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A straightforward kinetic evidence for coexistence of "induced fit" and "selected fit" in the reaction mechanism of a mutant tryptophan indole lyase Y72F from *Proteus vulgaris*

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ABSTRACT

The interaction of the mutant tryptophan indole-lyase (TIL) from *Proteus vulgaris* Y72F with the transition state analogue, oxindolyl-L-alanine (OIA), with the natural substrate, L-tryptophan, and with a substrate S-ethyl-L-cysteine was examined. In the case of wild-type enzyme these reactions are described by the same kinetic scheme where binding of holoenzyme with an amino acid, leading to reversible formation of an external aldimine, proceeds very fast, while following transformations, leading finally to reversible formation of a quinonoid intermediate proceed with measureable rates. Principally the same scheme ("induced fit") is realized in the case of mutant Y72F enzyme reaction with OIA. For the reaction of mutant enzyme with L-Trp at lower concentrations of the latter a principally different kinetic scheme is observed. This scheme suggests that binding of the substrate and formation of the quinonoid intermediate are at fast equilibrium, while preceding conformational changes of the holoenzyme proceed with measureable rates ("selected fit"). For the reaction with S-ethyl-L-cysteine the observed concentration dependence of k_{obs} agrees with the realization of both kinetic schemes, the "selected fit" becoming predominant at lower concentrations of substrate, the "induced fit"— at higher ones. In the reaction with S-ethyl-L-cysteine the formation of the quinonoid intermediate proceeds slower than does catalytic α,β-elimination of ethylthiol from S-ethyl-L-cysteine, and consequently does not play a considerable role in the catalysis, which may be effected by a concerted E2 mechanism.

1. Introduction

According to the prevailing classic notion the conformity between an enzyme and its substrate is ensured by the induced conformational changes of both the bound substrate in the active site, and the protein ("induced fit"), which result from the binding, and lead finally to the stabilization of the transition state as compared to the ground state [1]. Considerable attention is drawn in literature to the selection of the ground state conformations. Thus, in [2] the role of selection of the reactive conformations of the substrate in the overall mechanism is discussed in detail for the example of chorismate mutase. In this connection a principally different mechanism seems possible, which implies a certain preorganization of the enzyme, preceding the binding, and subsequent selection of the necessary enzyme conformation by the substrate. This mechanism was first suggested by F.B. Straub in sixties ("fluctuation fit"), and is attracting growing attention of researchers in the last years [3]. New terms like "conformational selection" or "selected fit", have been introduced to denote it [3]. A promising recent notion [4] is that "induced fit" and "selected fit" are two limiting mechanisms, which in the general case are operative together, and their relative contributions change depending on the concentration of the ligand and the rates of the conformational changes of the protein [3]. In the present work we have examined the interactions of a mutant form of tryptophan indole-lyase, TIL Y72F, with the substrate and its analogues, and we believe that the results may be considered as a good illustration of coexistence of different recognition mechanisms in the considered processes.

Tryptophan indole-lyase (tryptophanase, Trpase, EC 4.1.99.1) (TIL) may be found in many microorganisms. This enzyme catalyzes the

Abbreviations: TIL, tryptophan indole-lyase (tryptophanase) [EC 4.1.99.1]; TPL, tyrosine phenol-lyase [EC 4.1.99.2]; PLP, pyridoxal-5[1]-phosphate; OIA, oxindolyl-L-alanine

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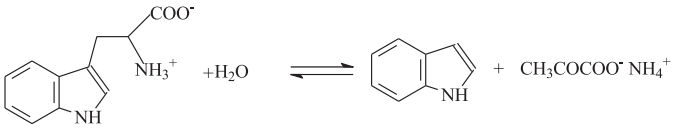
formation of indole and ammonium pyruvate from L-tryptophan [5] (see Eq. (1)), and uses pyridoxal 5[1]-phosphate (PLP) as a cofactor.



(1)

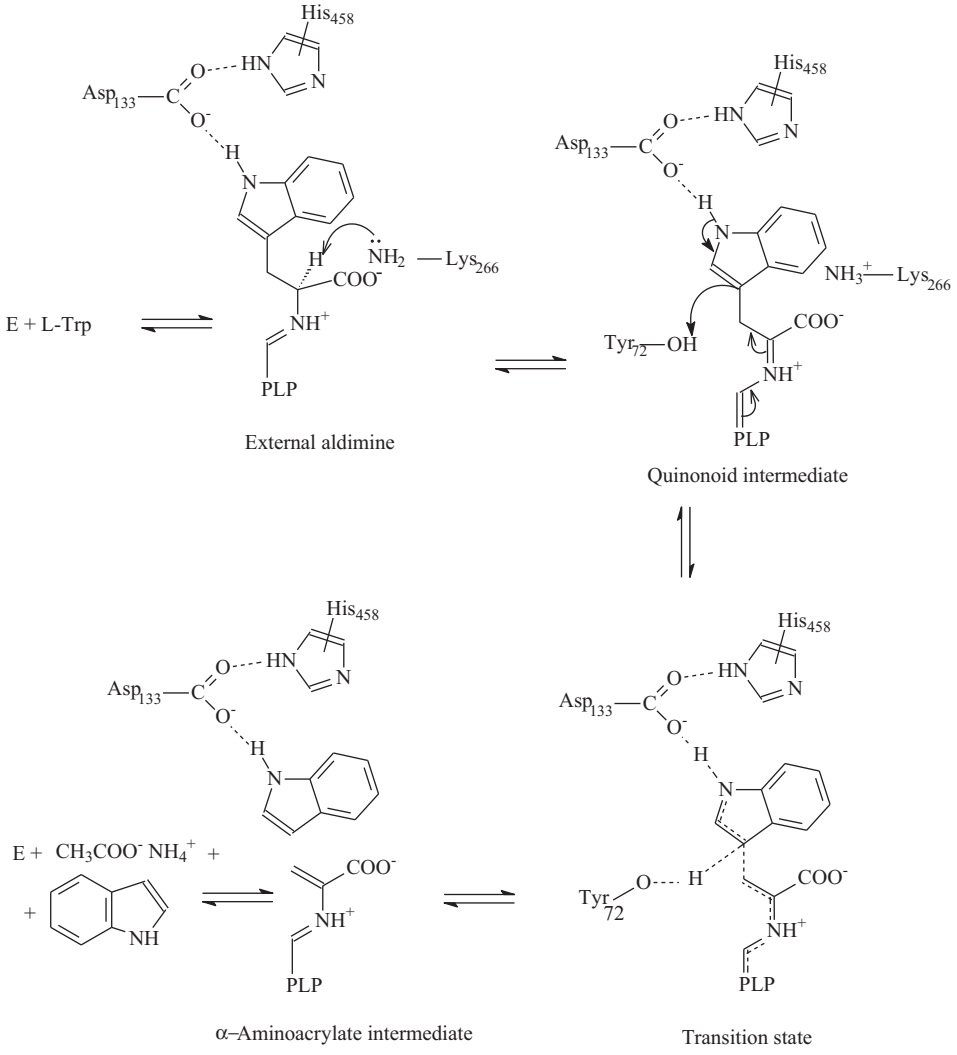
It is capable to catalyze α,β-elimination reactions with a number of other amino acids, containing better leaving groups [6,7].

The main reaction TIL catalyzes is reversible, thus the enzyme may be used for preparative synthesis of L-tryptophan and its physiologically active analogues [8–10]. It was shown that the gene, encoding TIL, is necessary for biofilm formation in *Escherichia coli* [12]. Interestingly, virulent properties of *Haemophilus influenzae* might be correlated with the production of indole in the cells [11]. TIL is composed of four identical subunits, each possessing a PLP-binding site. Crystallization and X-ray data of holoenzymes from *E. coli* and *Proteus vulgaris* have been reported [13,14].

It is known that the holoenzyme of TIL exists in different conformations, absorbing at 420 nm and 337 nm [15,16]. These conformations differ in hydrophobicities and in their accessibilities to the binding with substrates and inhibitors, and interconvert with measureable rates.

The data concerning the mechanism of action of TIL are summed up in [17,18]. The suggested mechanism is presented in Scheme 1. It was shown by Phillips et al. [19] for the wild type enzyme from *E. coli* that the key stage in the enzymatic mechanism is the simultaneous protonation of the C_3 atom of the indole moiety and the breakdown of the $C_3 - C_\beta$ bond in the quinonoid intermediate structure. Tyr 74 residue may be considered as the general acid catalyst performing the protonation of C_3. In TIL from *P. vulgaris* Tyr 72 is homologous to Tyr 74 in the enzyme from *E. coli*. The effect of the replacement of Tyr 72 to Phe on the catalytic properties of TIL was examined in [20]. As expected, the replacement has led to a drastic decrease in activity for L-tryptophan by 50,000-fold. On the other hand considerable activities were retained with respect to substrates bearing good leaving groups and, interestingly, to L-serine.

In continuation of these studies we examined in the present work, the interaction of the mutant tryptophan indole-lyase (TIL) from *P. vulgaris* Y72F with the transition state analogue, oxindolyl-L-alanine (OIA), with the natural substrate, L-tryptophan, and with a substrate bearing a better leaving group, S-ethyl-L-cysteine. We have found that



Scheme 1. The mechanism of TIL reaction with its natural substrate, L-Trp.