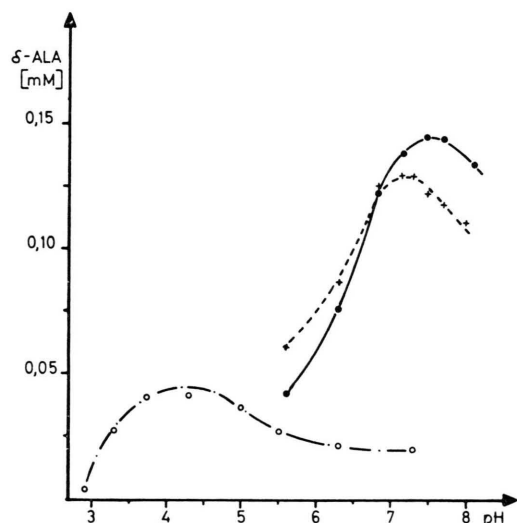
Fig. 5. δ-ALA formation by transamination from DOVA, Ala (10 mM each) and PLP (5 mM) at various pH in absence and in presence of VO^{2+} and VO_3^- ions (1 mM each) after 100 min at 40 °C. — · — · — without metal, — — — VO^{2+}, ——— VO_3^-.

investigations. The latter is in so far an exeption as it bears its amino function in 5-position with a vicinal oxo-group in 4-position. DOVA, being the corresponding keto acid, has even two vicinal oxo-groups. This fact gives rise to expectation of some pecularities during the transamination with respect to metal catalysis. A possible influence of metal ions, more especially that of VO^{2+} cations, which is of interest in connection with δ-ALA biosynthesis, has to be discussed in principle for both partial reactions leading to δ-ALA. In presence of the metal ions, the Schiff base intermediate with PLP and the amino acid is stabilized by forming a chelate:
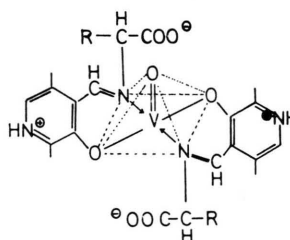
It is seen that the oxo-complex of the tetravalent vanadium is shaped like a square pyramid with its substituents in one plane, an arrangement which makes possible the transamination. The planarity of the system and a lower basicity of the imine-N in the chelate facilitate the removal of a proton during tautomeric rearrangement of the Schiff base [25] which is further hydrolized to the products.

The other metal complexes have normally planar structures, thus explaining the general high activity of the metals in transamination reactions [25]. Differences between kinetic stabilities of the complexes are possibly responsible for the extent of transamination. A certain influence of the substituents at the imine-N cannot be excluded because long-chain, electron-rich groups $(-CO-CH_2-CH_2-COOH$ from DOVA) may interact with the non-bonding d_{xy}-orbital of the vanadium atom. Such an interference would retard the transamination, as found by comparing the transamination rates Ala/PA to DOVA/δ-ALA.

(pH > 5) or citrate (pH < 5). As indicated by the graphs in Fig. 5, the optimal pH for the metal-free assay was found to be at pH 4, while in presence of both, VO^{2+} and VO_3^-, the optimum has shifted to pH 7.3-7.5.

In the work presented here, the complex transamination reaction leading to δ-ALA has been characterized for the first time by a non-enzymatic test. Thereby, a remarkable catalytic effect of vanadium has been detected. The only non-enzymatic conversion of DOVA to δ-ALA described in the literature [22] had been performed in the ethanol/KOH, but there were neither kinetic investigations nor any tests in connection with metal ions. Only a qualitative detection of δ-ALA by TLC was taken. Many non-enzymatic transaminations had been studied with α-amino acids, including tests with metal ions, such as Ni^{2+}, Cu^{2+}, Fe^{3+}, Al^{3+} [23, 24]. In all cases, the reactions were limited to a simple conversion of the α-amino acid to the corresponding α-keto acid with PLP or pyridoxal as reaction partners. δ-ALA, however, was not included in these

Furthermore, it is remarkable that the transaminations are also enhanced in presence of vanadate. Metal-dependent transaminations have so far been mentioned only in connection with metal cations [11, 17, 24]. The sole indication for a catalytic activity of vanadate is found with a non- enzymatic model for the enzyme desulfhydrase [26, 27], where, in a PLP-dependent reaction, cysteine is broken down with separation of H_2S. But also in this case, tetravalent vanadium (as VO^{2+}) was found to be far more effective. Because vanadium(V) is present as anionic vanadate in neutral aqueous solution, the structure of a possible Schiff base complex with vanadate must be discussed. It is known from reactions of vanadate with compounds of the salicylaldoxime-type that stable vanadato-complexes are formed, but the corresponding VO-complexes also exist, are, however, very sensitive to oxidation [12]. With respect to the proposed structure of those complexes, the analogous vanadato-Schiff base complex with PLP/amino acid would have the following structure:

The comparable catalytic behaviour of both, the vanadyl and the vanadato complexes, is possibly due to their similar structures. The difference in their activity compared to the other metal ions should arise from the presence of a stable VO-double-bond, and it is supposed that the rests of the amino acids may interact with the oxygen atoms, thereby causing a decreased kinetic stability of the complex which is favourable for transamination.

Another advantage for a biological transamination is the fact that the pH-dependence of the reaction is influenced in presence of metal ions. The metal complexes are able to displace the optimum of transamination, which is found in absence of metals in a weak acid range, to physiological pH-values [23]. This is particular seen in the case of δ-ALA formation in presence of vanadium, where the optimal pH is shifted from 4 to 7.4 (Fig. 5).

The high catalytic effectiveness of vanadium compared to other metals during the conversion of DOVA to δ-ALA under participation of Ala and PLP has completely confirmed the observed stimulation of δ-ALA formation *in vivo* [7, 8]. It indicates that the essential role of the trace element vanadium for the biosynthesis of the chlorophylls is attributed with high probability to a participation of the metal in DOVA transamination leading to δ-aminolevulinic acid.

[1] D. I. Arnon and G. Wessel, Nature **172**, 1039 (1953).
[2] H.-U. Meisch and H.-J. Bielig, Arch. Microbiol. **105**, 77 (1975).
[3] H.-U. Meisch, H. Benzschawel, and H.-J. Bielig, Arch. Microbiol. **114**, 67 (1977).
[4] D. I. Arnon, in Trace Elements (C. A. Lamb, O. G. Bentley, and J. M. Beattie, eds.), Academic Press, New York 1954.
[5] O. Warburg, G. Krippahl, and W. Buchholz, Z. Naturforsch. **10 b**, 422 (1955).
[6] L. L. Hopkins and H. E. Mohr, Fed. Proc. **33**, 1773 (1974).
[7] H.-U. Meisch and J. Bauer, Arch. Microbiol. 1978, in press.
[8] J. Bauer, Diplomarbeit Univ. Saarbrücken 1978.
[9] M. Gassman, J. Pluscec, and L. Bogorad, Plant Physiol. **43**, 1411 (1968).
[10] D. E. Metzler, M. Ikowa, and E. E. Snell, J. Am. Chem. Soc. **76**, 648 (1954).
[11] J. Cattanéo, J. Senez, and P. Beaumont, Biochim. Biophys. Acta **44**, 543 (1960).
[12] H.-J. Bielig and E. Bayer, Ann. Chem. **580**, 135 (1953).
[13] F. Henke, Dissertation Univ. Halle/S. 1891.
[14] H. J. Kissel and L. Heilmeyer, Biochim. Biophys. Acta **177**, 78 (1969).
[15] D. Mauzerall and S. Granick, J. Biol. Chem. **219**, 435 (1956).
[16] K. M. Chan and J. P. Riley, Anal. chim. Acta **34**, 337 (1966).
[17] G. Eichhorn and J. W. Dawes, J. Am. Chem. Soc. **76**, 5663 (1954).
[18] R. A. Morton, Biochemical Spectroscopy, **Vol. 2,** p. 436, A. Hilger Verlag, London 1975.
[19] D. E. Metzler and E. E. Snell, J. Am. Chem. Soc. **74**, 979 (1952).
[20] H. Oki, Bull. Chem. Soc. Japan **50**, 680 (1977).
[21] H. Hoffmann, Diplomarbeit Univ. Saarbrücken 1977.
[22] A. E. Braunstein, N. W. Gnuchev, and A. A. Poznanskaya, Dokl. Acad. Nauk SSSR **152**, 1239 (1963).
[23] J. B. Longenecker and E. E. Snell, J. Biol. Chem. **79**, 142 (1956).
[24] Y. Matsuo, J. Am. Chem. Soc. **79**, 2016 (1957).
[25] D. L. Leussing, Metal Ions in Biological Systems, H. Sigel, ed., **Vol. 5,** p. 1, Marcel Dekker Inc., New York 1976.
[26] F. Bergel, R. C. Bray, and K. R. Harrap, Nature **181**, 1654 (1958).
[27] F. Bergel, K. R. Harrap, and A. M. Scott, J. Chem. Soc. **1**, 1101 (1962).