Ligand-Induced Maturation of Threonine Deaminase

Abstract. *The dimeric intermediate substructures of threonine deaminase, which are obtained by alkaline dialysis of the native tetrameric enzyme, are inactive after reassembly unless they are subsequently exposed to maturation-inducing ligands for which the enzyme possesses stereospecific binding sites. The binding of the ligands at these sites precedes the full maturation of the enzyme.*

Threonine deaminase (E.C. 4.2.1.16) from *Salmonella typhimurium* has a molecular weight of 194,000 and is composed of four polypeptide chains, each with a molecular weight of 48,500 (*1*). Analysis of the tryptic digests of the enzyme suggests that the polypeptide chains are identical (*1*). Also, the native tetrameric structure contains two covalently bound pyridoxal 5′-monophosphate (PALP) residues and therefore is presumed to possess two active sites (*2*). The kinetic data of Maeba and Sanwal also suggest two active sites for the native enzyme (*3*). These results suggest that the functional protomer for this enzyme is a dimeric structure composed of two of the polypeptide chains.

Therefore, to elucidate the relation of structure to function for this enzyme, we examined the intermediate substructure of the enzyme. A stable dimeric structure was isolated by resolving the enzyme from its cofactor, PALP, by alkaline dialysis against a 0.1*M* tris(hydroxymethyl)aminomethane (tris)HCl buffer, *p*H 8.5, containing $5 \times 10^{-3}M$ dithiothreitol and $10^{-3}M$ ethylenediaminetetraacetate. This apodimer had a sedimentation coefficient ($S^0_{20,w}$) of 4.7; sedimentation equilibrium analysis revealed a molecular weight of 97,000. As expected, these apodimers were inactive in an assay mixture which lacked PALP, but which contained 80 μmole per milliliter of L-threonine, the substrate. However, a gradual first-order regain of activity was initiated when 0.02 μmole of PALP was added to this reaction mixture. The half-life ($t_{0.5}$) of the activation period was 45 to 50 seconds. It was subsequently observed that the apoprotein was not activated by prior incubation with PALP but instead required L-threonine to undergo the activation. Also, the addition of PALP promoted a rapid reassociation of the apodimers to form an inactive holotetramer (*4*). This holotetramer remained initially inactive up to 12 hours but immediately underwent an activation period when exposed to L-threonine (Fig. 1).

We then tested the capacity of the other two ligands, isoleucine and valine, for which threonine deaminase has stereospecific sites, to activate the enzyme (*3*). Either of these ligands at saturating concentrations could also activate the inactive holotetramer (Fig. 2). Several other amino acids were tested to establish the specificity of the activating ligands mentioned above. Among these were glycine, phenylalanine, tryptophan, and glutamate; none showed any activating ability. Serine, which is also a substrate for this enzyme, was able to activate the inactive holotetramer. Therefore, the activation phenomenon appeared to be specific.

We sought to determine whether the activation observed in the presence of the activating ligands was induced as a consequence of the binding of the ligands to the inactive holotetramer (that is, if the binding of the ligands was a required prerequisite for the maturation process), or whether the role of the ligands was to stabilize an unfavorable equilibrium which existed between the active and inactive forms of the holotetramer. Once the inactive holotetramer was activated it remained active even after the activating ligand was removed from the enzyme. We activated the inactive holotetramer with C^{14}-labeled isoleucine of a sufficient radiospecific activity so that any ligand that remained bound to the enzyme would be discernible after filtration through Sephadex G-25. When such a treatment was employed, no radioactivity remained associated with the enzyme; yet the enzyme remained active indefinitely. Therefore, if a spontaneous equilibrium does exist between the active and inactive forms of the holotetramer, then the equilibrium must lie far in the direction of the active species. If this were so, then the inactive holotetramer should become activated with time even in the absence of the activator ligands. This was not the case; the inactive enzyme remained inactive (Fig. 1). On the basis of these observations nothing can be concluded about the nature of the equilibrium between the two forms of the holotetramer except that the rates of approach to the equilibrium are extremely slow in the absence of one of the maturation-inducing ligands. Presumably, a high energy of activation for

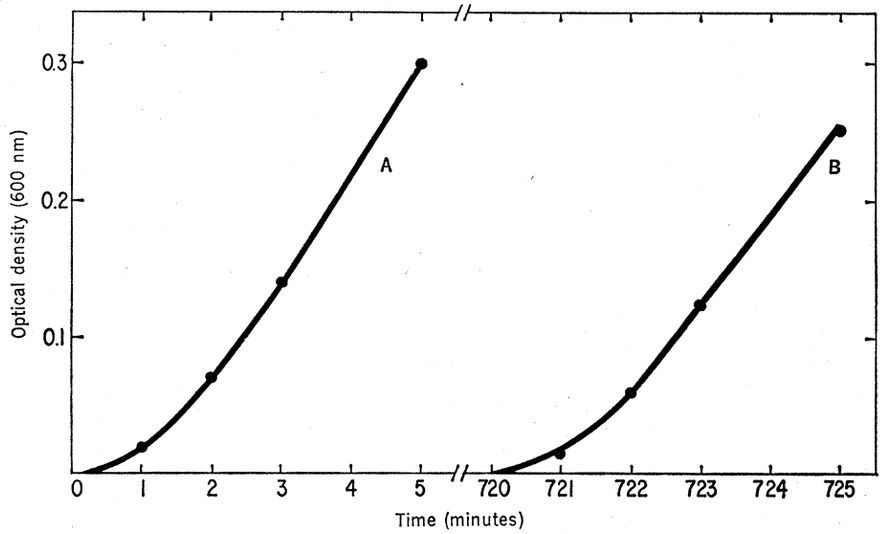

Fig. 1. Time course of threonine deaminase activation. The course of the reaction was followed by measuring the amount of α-ketobutyrate formed from L-threonine as the dinitrophenylhydrazone derivative. The reaction mixture contained per milliliter: tris-HCl, *p*H 8.0, 100 μmole; NH_4Cl, 100 μmole; pyridoxal 5′-monophosphate, 0.02 μmole; and enzyme, 3 μg. The reaction in curve A was initiated with the addition of 80 μmole of L-threonine immediately after the addition of the enzyme to the reaction mixture. The reaction in curve B was initiated with 80 μmole of L-threonine after the enzyme had been incubated in the reaction mixture for 12 hours at room temperature. Assays were performed at 37°C and terminated at the times indicated by transferring 0.1-ml portions of the reaction mixture directly into 3 ml of 0.25 percent 2,4-dinitrophenylhydrazine dissolved in 1*N* HCl. The absorbance was read at 600 nm. At this wavelength one optical density unit represents 1 μmole of α-ketobutyrate.

the transition of the inactive holotetramer to the active form is responsible for the slow spontaneous activation rate, but the binding of a maturation-inducing ligand to the inactive form overcomes this energy barrier and allows the transition to proceed; that is, in the classical sense the activation of threonine deaminase is catalyzed, or induced, by the binding of the ligand to the inactive form.

The concept of induced maturation is supported by the observation that a combination of isoleucine and valine or of isoleucine and threonine blocked the activation of the enzyme. Valine and threonine together, however, continued to promote normal maturation.

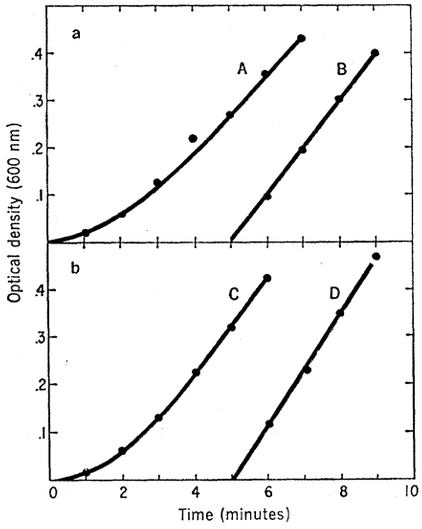

Fig. 2. (a) Activation of threonine deaminase by prior incubation with L-isoleucine. Enzyme (500 μg) was added to a reaction mixture (as described in Fig. 1) to which 1 μmole of L-isoleucine had been added. In curve A, 0.01 ml of this mixture was transferred immediately to another reaction mixture containing no L-isoleucine but containing 80 μmole of L-threonine (time 0). The dilution of the L-isoleucine was sufficient to eliminate most of its inhibiting effect at the concentration of L-threonine used. The activation period is observed. In curve B the enzyme was first incubated for 5 minutes in the buffer containing L-isoleucine before 0.01 ml of the mixture was diluted into the reaction mixture containing L-threonine. No activation period is observed. (b) Activation of threonine deaminase by prior incubation with L-valine. Curve C represents an assay performed as described for curve A, except that 1 μmole of L-valine was included in the first reaction mixture instead of L-isoleucine. Curve D represents an assay performed as described for curve B, except that 1 μmole of L-valine was substituted for the L-isoleucine in the first reaction mixture.

These results imply that at least two distinct stereospecific sites exist on the inactive holotetramer, one specific for either valine or threonine, and the other specific for isoleucine. The maturation process is effected when only one of these sites on the enzyme is filled, but when both sites are occupied this process is blocked. It is presumed that inhibition of the maturation process results from a steric hindrance which is imposed on the immature protein by ligands bound at both sites simultaneously and which prevents a conformational alteration required for the activation of the protein. An allosteric interpretation of this observation is also possible and cannot be easily ruled out at this time. Nevertheless, it is evident that the maturation-inducing ligands bind to the immature protein before the maturation process and that therefore the maturation of this enzyme represents a ligand-specific induction (*5*).

The functional protomer for threonine deaminase from *Salmonella typhimurium* is apparently a dimer composed of two identical polypeptide chains (*6*). A stable dimeric intermediate substructure of this enzyme can be obtained with the use of mild conditions (alkaline dialysis). These intermediate substructures are catalytically inactive even after they are reassembled into a form identical to the native enzyme in its gross molecular architecture. However, this immature, or inactive, protein can be irreversibly maturated, or activated, in the presence of those ligands for which there exist stereospecific binding sites on the enzyme. This maturation is apparently induced by the binding of these ligands before the maturation process itself. The existence in vivo of such a phenomenon would present an interesting correlation with the multiligand system (multivalent repression) involved in the regulation of the synthesis of this enzyme (*7*); it remains to be seen whether or not this process plays a significant regulatory role within the cell.

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Receptor Potentials from Hair Cells of the Lateral Line

Abstract. *Intracellular recordings from hair cells in the tail lateral line of mudpuppy* Necturus maculosus *show receptor potentials less than 800 microvolts, peak to peak, from stimuli that are considered large compared to natural stimuli. The hair cells are in neuromasts that are sensitive at the time of recording and are identified by both in vivo and in vitro examination of intracellular staining.*

Microphonic potentials from the inner ear of many different species have been recorded and studied (*1*). Similar potentials have been observed in other labyrinth organs (*2*) and in lateral line organs (*3*). The source of these potentials recorded extracellularly was hypothesized to be the hair cells that are found in the inner ear and in all other sensory epithelia of the acoustico-lateral system (*4*). Receptor potentials have been sought in hair cells without success. We have recorded intracellular potentials from hair cells in the lateral line organ of the tail of the mudpuppy *Necturus maculosus*, and we have found a small component that responds to motion of the cupula (a 27-hz displacement was used); this response appears to have the characteristics of a receptor potential. The magnitude of cupular motion was about 2 μm at a distance of about 80 μm above the cupula hair-cell junction but of variable and unknown magnitude at the junction. The receptor potentials ranged downward from a peak of 800 μv (peak to peak). These potentials were recordable only from hair cells, and they were not an artifact of motion.

The neuromasts on the mudpuppy tail occur in groups called stitches with two to six neuromasts per stitch (Fig. 1). During an experiment a mudpuppy was immobilized with Flaxedil (1 mg