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MINI-REVIEW

Optical Characteristics of Thiamine in Model Systems and in Holoenzyme

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Abstract—The optical properties of thiamine diphosphate-dependent enzymes change significantly on their interaction with cofactors (thiamine, bivalent metal ions) and substrates. These changes are connected with structural alterations of the active site and the mechanism of its functioning, and in some cases they reflect changes in the optical properties of the coenzyme itself within the protein. The use of optical characteristics, especially together with model systems, appeared to be a rather promising approach for investigation of the active site of thiamine diphosphate-dependent enzymes and the mechanism of its functioning. So, it seemed to be useful to summarize the literature data concerning the optical characteristics of thiamine (thiamine diphosphate) in model systems and the efficiency of their application for study of thiamine diphosphate-dependent enzymes.

Key words: thiamine, thiamine diphosphate, thiamine diphosphate-dependent enzymes, model systems, optical characteristics

Thiamine (vitamin B_1), or its pyrophosphorylated form, acts as the coenzyme in the reactions catalyzed by transketolase, pyruvate decarboxylase, pyruvate- and ketoglutarate dehydrogenase complexes, and many other enzymes. Interaction of thiamine diphosphate-dependent enzymes with the coenzyme results in specific changes in their optical properties: appearance of a positive absorption band in the absorption spectrum at 300-310 nm and a negative absorption band in the CD spectrum at 315-320 nm (the original components, thiamine diphosphate [1] and the apoenzyme, do not exhibit absorption bands in the indicated spectral regions). The new bands were originally revealed in the CD [2-4] and absorption [5, 6] spectra of yeast transketolase, and then in the CD [7-12] and absorption [10, 11] spectra of other thiamine diphosphate-dependent enzymes. The reason for the appearance of these bands was unknown for a long time. However, it was clear from the very beginning that they were directly connected with the catalytic activity of the enzyme. For example, it was shown that on the interaction of apo-transketolase with thiamine diphosphate, the intensity of the abovementioned induced bands in the absorption and CD spectra depended on the amount of the coenzyme bound to the protein and correlated strictly with the amount of the produced catalytically active holoenzyme [13-17]. Modification of thiamine diphosphate resulted in disappearance of the induced bands and in the loss of the catalytic activity of the enzyme [18]. Binding and cleavage of the donor substrate (the first step of the transketolase reaction) and the subsequent transfer of the glycol aldehyde residue on the acceptor substrate (the second step of the reaction) also resulted in corresponding changes in the induced absorption bands [5, 17, 19, 20]. Unclear nature of the discovered optical effects did not hamper their application, this yielding much valuable information in studies of the interaction of the coenzyme and the substrates with the apoenzyme (see review [21] and also [12, 17, 22-25]). The results obtained in model systems with thiamine and its derivatives have played an important role in elucidation of the nature of the induced absorption bands, which is discussed below.

OPTICAL CHARACTERISTICS OF THIAMINE IN MODEL SYSTEMS

The spectrum of thiamine[1] in neutral aqueous medium is the sum of the spectra of its thiazole and aminopyrimidine components (peaks with maxima at 265-267 and

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[1] The absorption spectra of thiamine and thiamine diphosphate are the same, so we do not specify the coenzyme form.

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233-235 nm, respectively). In media less polar than water, a new band with maximum at 310 nm appears in the absorption spectrum (Fig. 1, curve *1*) that is absent in the aqueous environment. A similar band was also observed when thiamine was dissolved in dioxane [27]. The induced absorption band with maximum at 310 nm is due to the imino form of the aminopyrimidine ring. In aqueous solutions, the amino form of thiamine (Fig. 2, 1.1) and its imino form with the quinoid structure of the aromatic core (Fig. 2, 1.2) are in equilibrium [28-30]. The equilibrium is virtually completely shifted towards the amino form (this being normally characteristic for aminopyrimidines [31]) that has no absorption bands in the near UV region [1]. In hydrophobic surrounding, the equilibrium is shifted towards the imino form [29] that exhibits a positive peak in the absorption spectrum in the near UV region (Fig. 1, curve *1*). It is also known that the imino form of aminopyrimidine compounds is characterized by absorption in the region of ≥300 nm that increases in hydrophobic environments [27, 32].

The intensity of the absorption band of the imino form of the aminopyrimidine ring of thiamine depends on pH, decreasing with decreasing pH value [26]. This is due to the fact, that the increase in concentration of hydrogen ions results in the protonation of the unshared electron pair of the aminopyrimidine ring, this breaking the coupling. As a result, form 1.2 (Fig. 2) transforms into form 2 that has no absorption bands in the near UV region.

In alkaline aqueous solutions, 4-aminopyrimidine methylated at N1 exhibits near UV spectra (Fig. 3) similar to those of thiamine in a hydrophobic medium [27] or in a medium less polar than water (Fig. 1, *1*). NMR studies demonstrated that N1-methylaminopyrimidine [10] as well as N1-methylated thiamine [33] has quinoid structure similar to that presented in Fig. 2 (form 1.2).

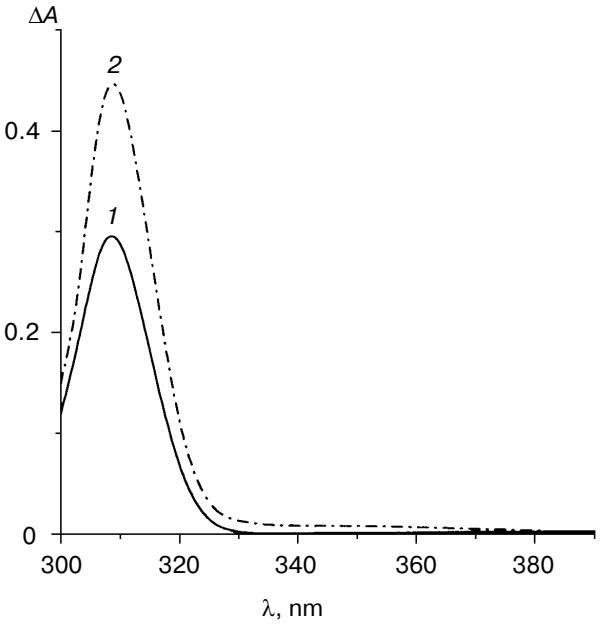

Fig. 1. Difference absorption spectrum of thiamine diphosphate in 50% ethanol solution in the absence (*1*) and in the presence (*2*) of phenylalanine [26].

The imino form of thiamine with the quinoid structure of the aromatic core is prone to interaction with aromatic rings, this resulting in an increase in the intensity of the absorption at 300-320 nm. This can be illustrated by the change in the absorption spectrum of the imino form of thiamine on its interaction with phenylalanine (Fig. 1, curves *1* and *2*). A peak with maximum at 310 nm is also observed on the interaction of thiamine with indolyl-propionic acid in 20% ethanol solution (pH 7.8) [34]. Based on the results of X-ray analysis of crystals of thiamine-indolyl-propionic acid, the authors demonstrated the presence of stacking interaction between the indolyl ring of the tryptophan and the pyrimidine ring of the thiamine. However, based on the analysis of the absorption spectra, they do not exclude the presence of a charge-transfer complex between the indolyl ring of the tryptophan and the pyrimidine and/or the thiazole ring of the thiamine.

NMR studies demonstrated the formation of a molecular complex between tryptophan and its derivatives and thiamine in aqueous solutions [35, 36]. The positively charged thiazole ring of thiamine and the benzene ring of tryptophan play a central role in this interaction. Analogs of thiamine with N3 of the thiazole ring lacking positive charge were incapable of interacting with tryptophan. The aminopyrimidine ring of thiamine at pH 3.0 has positively charged N1 atom and thus is also capable of interacting with tryptophan; however, at pH 6.5 the main contribution is made into this interaction by the thiazole ring. The pyrophosphate residue influences the interaction of thiamine with tryptophan. The negatively charged pyrophosphate group partially masks the positive charge of the tetravalent nitrogen atom of thiamine diphosphate, this reducing its acceptor characteristics [36].

The interaction of thiamine with tryptophan and its derivatives is accompanied by the appearance of a new absorption band that is absent in the spectra of the original components. The substitution of other amino acids (tyrosine, phenylalanine, or histidine) at different concentrations instead of tryptophan did not result in any changes in the absorption spectra [36, 37].

The induced absorption band of the complex of thiamine with tryptophan has diffuse character, indistinct maximum, and a long tail extended in the visible region of the spectrum. Such a spectrum is a characteristic feature for charge transfer complexes. In this case, the components of the complex are the benzene ring of the indolyl group of the tryptophan (donor) and the positively charged thiazole ring of the thiamine (acceptor). However, the experimental results of NMR spectroscopy suggested the equivalent interaction of the individual thi-

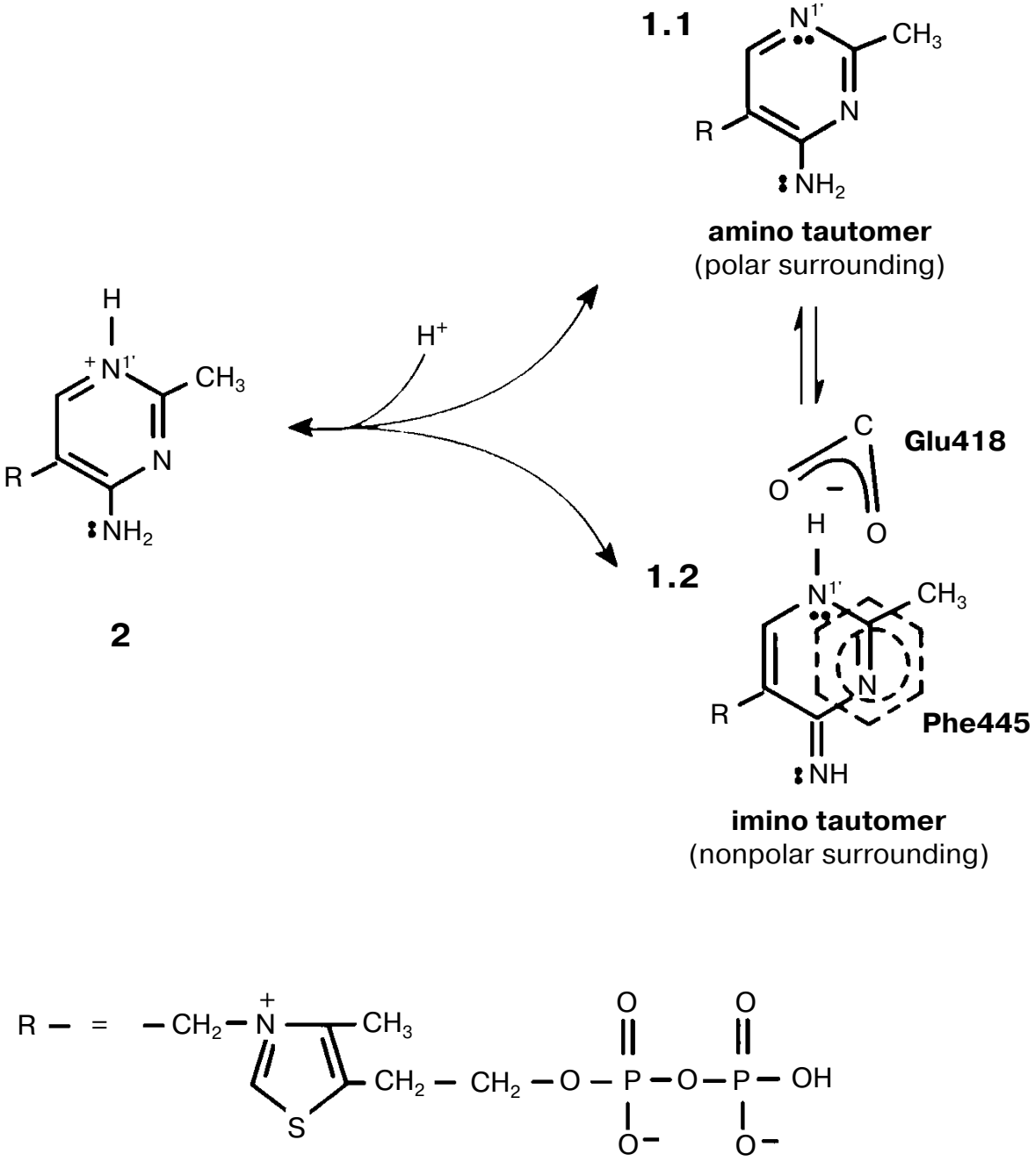

Fig. 2. Equilibrium forms of thiamine diphosphate.

amine rings with tryptophan [35, 38]. According to this assumption, both rings must be equidistant from the plane of the indolyl ring of the tryptophan molecule. Conformational mobility of the complex between thiamine and the indolyl ring of tryptophan results in oscillation between the interactions indole ring–pyrimidine ring and indolyl ring–thiazole ring.

The functional groups of thiamine diphosphate when functioning as a coenzyme are C2 of the thiazole ring and the amino group of the aminopyrimidine ring. The first stages of the catalysis are the same for all thiamine diphosphate-dependent enzymes. It is considered that the amino group of the aminopyrimidine ring takes part in deprotonation of C2 of the thiazole ring, which is necessary for the addition of the substrate (Fig. 4). A glutamate residue of the apoenzyme takes part in protonation of N1′ of the aminopyrimidine ring yielding the 1′,4′-imino tautomeric form of thiamine diphosphate.

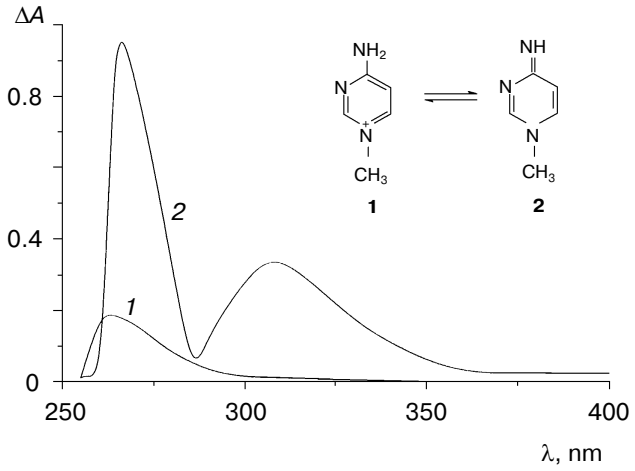

Fig. 3. Absorption spectrum of N1-methyl-4-aminopyrimidine salt of trifluoromethylsulfonic acid before (*1*) and after (*2*) the addition of NaOH (10 mM) [10].

Such a residue located at the distance of a hydrogen bond from N1′ of the aminopyrimidine ring has been identified in many thiamine diphosphate-dependent enzymes. For example, for transketolase it is Glu418, and for pyruvate decarboxylase it is Glu50. As a result of the protonation of N1′ of the coenzyme, the mobility of the proton of its amino group increases and the aminopyrimidine ring acquires the ability to function both as the donor and the acceptor of a proton. The possible participation of the aminopyrimidine ring of thiamine diphosphate (its 1′,4′-imino tautomeric form) in the catalysis has been discussed at various times [33, 40-44]; however, this is still not confirmed experimentally.

SPECTRAL CHARACTERISTICS OF THIAMINE DIPHOSPHATE IN HOLOENZYME

Figure 5 (spectrum *1*) presents the differential absorption spectrum of holo-transketolase. The negative peak with the extremum at 269-272 nm is probably due to hypochromism in the absorption region of the thiazole ring of the thiamine diphosphate bound to the holoenzyme [5]. As concerns the induced positive absorption band in the long-wavelength region of the spectrum, originally, based on the similarities of the absorption spectra in the systems thiamine diphosphate–tryptophan and thiamine diphosphate–apo-transketolase in aqueous solutions, it was considered to be connected with the formation of a charge transfer complex between the coenzyme and the tryptophan residue of the apoenzyme [3, 20]. When the X-ray analysis did not revealed a tryptophan residue in proximity to the thiamine diphosphate in the active site of transketolase, another hypothesis was suggested, the main idea of which is discussing below [26].

On binding with apo-transketolase, the thiamine diphosphate enters the hydrophobic surrounding, this resulting in the transition of the amino form of its amino pyrimidine ring into the imino form (1.1 → 1.2 in Fig. 2). The imino form is stabilized by the interaction of the coenzyme with phenylalanine 445 and N1 of the aminopyrimidine ring with the carboxyl group of Glu418 (Fig. 2). This hypothesis was confirmed experimentally.

Actually, on binding with apo-transketolase, the aminopyrimidine ring of the thiamine diphosphate enters the hydrophobic pocket of the active site [45]. As mentioned above, in a hydrophobic medium the equilibrium 1.1 ↔ 1.2 (Fig. 2) shifts towards form 1.2, i.e., towards the imino form. The absorption spectra of thiamine (thi-

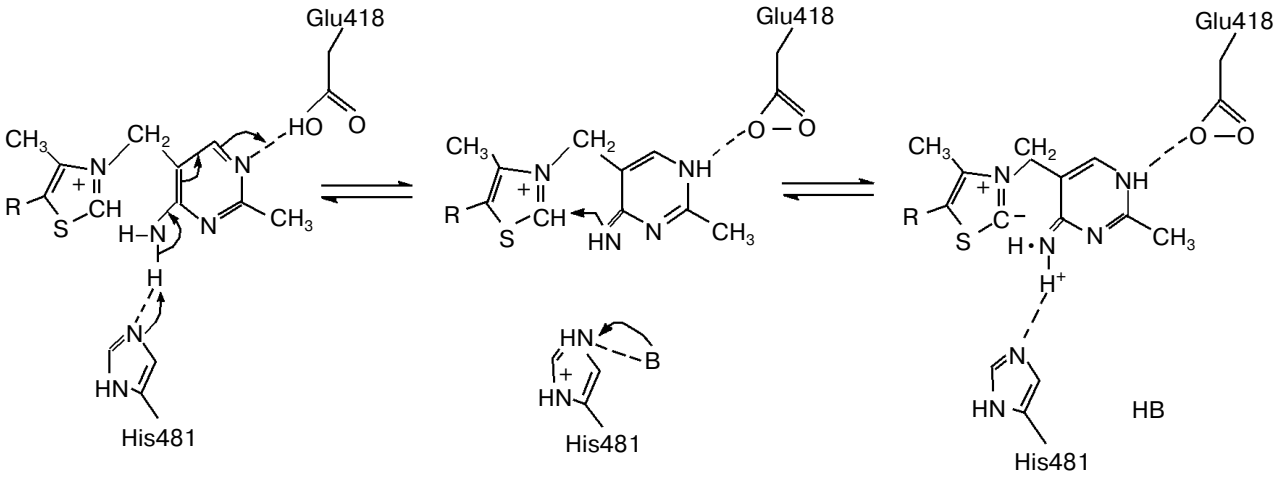

Fig. 4. Participation of the amino group of the pyrimidine ring in deprotonation of C2 of the thiazole ring (illustrated by the example of transketolase) [39].

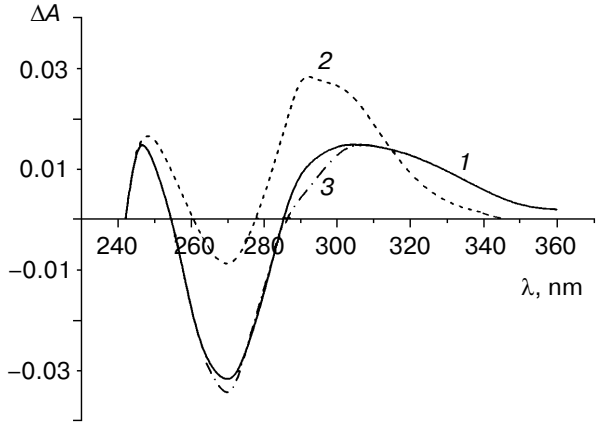

Fig. 5. Difference absorption spectra of holo-transketolase in the absence (*1*) and in the presence of the substrates—hydroxypyruvate alone (*2*) and hydroxypyruvate together with the acceptor substrate (*3*) [5].

amine diphosphate) in a hydrophobic medium [27] or in a medium less polar than water (Fig. 1, spectrum *1*) are similar to the absorption spectrum of holo-transketolase (Fig. 5), exhibiting in the near-UV region the induced absorption band with maximum at 300-315 nm.

The intensity of this band in the absorption spectrum of holo-transketolase decreases at pH values below 6.0 (Fig. 6, *1*), in the same way as the intensity of the analogous band in the spectrum of thiamine decreases on acidification in the model system [26], this corresponding precisely to the scheme in Fig. 2 that was discussed above. It should be noted that the pH dependence of the intensity of the induced band in the CD spectrum (Fig. 6, *2*) is analogous to the pH dependence of the induced band in the absorption spectrum of holo-transketolase (Fig. 6, *1*).

The transketolase mutated at Glu418 has no induced absorption band in the CD spectrum in the near-UV region [46], so as the carboxyl group interacting with N1′ of the coenzyme and stabilizing its imino form is absent (Figs. 2 (1.2) and 4). Stabilization of the imino tautomeric form in the presence of a carboxyl group was also demonstrated for 2-aminopyridine (Fig. 7). The N1′-methyl derivative of thiamine diphosphate (analogous to the N1′-protonated coenzyme in the active site) is characterized, as mentioned above, by the imino form of its aminopyrimidine ring [33] and has an absorption spectrum [10] analogous to that of transketolase in the near-UV region (Fig. 5, spectrum *1*).

The CD spectrum of the transketolase mutated at Phe445 retains the induced absorption band, but its intensity significantly decreases [9], this demonstrating the role of the phenylalanine residue in stabilizing the imino tautomeric form of thiamine diphosphate. An analogous observation was obtained in a model system: in a medium less polar than water, the intensity of the absorption band that is characteristic of the imino form of the aminopyrimidine ring increases in the presence of phenylalanine (Fig. 1, *2*).

In the presence of the donor substrate, the absorption spectrum of holo-transketolase changes both in the region of the absorption of the thiazole ring (negative peak with extremum at 270 nm) and in the long-wavelength region (compare curves *1* and *2* in Fig. 5). These changes gradually disappear and the original spectrum is restored. If the acceptor substrate (in this case, glycol aldehyde) is added after the addition of the donor sub-

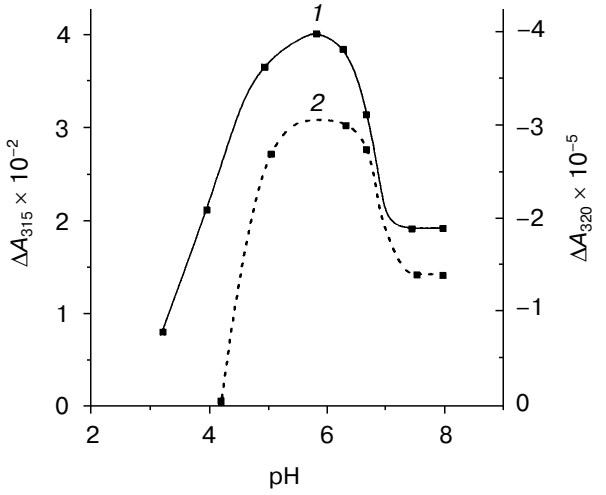

Fig. 6. Effect of pH on the intensity of the band induced by the binding of thiamine diphosphate in the absorption (*1*) and CD spectra (*2*) of holo-transketolase [26].

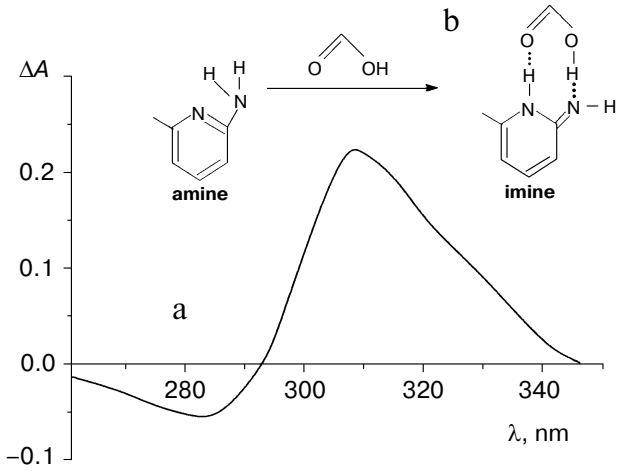

Fig. 7. a) Difference absorption spectrum of 2-aminopyridine in isooctane obtained by the subtraction of the 2-aminopyridine spectrum in the absence of acetic acid from that in the presence of acetic acid [47]. b) Pyridinimine stabilized by acetic acid.

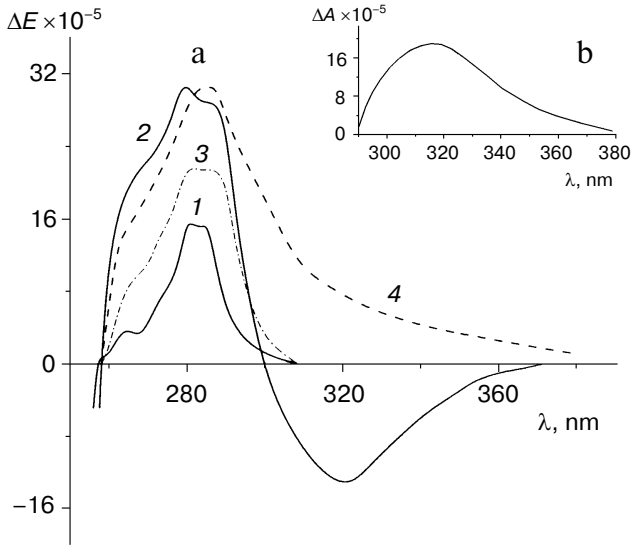

Fig. 8. a) CD spectra of transketolase in the presence of thiamine diphosphate and the substrates: apo-transketolase (*1*), holo-transketolase (*2*), holo-transketolase and fructose-6-phosphate (*3*) or hydroxypyruvate (*4*) [43]. b) Difference CD spectrum: holo-transketolase with hydroxypyruvate minus holo-transketolase.

strate, the original spectrum of holo-transketolase is restored instantly (Fig. 5, curve *3*).

It is still rather difficult to explain precisely the changes observed in the spectrum of holo-transketolase on the addition of the substrates. However, these changes characterize definitely the processes of binding of the donor substrate to C2 of the thiazole ring of the coenzyme, its transformation through the formation of the intermediate products, release of the products into the medium, and restoration of the original form of the coenzyme.

Figure 8 (curve *1*) demonstrates the CD spectrum of apo-transketolase. The induced band arising on the interaction of apo-transketolase with thiamine diphosphate is due to the imino tautomeric form of the coenzyme (Fig. 8, curve *2*). This band has a maximum in the same region as that in the absorption spectrum (Fig. 5, curve *1*). To some extent, the changes observed in the CD spectrum on the addition of the substrates to holo-transketolase are similar to those observed in the absorption spectra. The reversibly cleaving substrate, fructose-6-phosphate, causes disappearance of the induced band (Fig. 8, curve *3*), while hydroxypyruvate (irreversibly cleaving substrate) causes its inversion (Fig. 8, curve *4*). In both cases, the changes in the spectrum are due to the formation of the intermediate product of the transketolase reaction, dihydroxyethyl-thiamine diphosphate [5, 48], but in the case of the reversibly cleaving substrate, only a part of the thiamine diphosphate is bound to the glycol aldehyde residue, the other part being bound to the uncleaved substrate. In the case of hydroxypyruvate, all the thiamine diphosphate is converted into dihydroxyethyl-thiamine diphosphate [48].

The difference CD spectrum of holo-transketolase in the presence of glycol aldehyde [25] does not differ from the difference CD spectrum of the transketolase–dihydroxyethyl-thiamine diphosphate complex (insert to Fig. 8). The difference between these two experiments is that in the second case the complex is formed as a result of the cleavage of the substrate by transketolase, and in the first case it is formed due to the direct interaction of free glycol aldehyde with the coenzyme within holo-transketolase.

In the case of transketolase, the imino tautomeric form of thiamine diphosphate is formed on the formation of the holoenzyme, during the interaction of the coenzyme with the apoprotein, i.e., before the catalysis [26]. In the same work, we suggested that in the case of the thiamine diphosphate-dependent enzymes, whose spectral characteristics on their binding to thiamine diphosphate

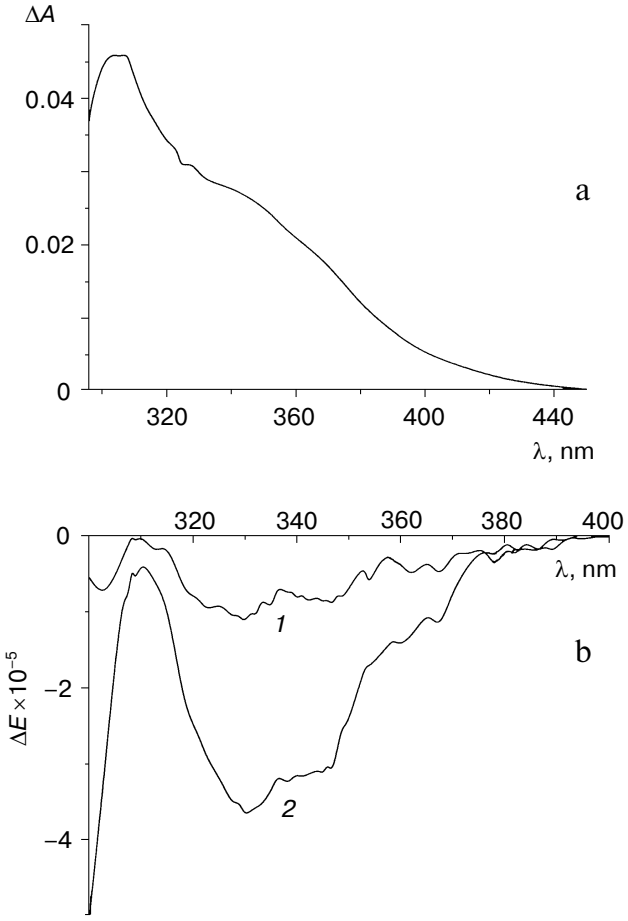

Fig. 9. Difference absorption (a) and CD (b) spectra of the yeast E477Q mutant pyruvate decarboxylase in the presence of pyruvate (after preincubation of the enzyme with pyruvamide) and acetaldehyde, respectively, in the absence (*1*) and in the presence (*2*) of pyruvamide [10].

differ from those for transketolase, the imino form of thiamine diphosphate is produced only during the catalysis. The experiments with pyruvate decarboxylase and E1 of the pyruvate decarboxylase complex (see below) confirmed our assumption.

The addition of pyruvate in the presence of pyruvamide (which activates the enzyme, binding to its regulatory site) results in the appearance of a peak with a maximum at 300-310 nm in the absorption spectrum of the mutant E477Q yeast pyruvate decarboxylase (Fig. 9a). The same peak appears in the CD spectrum of the enzyme on the addition of acetaldehyde (Fig. 9b). A peak with maximum at 300-310 nm also arises in the CD spectrum of the first component (E1) of the pyruvate dehydrogenase complex from *E. coli* on the addition of phosphonolactyl-thiamine diphosphate, a stable analog of the lactyl-thiamine diphosphate (intermediate product of the pyruvate dehydrogenase reaction) that is formed in the course of the interaction of the substrate (pyruvate) with thiamine diphosphate [11].

Based on the experimental data, the authors of the works [10, 11, 49] concluded that the appearance of the band with λ_{max} of 300-310 nm in the absorption and CD spectra of pyruvate decarboxylase and pyruvate dehydrogenase is due to the formation of the 1′,4′-imino form of thiamine diphosphate.

The addition of acetaldehyde to pyruvate decarboxylase also induces a negative band with a maximum at 320-330 nm in the CD spectrum of the enzyme [10]. The authors do not make a concrete interpretation of this phenomenon because of the absence of the corresponding model system. However, they assumed that the negative band is a characteristic of the coenzyme itself within the holoenzyme [10, 49].

For the last decade, thiamine diphosphate-dependent enzymes have been intensively studied using NMR spectroscopy and mutagenesis. This has yielded a rather complete picture of the active site structure that appears to be common for all investigated representatives of the given group of enzymes and allows for prediction of the mechanism of its functioning. The knowledge and the use of the optical characteristics of thiamine diphosphate-dependent enzymes, free thiamine (thiamine diphosphate), and its derivatives played an important role in experimental tests and was used to confirm suggested hypotheses. This was illustrated by an example of identification of the catalytically active imino tautomeric form of the coenzyme within the holoenzyme.

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REFERENCES

1. Metzler, D. E. (1960) *Enzymes*, **2**, 295-337.
2. Kochetov, G. A., Usmanov, R. A., and Merzlov, V. P. (1970) *FEBS Lett.*, **9**, 265-266.
3. Kochetov, G. A., and Usmanov, R. A. (1970) *Biochem. Biophys. Res. Commun.*, **41**, 1134-1140.
4. Kochetov, G. A., Usmanov, R. A., and Merzlov, V. P. (1971) *FEBS Lett.*, **14**, 14-16.
5. Kochetov, G. A., Usmanov, R. A., and Mevkh, A. T. (1973) *Biochem. Biophys. Res. Commun.*, **54**, 1619-1626.
6. Usmanov, R. A., and Kochetov, G. A. (1978) *Biokhimiya*, **43**, 1796-1804.
7. Khailova, L. S., and Korochkina, L. G. (1982) *Biochem. Int.*, **5**, 525-532.
8. Ali, M. S., Shenoy, B. C., Eswaran, D., Andersson, L. A., Roche, T. E., and Patel, M. S. (1995) *J. Biol. Chem.*, **270**, 4570-4574.
9. Meshalkina, L. E., Wikner, Ch., Nilsson, U., Lindqvist, Y., and Schneider, G. (1996) in *Biochemistry and Physiology of Thiamine Diphosphate Enzymes* (Bisswanger, H., and Schellenberger, A., eds.) A. u. C. Intemann, Wissenschaftlicher Verlag, Prien, Germany, pp. 532-542.
10. Jordan, F., Zhang, Z., and Sergienko, E. (2002) *Bioorg. Chem.*, **30**, 188-198.
11. Jordan, F., Nemeria, N. S., Zhang, S., Yan, Y., Arjunan, P., and Furey, W. (2003) *J. Am. Chem. Soc.*, **125**, 12732-12738.
12. Kovina, M. V., de Kok, A., Sevostyanova, I. A., Khailova, L. S., Belkina, N. V., and Kochetov, G. A. (2004) *Proteins: Structure, Genetics and Function*, accepted for publication.
13. Kochetov, G. A., Meshalkina, L. E., and Usmanov, R. A. (1976) *Biochem. Biophys. Res. Commun.*, **69**, 839-843.
14. Meshalkina, L. E., and Kochetov, G. A. (1979) *Biochim. Biophys. Acta*, **571**, 218-223.
15. Meshalkina, L. E., and Kochetov, G. A. (1979) *Dokl. Akad. Nauk SSSR*, **248**, 1482-1486.
16. Usmanov, R. A., and Kochetov, G. A. (1982) *Biochem. Int.*, **5**, 727-734.
17. Usmanov, R. A., and Kochetov, G. A. (1983) *Biokhimiya*, **48**, 772-781.
18. Kochetov, G. A., and Usmanov, R. A. (1972) *Dokl. Akad. Nauk SSSR*, **202**, 471-474.
19. Usmanov, R. A., and Kochetov, G. A. (1983) *Biokhimiya*, **48**, 550-558.
20. Heinrich, C. P., Noak, K., and Wiss, O. (1971) *Biochem. Biophys. Res. Commun.*, **44**, 275-279.
21. Kochetov, G. A. (2001) *Biochemistry* (Moscow), **66**, 1077-1085.
22. Bykova, I. A., Solovjeva, O. N., Meshalkina, L. E., Kovina, M. V., and Kochetov, G. A. (2001) *Biochem. Biophys. Res. Commun.*, **280**, 845-847.
23. Fiedler, E., Golbik, R., Schneider, G., Tittmann, K., Neef, H., König, S., and Hübner, G. (2001) *J. Biol. Chem.*, **276**, 16051-16058.
24. Fiedler, E., Theorell, S., Sandalova, T., Golbik, R., König, S., and Schneider, G. (2002) *Proc. Natl. Acad. Sci. USA*, **99**, 591-595.
25. Sevostyanova, I. A., Solovjeva, O. N., and Kochetov, G. A. (2004) *Biochem. Biophys. Res. Commun.*, **313**, 781-784.
26. Kovina, M. V., Bykova, I. A., Solovjeva, O. N., Meshalkina, L. E., and Kochetov, G. A. (2002) *Biochem. Biophys. Res. Commun.*, **294**, 155-160.
27. Ostrovskii, Yu. M., Stepuro, I. I., Schellenberger, A., and Hubner, H. (1971) *Biokhimiya*, **36**, 1222-1227.

28. Suchy, J., Mieyal, J. J., Bantle, G., and Sable, H. (1972) *J. Biol. Chem.*, **247**, 5905-5912.
29. Schneider, G., and Lindqvist, Y. (1993) *Bioorg. Chem.*, **21**, 109-117.
30. Schellenberger, A. (1998) *Biochim. Biophys. Acta*, **1385**, 177-186.
31. Brown, D. J., Hoerger, E., and Mason, S. F. (1955) *J. Chem. Soc.*, 4035-4040.
32. Pietrzycki, W., Sepiol, J., Tomasik, P., and Brzozka, L. (1993) *Bull. Soc. Chim. Belg.*, **102**, 709-717.
33. Jordan, F., and Mariam, Y. H. (1978) *J. Am. Chem. Soc.*, **100**, 2534-2541.
34. Inoue, M., Hirano, H., Sugiyama, K., and Ishida, T. (1982) *Biochem. Biophys. Res. Commun.*, **108**, 604-609.
35. Biaglow, J. E., Mieyal, J. J., Suchy, J., and Sable, H. Z. (1969) *J. Biol. Chem.*, **244**, 4045-4062.
36. Mieyal, J. J., Suchy, J., Biaglow, J. E., and Sable, H. Z. (1969) *J. Biol. Chem.*, **244**, 4063-4071.
37. Usmanov, R. A. (1970) *Study of the Transketolase Structure by Measuring the Dispersion of the Optical Rotation and Circular Dichroism*: Author's abstract of Candidate's dissertation [in Russian], Moscow State University, Moscow.
38. Ishida, T., Matsui, M., Inoue, M., Hirano, H., Yamashita, M., and Sugiyama, K. (1985) *J. Am. Chem. Soc.*, **107**, 3305-3314.
39. Lindqvist, Y., Schneider, G., Ermler, U., and Sundström, M. (1992) *EMBO J.*, **11**, 2373-2379.
40. Schellenberger, A., Wendler, K., Crentzburg, P., and Hübner, G. (1967) *Hoppe-Seyler's Z. Physiol. Chem.*, **348**, 501-510.
41. Schellenberger, A. (1982) *Ann. N. Y. Acad. Sci.*, **378**, 51-62.
42. Jordan, F., Chen, G., Nishikawa, S., and Sundoro-Wu, B. (1982) *Ann. N. Y. Acad. Sci.*, **378**, 14-31.
43. Pustynnikov, M. G., Neif, H., Usmanov, R. A., Schellenberger, A., and Kochetov, G. A. (1986) *Biokhimiya*, **51**, 1003-1016.
44. Golbik, R., Neef, H., Hübner, G., König, S., Seliger, B., Meshalkina, L., Kochetov, G., and Schellenberger, A. (1991) *Bioorg. Chem.*, **19**, 10-17.
45. Nikkola, M., Lindqvist, Y., and Schneider, G. (1994) *J. Mol. Biol.*, **238**, 387-404.
46. Wikner, C., Meshalkina, L., Nilsson, M., Nikkola, M., Lindqvist, Y., Sundström, M., and Schneider, G. (1994) *J. Biol. Chem.*, **269**, 32144-32150.
47. Inuzuka, K., and Fujimoto, A. (1990) *Bull. Chem. Soc. Jpn.*, **63**, 971-975.
48. Solov'eva, O. N., Bykova, I. A., Meshalkina, L. E., Kovina, M. V., and Kochetov, G. A. (2001) *Biochemistry* (Moscow), **66**, 932-936.
49. Jordan, F. (2003) *Nat. Prod. Rep.*, **20**, 184-201.