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*phe*A (Rv3838c) of *Mycobacterium tuberculosis* Encodes an Allosterically Regulated Monofunctional Prephenate Dehydratase That Requires Both Catalytic and Regulatory Domains for Optimum Activity*

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Prephenate dehydratase (PDT) is a key regulatory enzyme in L-phenylalanine biosynthesis. In *Mycobacterium tuberculosis,* expression of *phe*A, the gene encoding PDT, has been earlier reported to be iron-dependent (1, 2). We report that *M. tuberculosis phe*A is also regulated at the protein level by aromatic amino acids. All of the three aromatic amino acids (phenylalanine, tyrosine, and tryptophan) are potent allosteric activators of *M. tuberculosis* PDT. We also provide *in vitro* evidence that *M. tuberculosis* PDT does not possess any chorismate mutase activity, which suggests that, unlike many other enteric bacteria (where PDT exists as a fusion protein with chorismate mutase), *M. tuberculosis* PDT is a monofunctional and a non-fusion protein. Finally, the biochemical and biophysical properties of the catalytic and regulatory domains (ACT domain) of *M. tuberculosis* PDT were studied to observe that, in the absence of the ACT domain, the enzyme not only loses its regulatory activity but also its catalytic activity. These novel results provide evidence for a monofunctional prephenate dehydratase enzyme from a pathogenic bacterium that exhibits extensive allosteric activation by aromatic amino acids and is absolutely dependent upon the presence of catalytic as well as the regulatory domains for optimum enzyme activity.

The absence of a human counterpart of the aromatic amino acid biosynthesis pathway makes the member enzymes promising targets for therapeutic interventions against pathogenic microbes. In the case of *Mycobacterium tuberculosis* as well, the aromatic amino acid pathway has been proven to be essential for the survival of this pathogen (3, 4). Furthermore, amino acid auxotrophs of *M. tuberculosis* do not survive or multiply in macrophages (5, 6), which suggests that these amino acids are not available within the compartment of the macrophage in which the bacteria reside.

Prephenate dehydratase (PDT)[1] is a terminal enzyme of the aromatic amino acid biosynthesis pathway that catalyzes the conversion of prephenate to phenylpyruvate with the elimination of water and carbon dioxide. Further, with the action of aromatic aminotransferase, phenylpyruvate is converted to phenylalanine (Fig. 1). In enteric bacteria, PDT usually exists as a fusion protein with chorismate mutase (CM), thereby catalyzing the first two steps in the biosynthesis of phenylalanine. In *Escherichia coli* as well, CM and PDT are fusion partners of the bifunctional P-protein, coded by *phe*A (7). The N-terminal end of the bifunctional P-protein of *E. coli* specifies the chorismate mutase activity, whereas the remainder of the sequence specifies the prephenate dehydratase enzymatic activity (8). In few bacteria, PDT is a monofunctional protein that aligns well with the C-terminal part of P-proteins (9).

PDT is a highly regulated enzyme in bacterial systems (10). Regulatory domains or the ACT domains (named after the regulatory domains of aspartate kinase, chorismate mutase, and TyrA (prephenate dehydrogenase) are present in many metabolic enzymes, including enzymes of the aromatic amino acid biosynthesis family (11, 12). The prephenate dehydratase enzyme of *M. tuberculosis* also possesses a discrete ACT domain that is predicted to impart regulatory properties to the enzyme.

In *M. tuberculosis*, expression of *phe*A (the gene encoding PDT) has earlier been reported to be dependent on the transcription factor IdeR (1, 2). This study was initiated to determine whether, apart from genetic regulation, *M. tuberculosis phe*A is also regulated at the enzymatic level. The experimental approach involved expression of the *M. tuberculosis* ORF Rv3838c in *E. coli* and determination of the biochemical parameters of the encoded protein. We have described the kinetic and regulatory properties of *M. tuberculosis* PDT and also dissected the role of the catalytic and regulatory domains of the recombinant protein. The significance of a differential feedback regulation of the terminal enzymes of the aromatic amino acid biosynthesis pathway of *M. tuberculosis* has also been discussed.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Plasmids—E. coli XL1Blue and *E. coli* BL21 DE3 strains were used, respectively, as hosts for cloning and recombinant protein expression purposes using the pET23a (Novagen) expression vector.

Cloning, Expression, and Purification of M. tuberculosis PDT and PDT-N and PDT-C—The ORF Rv3838c encoding the prephenate dehydratase enzyme of *M. tuberculosis* H37Rv was amplified from the bacterial genomic DNA using primers carrying specific restriction enzyme

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[1] The abbreviations used are: PDT, prephenate dehydratase; CM, chorismate mutase; ACT, aspartate kinase, chorismate mutase, and TyrA; ORF, open reading frame; MES, 4-morpholinoethanesulfonic acid; CPB, citrate phosphate buffer.

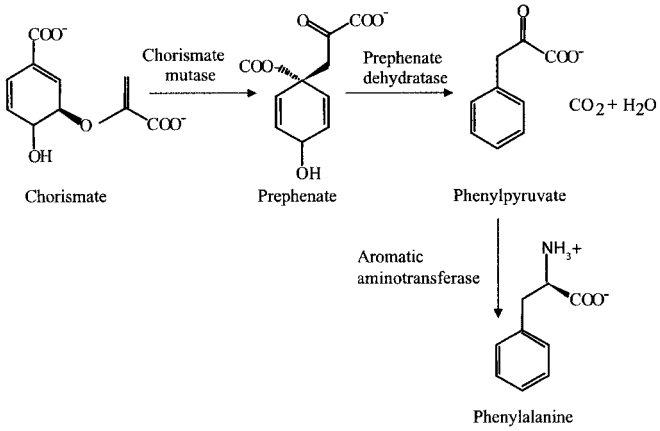

FIG. 1. **Terminal steps in the biosynthesis of phenylalanine.** Chorismate mutase catalyzes the conversion of chorismate to prephenate, and prephenate dehydratase converts prephenate to phenylpyruvate with the elimination of water and carbon dioxide. Further, an aromatic aminotransferase converts phenylpyruvate to phenylalanine.

sites (Table I). The amplicons were digested with NdeI/XhoI enzymes and cloned into the corresponding sites of pET23a expression vector. The N and the C terminus of *M. tuberculosis* PDT, corresponding to the catalytic and regulatory domains, were cloned separately using other sets of primers described in Table I. The resultant plasmids were labeled as pET3838, pET3838N, and pET3838C.

The chimeric constructs were used to transform *E. coli* BL21DE3 cells, and the transformants were grown in 1 liter of LB medium with 100 μg/ml ampicillin and 4% glycerol. Isopropyl 1-thio-β-D-galactopyranoside was added to a final concentration of 0.1 mM, and the culture was further incubated for 5 h at 27 °C to allow protein expression. The cells were harvested by centrifugation and resuspended in 20 ml of lysis buffer (10 mM Tris-HCl, 100 mM NaCl, and 10% glycerol, pH 7.5) with 0.1 mM phenylmethylsulfonyl fluoride and disrupted using a sonicator. A second round of centrifugation at 10,000 $\times$ *g* for 10 min was carried out to remove particulate matter and cell debris. The resulting supernatant was applied to Talon resin (Clontech). The supernatant containing a mixture of soluble protein was allowed to bind to Talon resin packed in a polypropylene column. The column was washed with 5 bed volumes of lysis buffer containing 30 mM imidazole followed by elution with 250 mM imidazole. The purified protein fractions were analyzed by SDS-PAGE and dialyzed against Tris buffer to remove salts and imidazole.

Enzyme Assays and Kinetic Studies—Prephenate dehydratase activity assays were carried out as described earlier (13) with a few modifications. Essentially, the dehydratase activity of rRv3838c was assayed by measuring the rate of conversion of prephenate to phenylpyruvate. The reaction mixture contained 20 mM Tris-HCl, pH 8.2, 1 mM EDTA, 0.01% bovine serum albumin, 1 mM dithiothreitol, and 0.1–2 mM barium prephenate in a total volume of 400 μl. The sample was pre-incubated at 37 °C followed by the addition of 20–100 pmol of purified recombinant *M. tuberculosis* PDT. After a second round of incubation at 37 °C for 5 min, the reaction was terminated by the addition of 800 μl of 1.5 M NaOH. Phenylpyruvate was measured spectrophotometrically at 320 nm. Appropriate blanks without the enzyme were kept as controls. The optimum pH for the activity of *M. tuberculosis* PDT was determined using buffers of different pK_a (CPB, pH values 4 and 4.5; MES, 6; HEPES, 7; and Tris-HCl, pH values 7.5 and 8). PDT activity was also assessed at different temperatures (15–80 °C). One unit of enzyme was defined as the amount of enzyme required to form 1 μmol of product/min at 37 °C. Allosteric regulation of PDT activity by L-Phe, L-Tyr, and L-Trp was measured at 100–500 μM concentrations of the effectors. Chorismate mutase activity assays were carried out exactly as described by Davidson and Hudson (14).

Analytical Size Exclusion Chromatography—Gel filtration or size exclusion chromatography was used to determine the oligomeric state of *M. tuberculosis* PDT, as well as the individually expressed catalytic and regulatory domains. The chromatography experiment was performed on Superdex 200 (for PDT) and Superdex 75 (for PDT-N and PDT-C) fast protein liquid chromatography columns from Amersham Biosciences using 10 mM Tris, 100 mM NaCl, and 1 mM dithiothreitol as the running buffer. Void volume of the column was determined using blue dextran 2000. Elution time of all the recombinant proteins was recorded, and the molecular weight was calculated by estimating the elution volumes of standards of known molecular weight. The recombinant proteins were loaded on the gel filtration column at a concentration of 2 mg/ml in the presence of 1 mM dithiothreitol.

Phenylalanine Binding Assays/Fluorimetric Procedures—Binding of phenylalanine to the recombinant proteins was monitored using fluorescence spectroscopy. Fluorescence spectra of individual proteins were recorded in the presence and absence of phenylalanine on a PerkinElmer LS-3B spectrofluorometer. Briefly, the recombinant proteins were excited using an excitation wavelength of 295 nm, and the tryptophan emission spectra were recorded from 305 to 440 nm at 37 °C. The fluorescence slit width of excitation and emission were kept at 4 nm, and the scan speed was 50 nm/s. Protein concentrations were kept at 2.5 μM in 10 mM Tris, pH 8, and the buffer signal was subtracted from the spectrum of recombinant proteins.

RESULTS

pheA (ORF Rv3838c) of M. tuberculosis Encodes a Functional Prephenate Dehydratase Enzyme—The recombinant protein corresponding to ORF Rv3838c, predicted to be the PDT enzyme of the bacterium, was purified using affinity chromatography procedures (Fig. 2, Inset). The purified protein was quantified using the Bradford assay and used for prephenate dehydratase activity assays. As evident from the substrate saturation plot, *M. tuberculosis* PDT followed Michaelis-Menten's kinetics. The double reciprocal plot of the enzyme was linear, and K_m for the enzyme was calculated as 500 μM and V_{max} as 125 μmoles/min/mg (Fig. 2). Specific activity of the enzyme was determined as 125 units/mg pure protein. The effect of temperature and pH on enzyme activity was also studied. It was observed that the enzyme is maximally active between temperatures 37 and 42 °C and pH 6 and 7 (Fig. 3). Although other bacterial PDTs are active at an acidic pH (15), *M. tuberculosis* PDT was found to be active in mildly acidic to near neutral pH.

Ionic Interactions Are Required for Optimum Prephenate Dehydratase Activity—To determine whether ionic interactions are important for the activity of *M. tuberculosis* PDT, the enzyme was assayed in the presence of various concentrations of NaCl. It was observed that the enzyme activity was completely abolished in the presence of 200 mM and higher concentrations of NaCl (Fig. 4). This suggests that enzyme substrate interactions are brought about by ionic interactions, and disruption of the same leads to inhibition of enzyme activity.

Aromatic Amino Acids Are Potent Allosteric Activators of M. tuberculosis Prephenate Dehydratase—Control of the terminal enzymes in any biosynthetic pathway is crucial for maintaining the correct balance of the end product in accordance with the requirement of the organism. This holds true for the aromatic amino acid biosynthesis as well. PDT being a terminal enzyme in phenylalanine biosynthesis, we decided to determine the effect of phenylalanine as well as other cross-pathway-specific aromatic amino acids (tyrosine and tryptophan) on prephenate dehydratase activity. Results showed that *M. tuberculosis* PDT was inhibited by low concentrations of aromatic amino acids (up to ~100 μM) and highly activated at higher concentrations (Fig. 5). This property of *M. tuberculosis* PDT was exactly opposite to that of *M. tuberculosis* CM, as recent work from our laboratory has shown that *M. tuberculosis* CM is activated by lower concentrations of aromatic amino acids (up to ~100 μM) and inhibited by higher concentrations (16).

M. tuberculosis Prephenate Dehydratase Does Not Display Any Chorismate Mutase Activity—In *E. coli*, *phe*A codes for a bifunctional P-protein carrying both CM and PDT activities, whereas in several other bacteria, PDT is a monofunctional protein. *M. tuberculosis* PDT does not have a predicted CM domain. However, on account of several examples of convergent evolution of enzyme reaction mechanisms, it was decided to determine whether PDT has any associated CM activity. Recombinant

TABLE I

Sequences of primers used for PCR amplification of M. tuberculosis prephenate dehydratase (coded by ORF Rv3838c) and its N and C termini (catalytic and regulatory domains)

Nucleotides in bold italics represent the restriction enzyme sequence appended to the primers to enable directional cloning in pET23a expression vector.

Gene	Forward primer sequence	Reverse primer sequence
Rv3838c	NdeI ATA***CATATG***GTGGTGCGTATCGCTTACCTCGGT	XhoI AT***CTCGAG***TGCTTGCGCCCCCTGGT
Rv3838N	NdeI ATA***CATATG***GTGGTGCGTATCGCTTACCTCGGT	XhoI AT***CTCGAG***ATCGGCTCCGGTGCGCG
Rv3838C	NdeI ATA***CATATG***CGCACGTCTGCAGTGCTGC	XhoI AT***CTCGAGT***TGCTTGCGCCCCCTGGT

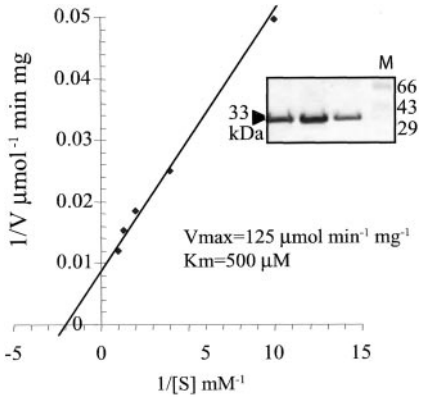

FIG. 2. **Recombinant protein coded by ORF Rv3838c is a functional PDT enzyme.** A double reciprocal plot of *M. tuberculosis* PDT enzyme activity was used to calculate K_m and V_{max}. The specific activity of recombinant *M. tuberculosis* PDT was calculated as 125 units/mg pure protein. *Inset*, *M. tuberculosis phe*A corresponding to ORF Rv3838c was cloned in the NdeI and XhoI sites of the pET23a vector with a C-terminal histidine tag and expressed in *E. coli* BL21 cells. Affinity purification of recombinant protein was carried out using Talon resin (Clontech). Purified protein was resolved on a 10% Tris-tricine gel and stained with Coomassie Brilliant Blue dye. *M* represents the protein molecular size marker. The *arrowhead* indicates the position of the 33-kDa protein, the predicted mass of *M. tuberculosis* PDT.

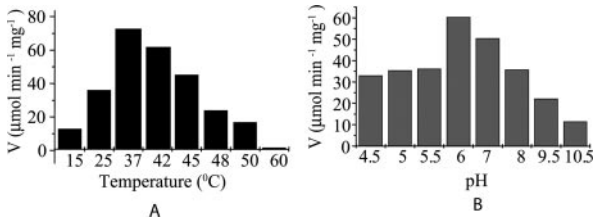

FIG. 3. **The PDT activity of rRv3838c is pH- and temperature-dependent.** Maximal enzyme activity was obtained in a mildly acidic pH. Temperatures above 50 °C completely inactivate the enzyme.

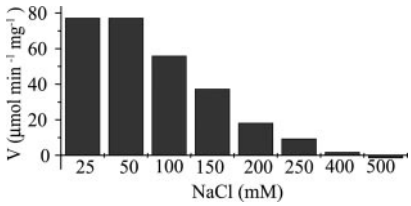

FIG. 4. **Ionic interactions are required for optimum catalytic activity of *M. tuberculosis* PDT.** Higher concentrations of NaCl (200 mM and above) completely abolish the PDT activity of *M. tuberculosis* ORF Rv3838c.

M. tuberculosis PDT was therefore used in a chorismate mutase activity assay. It was observed that *M. tuberculosis* PDT is completely devoid of CM activity (Table II), confirming that *M. tuberculosis* PDT is a monofunctional protein.

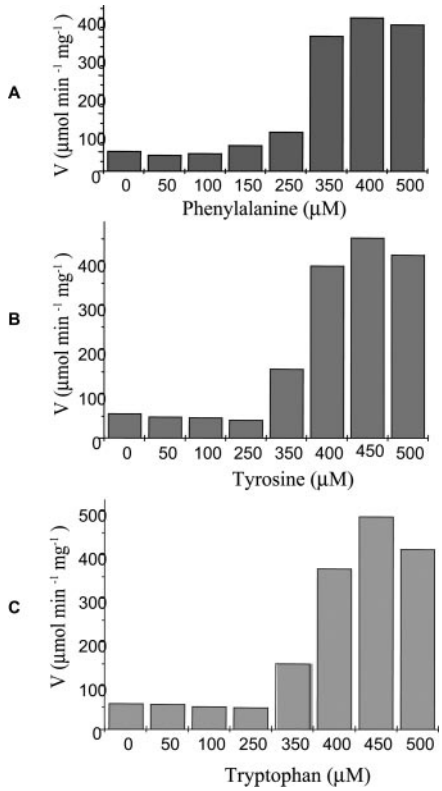

FIG. 5. **Aromatic amino acids, as a function of concentration, allosterically regulate *M. tuberculosis* PDT.** Up to 100 µM concentrations of aromatic amino acids, phenylalanine (*A*), tyrosine (*B*), and tryptophan (*C*) negatively regulate *M. tuberculosis* PDT. At higher concentrations (300 µM and above), these amino acids increase the enzyme activity.

TABLE II

Activity profile of the engineered M. tuberculosis PDT proteins

Clones	Protein	Amino acid residues	Purified protein activity	
			PDT	CM
pET3838	PDT	1–321	125 units/mg	—
pET3838N	PDT-N	4–192	—[a]	—
pET3838C	PDT-C	202–279	—	—
	PDT-C + PDT-N		—	—

[a] —, not detectable.

The Individually Cloned, Expressed, and Purified Catalytic and Regulatory Domains of M. tuberculosis Prephenate Dehydratase Are Inactive in Catalyzing the Conversion of Prephenate to Phenylpyruvate—M. tuberculosis PDT has a distinct predicted catalytic and regulatory domain (Fig. 6*A*). Hence, it was decided to test whether the catalytic activity of *M. tuberculosis* PDT is independent of the regulatory domain or it requires both the domains for optimum activity. For this purpose, the catalytic and regulatory domains of *M. tuberculosis* PDT were

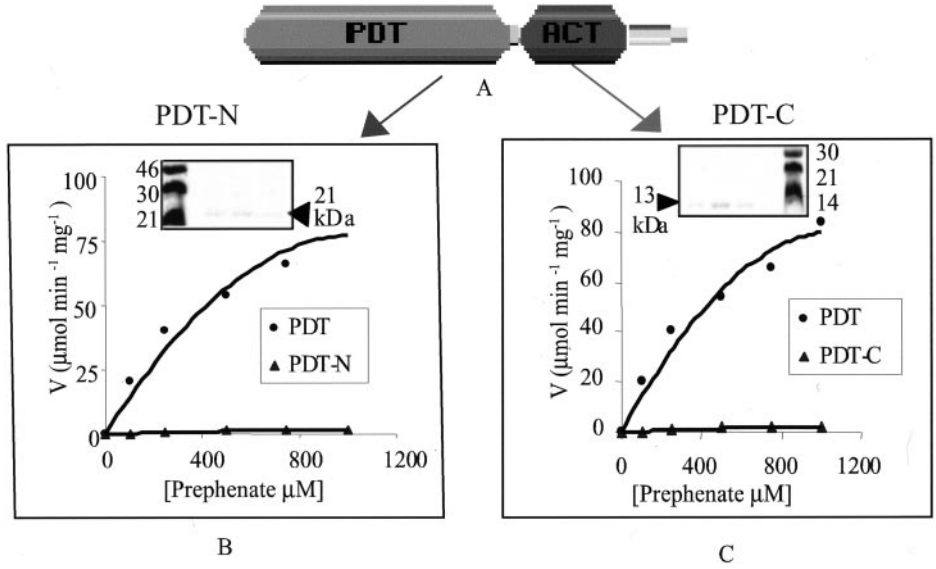

FIG. 6. **The catalytic (PDT-N) as well as the regulatory (PDT-C) domains of *M. tuberculosis* PDT in isolation cannot catalyze the conversion of prephenate to phenylpyruvate.** *A*, schematic representation of the catalytic and regulatory domains of *M. tuberculosis* PDT. *B* and *C*, lack of PDT activity of the individually purified catalytic and regulatory domains, as evident from the substrate saturation plots, suggest that interactions between both of the domains are essential for enzyme activity. *Insets*, the individual catalytic (PDT-N) and regulatory domains (PDT-C) of *M. tuberculosis* prephenate dehydratase were cloned in the NdeI/XhoI sites of pET23a expression vector, and recombinant proteins were purified using affinity chromatography procedure. The Coomassie Blue-stained polyacrylamide gel shows the eluted fractions of the pure recombinant proteins corresponding to catalytic (*PDT-N*) and regulatory (*PDT-C*) domains.

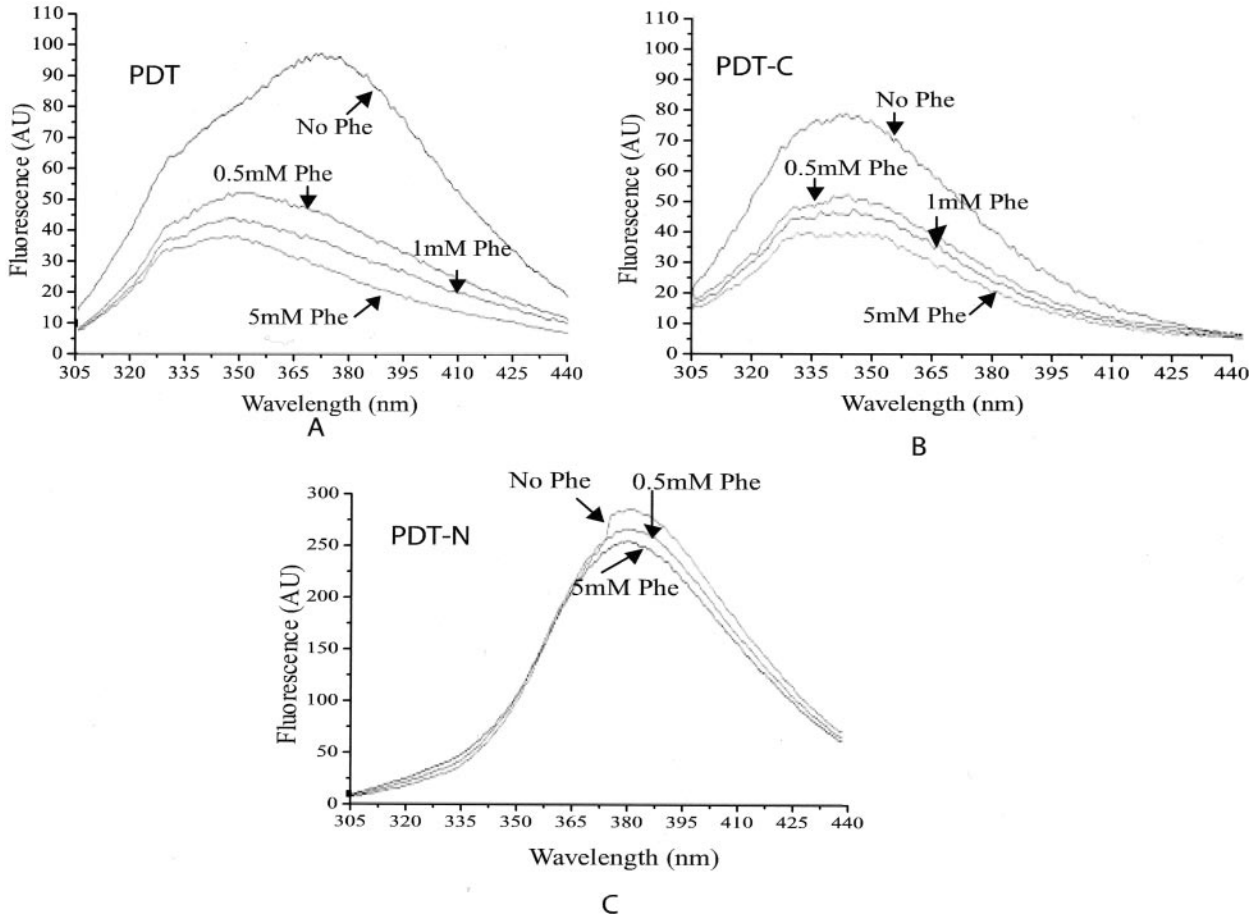

FIG. 7. **Phenylalanine binding leads to a conformational change in *M. tuberculosis* prephenate dehydratase, and the regulatory domain resides within the C terminus.** *A*, the intrinsic fluorescence intensity of full-length prephenate dehydratase decreased in the presence of phenylalanine, and the emission maxima shifted from 380 to 350 nm. A blue shift indicates that tryptophan residues of the protein are buried inside upon phenylalanine binding. *B*, fluorescence emission spectrum of the PDT-C (regulatory domain of *M. tuberculosis* prephenate dehydratase) is altered in the presence of phenylalanine. The intrinsic fluorescence intensity decreased, although the emission maxima remained the same. *C*, fluorescence emission spectrum of PDT-N (catalytic domain) remains unchanged upon the addition of phenylalanine. These experiments suggest that the phenylalanine regulatory domain resides only in the C terminus of *M. tuberculosis* PDT.

individually cloned in the NdeI/XhoI sites of pET23a vector, expressed in *E. coli* BL21 DE3 cells and purified as recombinant proteins (Fig. 6, *B* and *C*, *insets*). These proteins were labeled as PDT-N and PDT-C and were used in prephenate dehydratase activity assay. Although the regulatory domain was enzymatically completely inactive, surprisingly even the individually cloned catalytic domain of *M. tuberculosis* PDT did not show any PDT activity (Fig. 6). Reconstitution of the enzyme was attempted by taking equimolar ratios of the catalytic and regulatory domains and using it in PDT activity assays.

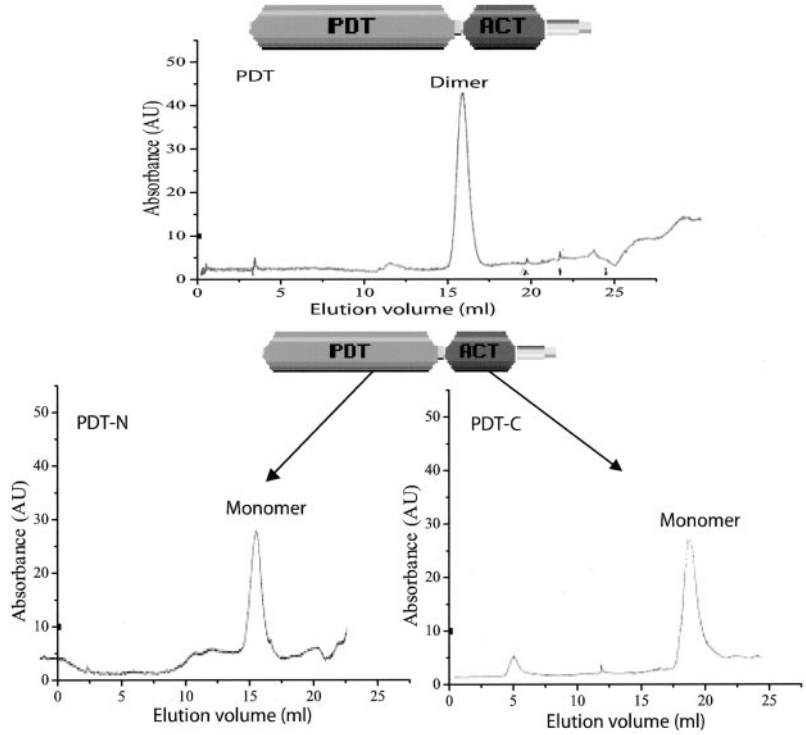

FIG. 8. **Size exclusion chromatography reveals that *M. tuberculosis* PDT is a dimeric protein and its individually cloned catalytic (PDT-N) and regulatory domains (PDT-C) exist as monomers.** The recombinant proteins were individually loaded on a Superdex column (Superdex 200 for PDT and Superdex 75 for PDT-N and PDT-C, Amersham Biosciences) and absorbance units (*AU*) at 280 nm was plotted as a function of elution volume. The elution parameter, Kav *versus* log molecular weight plot of protein standards, was used to calculate the actual MW of these proteins.

However, the activity assay demonstrated that the catalytic and regulatory domains cannot be reconstituted *in vitro* to generate an active enzyme. These results (Table II) demonstrate that, despite having a predicted catalytic and regulatory domain, in *M. tuberculosis* PDT, these domains individually did not display enzyme activity, even when the two are supplied in *trans*.

Phenylalanine Binding Leads to a Conformational Change in M. tuberculosis Prephenate Dehydratase Enzyme and Its Regulatory Domain—The chorismate mutase enzyme of *M. tuberculosis* undergoes a conformational change as a consequence of ligand binding (16). We therefore addressed the question of whether *M. tuberculosis* PDT (which is activated by pathway-specific as well as cross-pathway-specific amino acids) also undergoes conformational change after treatment with these ligands. Fluorescence spectroscopy was used to determine such conformational change in *M. tuberculosis* PDT using full-length *M. tuberculosis* PDT as well as individual catalytic and regulatory domains. Tryptophan emission spectra for all the three recombinant proteins were monitored in the presence and absence of phenylalanine. *M. tuberculosis* PDT possesses four tryptophan residues, and the individual catalytic and regulatory domains have two tryptophan residues each. As evident from the graph, the addition of phenylalanine leads to a change in the fluorescence emission spectrum of only the full-length *M. tuberculosis* PDT and PDT-C (Fig. 7, *A* and *B*).

The fluorescence emission spectrum of the catalytic domain or PDT-N remained unchanged even in the presence of phenylalanine (Fig. 7*C*). In case of full-length *M. tuberculosis* PDT, a blue shift in the fluorescence spectrum, along with quenching of fluorescence, was seen (Fig. 7*A*), whereas in the case of PDT-C only, quenching of fluorescence was seen (Fig. 7*B*). A blue shift in the fluorescence spectrum and quenching indicates that the tryptophan residues of the native protein are buried inside as a consequence of the conformational change that results from the binding of phenylalanine.

Size Exclusion Chromatography Reveals That M. tuberculosis PDT Is an Oligomer and PDT-N and PDT-C are Monomers—Size exclusion chromatography was performed to determine the oligomeric state of *M. tuberculosis* PDT and its individual catalytic and regulatory domains. As the individual domains were catalytically inactive, this experiment was expected to give insights into the possible reasons behind the catalytic insufficiency of the individual domain. All the three proteins eluted as a single peak (Fig. 8). The molecular weight of all the proteins were calculated using a standard curve using the elution parameters of proteins of known molecular weights. The results showed that *M. tuberculosis* PDT is a dimeric protein and its individual catalytic and regulatory domains elute as monomers.

DISCUSSION

PDT exists both as a monofunctional protein and as a fusion protein with chorismate mutase. In *E. coli*, both CM and PDT activities reside in a single, bifunctional protein known as the P-protein, encoded by *phe*A (7). Among the monofunctional PDTs, almost all possess an ACT or amino acid binding domain that brings about allosteric regulation of the enzyme.

In this work, *M. tuberculosis* PDT, an apparently monofunctional protein, was proved to be so using a biochemical approach. The specific activity of full-length recombinant PDT was very high (125 units/mg pure protein), and the enzyme was extensively allosterically activated by all of the three aromatic amino acids (Phe, Tyr, and Trp). Earlier reports have shown that *M. tuberculosis phe*A is an IdeR-regulated gene (1, 2). The present work demonstrates that *M. tuberculosis phe*A is also regulated at the protein level by aromatic amino acids. The high energy cost for the biosynthesis of aromatic amino acids (17) could be responsible for the dual regulation (gene level as well as protein level) of *M. tuberculosis* PDT. However, dual regulation (specifically by IdeR) also suggests the involvement of the aromatic amino acid biosynthesis pathway in the biosynthesis of iron acquisition systems. Although phenylalanine, as a precursor of salicylate *via* the phenyl ammonia lyase pathway, is well documented in plants, this route of salicylate biosynthesis is not at all reported in bacteria (18). It is therefore possible that *M. tuberculosis* could have an additional mechanism of salicylate biosynthesis that involves PDT. The

dual regulation of *M. tuberculosis* PDT and, specifically, activation by aromatic amino acids differentiates it from all other bacteria.

When the regulatory properties of *M. tuberculosis* CM and *M. tuberculosis* PDT were compared, the first observation was that both the enzymes display an exactly opposite pattern of regulation. Although *M. tuberculosis* CM shows moderate activation by low concentrations of aromatic amino acids, activity was greatly inhibited at higher concentrations. The reverse holds true for *M. tuberculosis* PDT, *i.e.* the enzyme is inhibited by low concentrations of aromatic amino acids and highly activated by higher concentrations. These results demonstrate for the first time that, despite the occurrence of CM and PDT as monofunctional proteins in *M. tuberculosis*, correct balance of aromatic amino acids is brought about by opposite regulation of these two enzymes at the protein level.

Another important goal of the present study was to ascertain whether the predicted catalytic domain of *M. tuberculosis* PDT, which lies in the N terminus, is independent of the regulatory domain that is part of the C terminus. Results presented in this report indicate that a discrete regulatory (ACT) domain along with a catalytic domain is required for an optimally active *M. tuberculosis* PDT. These observations were found to be unlike what was reported for *E. coli* P-protein, where discrete domains of the complex P-protein retain their original activity (8). However, in the case of *E. coli* T-protein, wherein although CM and PDH domains can be expressed independently as functional proteins, the efficiency of the enzymes in isolation is much reduced as compared with the entire fusion protein (19). In neither case, however, is the activity completely abolished when the PDH or PDT domains were taken separately.

Various possible explanations can be considered for the complete loss of PDT activity upon removal of the regulatory domain. The dissociation of the oligomeric state of the enzyme upon removal of the C terminus could be one possible reason. It is also possible that in the individually expressed and purified N terminus of *M. tuberculosis* PDT, the substrate binding site is mechanistically altered, leading to a loss of activity.

Although the catalytic domain of *M. tuberculosis* PDT in isolation was functionally inactive, at least the individually cloned C terminus of *M. tuberculosis* PDT retained its regulatory properties. Proof of the same was obtained using fluorimetric assays. These experiments generated a sufficient line of evidence to conclude that there is a definite conformational change in the regulatory domain of *M. tuberculosis* PDT in the presence of phenylalanine. On the other hand, fluorescence emission spectra of the catalytic domain of *M. tuberculosis* PDT remained unchanged, even in the presence of phenylalanine. It is possible that the C terminus of *M. tuberculosis* PDT may serve as a modular regulatory domain that can be used to impart regulatory properties to other proteins of interest. These proteins can, in turn, be used as biosensors.

In summary, this study is the first report of a prephenate dehydratase enzyme that shows allosteric activation by aromatic amino acids. Furthermore, the catalytic as well as the regulatory domains are absolutely required for optimum enzyme activity. The various levels at which the regulation of aromatic amino acid biosynthesis is brought about in *Mycobacterium tuberculosis* suggest that expression of the corresponding genes are strictly dependent upon the requirement of the bacterium. Additionally, considering the absence of a human homologue of PDT, the enzyme might serve as a novel target for the design of novel chemotherapeutic compounds.

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