

Kinetic and structural characterization of a product complex of 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase from *Escherichia coli*

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HPPK (6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase) catalyses the transfer of pyrophosphate from ATP to HMDP (6-hydroxymethyl-7,8-dihydropterin), to form AMP and DHPPP (6-hydroxymethyl-7,8-dihydropterin pyrophosphate). This transformation is a key step in the biosynthesis of folic acid, and HPPK is consequently a target for antimicrobial drugs. The substrates are known to bind to HPPK in an ordered manner, with ATP binding first followed by HMDP. In the present study we show by isothermal titration calorimetry that the product, DHPPP, can bind to the HPPK apoenzyme with high affinity (equilibrium dissociation constant, $K_d = 0.2 \mu\text{M}$), but without the enhancement of pterin fluorescence that occurs on binding of HMDP. The transient kinetics of the enzyme can be monitored by measuring the change in the fluorescence of the pterin ring using stopped-flow methods. The fluorescence exhibits a pronounced biphasic behaviour: it

initially rises and then declines back to its original level. This behaviour is in agreement with a two-state kinetic model, with the first phase of fluorescence increase associated with HMDP binding to the enzyme, and the second phase with a slow event that occurs after the reaction has taken place. The HPPK–DHPPP and HPPK–DHPPP–AMP complexes were examined by NMR, and the binding site for DHPPP partially mapped from changes in chemical shifts identified from two dimensional $^1\text{H}/^{15}\text{N}$ heteronuclear single-quantum coherence spectra. The results demonstrate that DHPPP, in contrast to HMDP, is able to bind to the HPPK apoenzyme and suggest that the pyrophosphate moieties on the ligand play an important role in establishment of a high affinity binding site for the pterin ring.

Key words: folic acid, ligand binding, NMR, pyrophosphokinase.

INTRODUCTION

HPPK (6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase; EC 2.7.6.3) catalyses the pyrophosphorylation of HMDP (6-hydroxymethyl-7,8-dihydropterin) by ATP to form DHPPP (6-hydroxymethyl-7,8-dihydropterin pyrophosphate; see Figure 1). This is a vital step in the folic acid biosynthesis pathway, which is a target for antimicrobial drugs [1]. The three-dimensional structures of HPPK from *Haemophilus influenzae* and *Escherichia coli* have been determined by X-ray crystallography [2–4]. The structures of the enzymes from both organisms are similar: they adopt an α/β structure, with five helices packed around a four-strand antiparallel β -sheet. The binding sites for ATP and HMDP have been identified by co-crystallisation with substrates or analogues and reveal several structural changes in the enzyme on binding of ligands [2,3,5]. The apoenzyme adopts an open structure, with the active site located within a large crevice in the enzyme surface. Comparison of this structure with two ternary complex structures has revealed that three loop regions undergo substantial shifts in position to accommodate ATP and HMDP. Two highly conserved aspartic acid residues are involved in chelating two magnesium ions, which interact with the α -, β - and γ -phosphates within ATP. The crystal structure of a non-productive ternary complex has provided evidence that the mechanism involves direct transfer of the pyrophosphoryl moiety from ATP to HMDP [5]. The position of the hydroxy group of HMDP with respect to the β -phosphorus of ATP is optimized for an in-line single displacement mechanism. Recent work has established that two arginine re-

sidues, Arg-82 and Arg-92, make an important contribution to catalysis [6,7].

The results of kinetic studies on HPPK have established that the enzyme adopts a preferred order of substrate binding, with ATP binding first, followed by HMDP [8]. This behaviour can be rationalized in terms of the X-ray crystallographic studies on the enzyme, which show that three loop regions are involved in a structural change on binding of ATP, and this change leads to formation of the HMDP binding site. HMDP does not bind to the HPPK apoenzyme at low concentrations [8]. Stopped flow fluorimetry data has shown that the binding of ATP is relatively slow and is followed by the faster addition of HMDP [8,9]. Studies of single-turnover and burst kinetics, using quenched-flow methods to monitor AMP formation, demonstrated that the rate constant for product formation on the enzyme is rapid (16 s^{-1}), compared with the overall k_{cat} for the reaction (0.71 s^{-1}) [9]. The authors concluded that a slow step, possibly involving product departure from the enzyme, was limiting the overall rate of the reaction.

The preceding enzymological and structural studies have focused largely on the interaction of HPPK with substrates, rather than products. Here we show that the pterin-containing product of the HPPK reaction, DHPPP, binds with high affinity to the apoenzyme, in contrast with the substrate HMDP. Furthermore, binding is not associated with an increase in DHPPP fluorescence. This observation agrees with transient kinetic analysis of pterin fluorescence during the HPPK reaction, which declines after DHPPP is formed, but at a rate slower than formation of DHPPP itself. Therefore, the pyrophosphate moiety, which is the difference

Abbreviations used: HPPK, 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase; HMDP, 6-hydroxymethyl-7,8-dihydropterin; DHPPP, 6-hydroxymethyl-7,8-dihydropterin pyrophosphate; AMPCPP, α,β -methyleneadenosine triphosphate; HSQC, heteronuclear single-quantum coherence.

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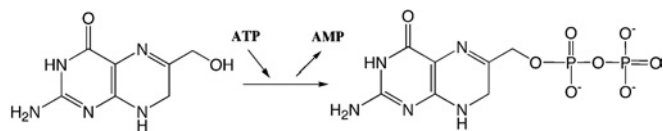


Figure 1 Reaction catalysed by HPPK

between DHPPP and HMDP, plays an important part in formation of the pterin binding site.

EXPERIMENTAL

Materials

ATP and AMP were purchased from Sigma. 6-hydroxymethyl pterin and 6-hydroxymethyl pterin pyrophosphate were obtained from Schirks Laboratories (Jona, Switzerland) and reduced using sodium dithionite [8] to form HMDP and DHPPP respectively.

Expression and purification of HPPK

For ease of purification, and subsequent analysis, it was found convenient to express *E. coli* HPPK with a poly-histidine tag so that the enzyme could be purified by metal chelate affinity chromatography. Purified plasmid pEH214 (1 μ g) [8] was digested with *Bam*H1 and *Nde*I restriction enzymes (Roche Diagnostics, Lewes, East Sussex, U.K.) and the product was ligated into the *Bam*H1 and *Nde*I sites of the pET16b expression vector (Novagen, Madison, WI, U.S.A.). This procedure effectively added the amino acid sequence MGHHHHHHHHSSGHIIEGRH to the N-terminus of the *E. coli* HPPK. For expression, the modified pET16b plasmid was transformed into *E. coli* BL21 (DE3) (Novagen): growth and induction of expression was carried out as described previously [8]. For the preparation of 15 N-labelled HPPK, growth was carried out in minimal medium containing [15 N]ammonium chloride (99% 15 N; Cambridge Isotope Laboratories, Andover, MA, U.S.A.) as sole nitrogen source. The cells were harvested by centrifugation at 3100 *g* for 10 min and the pellet was resuspended in 50 ml of 20 mM $\text{NaH}_2\text{PO}_4/\text{NaOH}$, 500 mM NaCl (pH 7.4). The cells were then lysed by sonication before centrifugation at 11300 *g* for 10 min to sediment non-soluble cellular debris. The supernatant was further cleared of particulates by passing through a 0.44 μm filter before application to a nickel-NTA affinity column (Qiagen), pre-equilibrated in 20 mM $\text{NaH}_2\text{PO}_4/\text{NaOH}$, 500 mM NaCl and 10 mM imidazole (pH 7.4). After washing with 5 column volumes of this buffer, HPPK was eluted with 3 column volumes of 20 mM $\text{NaH}_2\text{PO}_4/\text{NaOH}$, 500 mM NaCl and 500 mM imidazole (pH 7.4). Peak fractions were pooled and dialysed against three changes of 20 mM $\text{NaH}_2\text{PO}_4/\text{NaOH}$ buffer (pH 7.4). The protein was concentrated by ultrafiltration (Millipore) and stored at a working concentration of 10 mg/ml with the addition of 0.05% sodium azide, at 4 °C. The His-tagged HPPK had identical kinetic properties to the enzyme used in previous studies [8].

NMR spectroscopy

Samples of HPPK for NMR were prepared by gel filtration of the concentrated sample, prepared as described above, over a Superdex 200 FPLC column, equilibrated in 100 mM $\text{NaH}_2\text{PO}_4/\text{NaOH}$ (pH 7.4) plus 100 mM NaCl. Peak fractions of HPPK were pooled; MgCl_2 was added to a final concentration of 2.5 mM, and L-glutamic acid and L-arginine were added to final concentrations

of 50 mM each, which were found to enhance solubility. The samples were then concentrated by ultrafiltration, followed by a Centricon device (Amicon, Stonehouse, Gloucestershire, U.K.) to a final concentration of 0.6 mM. Dissolved oxygen was removed by bubbling with a gentle stream of oxygen-free nitrogen, before transfer of the sample to an anaerobic chamber (Belle Technology, Portesham, Dorset, U.K.) which maintained the oxygen level at less than 1.0 p.p.m. Additions of DHPPP and AMP were also carried out in the anaerobic chamber. The pH of the sample was monitored to ensure that it did not change on addition of DHPPP or AMP. The NMR spectra obtained were stable, indicating that this method was effective in excluding oxygen and hence oxidation of DHPPP. One-dimensional ^1H and two-dimensional [^1H , ^{15}N] HSQC (heteronuclear single-quantum coherence spectra) were acquired at 20 °C on a Bruker DRX600 NMR spectrometer. Free induction decay (acquisition time 0.256 s) was multiplied by square sine window function shifted by 72 degrees, and zero filled before Fourier transformation to 8 K and 2 K points for one-dimensional and two-dimensional spectra respectively. The identification of amino acid residues of HPPK involved in interactions with pterin and folate was carried out on separate pre-prepared samples of the HPPK-ligand complexes, by monitoring amide ^{15}N and ^1H chemical shift changes in the HSQC spectra at protein:ligand ratios of 1:4.

Calorimetry

Calorimetric measurements were carried out using a VP-ITC Isothermal Titrating Calorimeter (MicroCal, Maryland, U.S.A.). Solutions of DHPPP and HPPK were prepared by dilution into 25 mM Hepes/NaOH (pH 7.5) plus 2.5 mM MgCl_2 . Immediately prior to the start of each experimental run, the DHPPP and enzyme solutions were thoroughly de-gassed to avoid the formation of bubbles in the sample cell during measurement. All measurements were carried out at 25 °C. For each experimental run, an identical control experiment was carried out in the absence of enzyme. This measured the heat of dilution of DHPPP, which was later subtracted from the experimental data at each corresponding injection point to determine the heat response from DHPPP binding only. Data acquisition and processing were controlled by a PC workstation. Analysis of binding data was carried out using the Origin[®] software package supplied by the manufacturer (OriginLab, Northampton, MA, U.S.A.). A typical experiment was conducted as follows. The 1.4 ml calorimetry cell contained 15 μM HPPK in 25 μM Hepes/NaOH, 2.5 mM MgCl_2 (pH 7.5). Into this solution in the cell, 20 \times 10 μl aliquots of a 250 μM DHPPP stock solution were injected every 400 s. The energy required to maintain the temperature within the cell was measured relative to a reference cell filled with distilled water. The data were fitted to a model for single site binding using the Origin[®] data analysis suite.

Stopped-flow transient kinetics

Stopped-flow fluorescence measurements were carried out using an SX.18 MV spectrometer (Applied Photophysics, Leatherhead, Surrey, U.K.). The excitation wavelength was 330 nm and a 3.0 mm slit width was used. A cut-off filter at 400 nm was used in line with the emission photomultiplier. The temperature of the drive syringes and observation chamber were controlled by water circulation, and all experiments were carried out at 25 °C. The mixing ratio was 1:1 for all experiments. Solutions of ligands and enzyme were prepared by dilution into 25 mM Hepes/NaOH and 2.5 mM MgCl_2 (pH 7.5). Precautions were taken to maintain an anaerobic environment as described previously [8]. Data

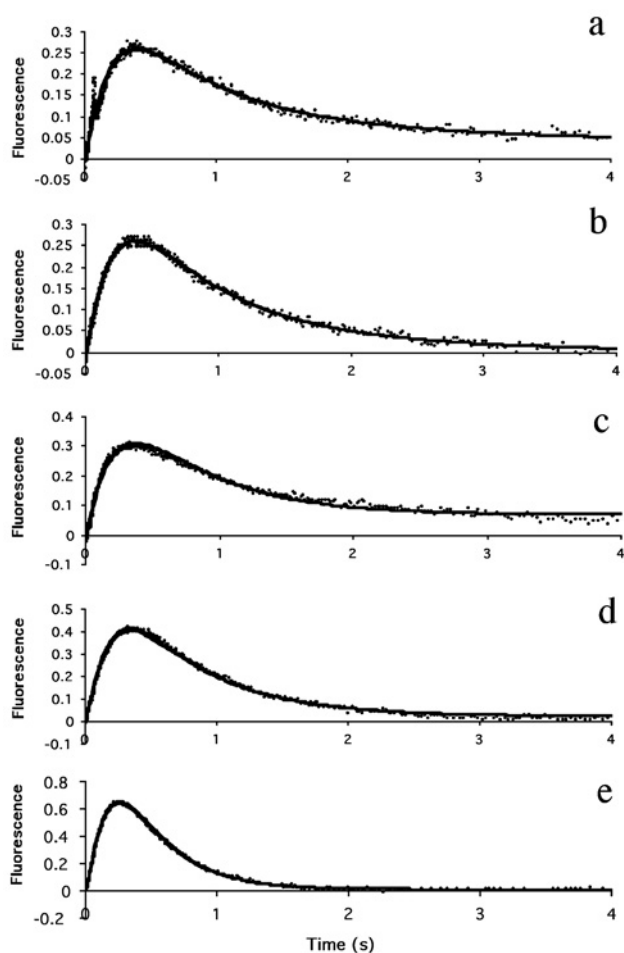


Figure 3 Transient kinetics of fluorescence change during HPPK-catalysed reaction

The concentration of HPPK was fixed at 25 μM , after mixing. The reaction was initiated by rapid mixing of HPPK in one syringe with various concentrations of ATP and HMDP in the second syringe. Concentrations of ATP and HMDP used, after mixing, were: (a) 1 μM ATP, 1 μM HMDP; (b) 1 μM ATP, 2 μM HMDP; (c) 1 μM ATP, 4 μM HMDP; (d) 2 μM ATP, 1 μM HMDP; (e) 4 μM ATP, 1 μM HMDP. The solid lines were obtained by global fitting using Dynafit [10] and show predicted behaviour for the kinetic model in Scheme 1. For details of the fitting parameters used, see Table 1.

Table 1 Kinetic constants of the HPPK reaction

Rate constant*	Value
k_1	$98\,300 \pm 2400 \text{ M}^{-1}\text{s}^{-1}$
k_{-1}	$17.9 \pm 0.4 \text{ s}^{-1}$
k_2^\dagger	$1.6 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$
k_{-2}^\dagger	0.92 s^{-1}
k_3	$4.2 \pm 0.1 \text{ s}^{-1}$

* Rate constants are for the kinetic scheme shown in Scheme 1.

† Values for k_2 and k_{-2} were determined by independent transient kinetic analysis of HMDP binding to the HPPK-AMPCPP binary complex, as described by Li et al. [9], but using the slightly different conditions given in the Experimental section to ensure consistency; k_2 and k_{-2} were then fixed during fitting of the data shown in Figure 3.

arbitrarily set at zero), confirming that the fluorescence enhancement seen on binding of HMDP is no longer present after formation of DHPPP.

NMR analysis of DHPPP binding

In order to obtain information on the nature of the DHPPP binding site, the product binary and ternary complexes with HPPK were analysed by NMR. HPPK was prepared with uniform ^{15}N incorporation and the two dimensional ^{15}N - ^1H HSQC spectrum recorded (Figure 4). Assignments for the HPPK ^{15}N and ^1H NMR spectra have been published previously [11] and, in principle, should be transferable to the spectra presented here. In practice we found that there were some differences which meant that not all cross peaks could be assigned with confidence. We observed more cross peaks than reported by Shi et al. [11]. Some residues on the long loop from Glu-77 to Leu-96 were missing from the previous assignment but may be detectable here due to the slightly different experimental conditions employed. It is also possible that some of the extra signals in our spectrum may originate from the 21-residue poly-histidine tag at the N-terminus of the protein. Nevertheless, there were a sufficient number of cross peaks in common to permit an analysis of a subset of defined residues within the protein where amide ^{15}N and ^1H chemical shifts were sensitive to the binding of DHPPP. Formation of the HPPK-DHPPP binary complex caused selective broadening of some of the cross peaks originating from these residues, indicating a change in the dynamics of the polypeptide chain in those regions (Figure 4a). For example, the cross peaks from Ala-57 and Val-58 were significantly weaker in the HPPK-DHPPP complex than with HPPK alone. In other cases, the new position of the shifted cross-peak was not apparent, either because it had broadened to the point where it was difficult to detect or had undergone large changes in the ^{15}N and ^1H chemical shifts. This was the case for Thr-42 and Asn-55, for example. The precise position of the resonances from these residues would require a reassignment of the HPPK-DHPPP complex. Nevertheless, an analysis of the residues where resonances were affected on ligand binding without making residue-specific assignments was sufficient to allow us to map these residues on to the crystal structures of the HPPK apoenzyme and the HPPK-AMPCPP-HMDP ternary complex (Figure 5). The 'localized' nature of the residues affected gave confidence that these are specific effects due to ligand binding. Residues Leu-54 to Val-58 lie on a β -strand that extends from the HMDP binding pocket. The side chain of Asn-55 forms two hydrogen bonds to the pterin ring, so it is conceivable that binding of DHPPP could alter the dynamics and environment of this part of the protein. Similarly, the side chain of Thr-42 also forms a hydrogen bond to the pterin ring, as well as to Asn-55. Tyr-40 is located on a second β -strand, aligned in an anti-parallel manner to Asn-55, and Ser-38 on the same strand lies immediately adjacent to Ala-56. Therefore the affected residues lie in a cluster which is linked to the HMDP-binding site, suggesting that this site is also used by DHPPP. Ile-98 is not connected to this group but is close to the ATP-binding site; its main chain atoms form two hydrogen bonds to the adenine ring [5]. It is not immediately apparent why this region of the enzyme would be affected by the binding of DHPPP, although it is clear from the comparison of the two spectra in Figure 4(a) that the effects of DHPPP binding are extensive.

The experiment was extended by addition of AMP to the HPPK-DHPPP binary complex, to form the product ternary complex (Figure 4b). The effects of AMP addition were more subtle than DHPPP addition but it was clear that binding of AMP led to a narrowing of the linewidths of several cross peaks in the HSQC spectrum which had previously broadened on addition of DHPPP. For example, the cross peaks of Ser-38 and Val-58 were sharper after addition of AMP. We conclude that the product ternary complex is very similar in structure to the HPPK-DHPPP binary complex.

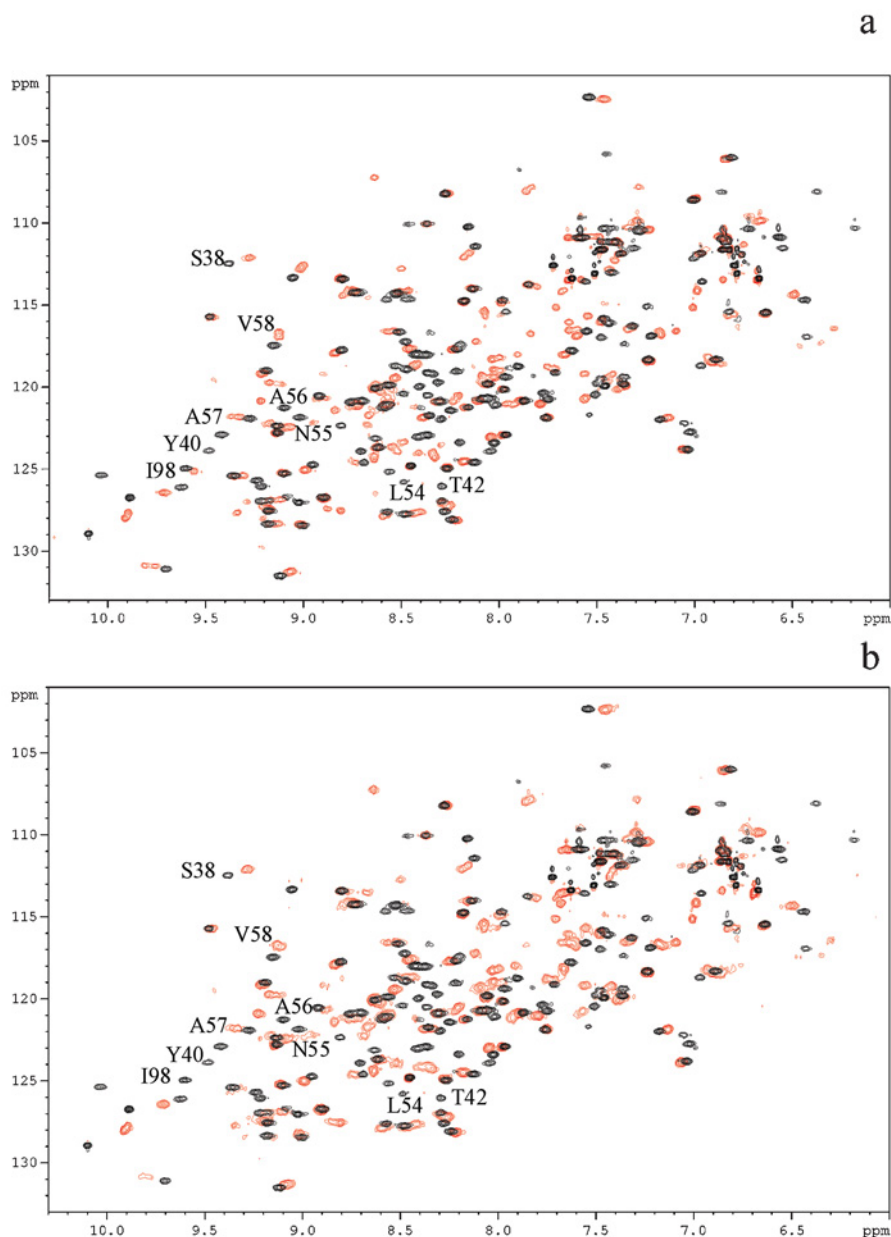


Figure 4 Two dimensional $^1\text{H}/^{15}\text{N}$ HSQC spectra of HPPK alone and in complex with DHPPP and AMP

(a) HPPK alone (black) and HPPK plus DHPPP (red), (b) HPPK alone (black) and HPPK plus DHPPP and AMP (red). Single-letter codes for amino acids have been used.

DISCUSSION

To date, structural and enzymological studies on HPPK have focused on the binding of substrates and substrate analogues. This previous work has revealed a structural rationale for the compulsory binding order of substrates: the binding of ATP induces changes in three loop regions which lead to formation of the HMDP binding site [3,5,12,13]. HMDP does not bind to the apoenzyme at low concentrations [8]. Furthermore, the comparatively slow rate constant for association of ATP with the apoenzyme suggests that this initial structural reorganization may be slow but that, once the HMDP site is formed, binding of HMDP is relatively rapid [8,9]. It is more difficult to rationalize the high affinity binding of DHPPP to the HPPK apoenzyme. How does

pterin recognition occur without the structural change induced by ATP? The lack of an enhancement of the pterin fluorescence on binding of DHPPP provides a clue. Enhancement of fluorescence of HMDP on binding to the HPPK-ATP or AMPCPP binary complexes is understandable, given that the environment of the pterin ring in the ternary complex is hydrophobic. The environment of the pterin ring in the HPPK-DHPPP complex may be much more hydrophilic. It is shown from the results of the transient kinetics studies above, that the fluorescence enhancement declines after formation of the product ternary complex at a rate of 4.2 s^{-1} ; this is slower than the rate of product formation (16 s^{-1}), but faster than the overall k_{cat} for the reaction (0.71 s^{-1}) [9]. We infer, therefore, that a structural transition occurs immediately after product formation, from a 'substrate-like' complex to a

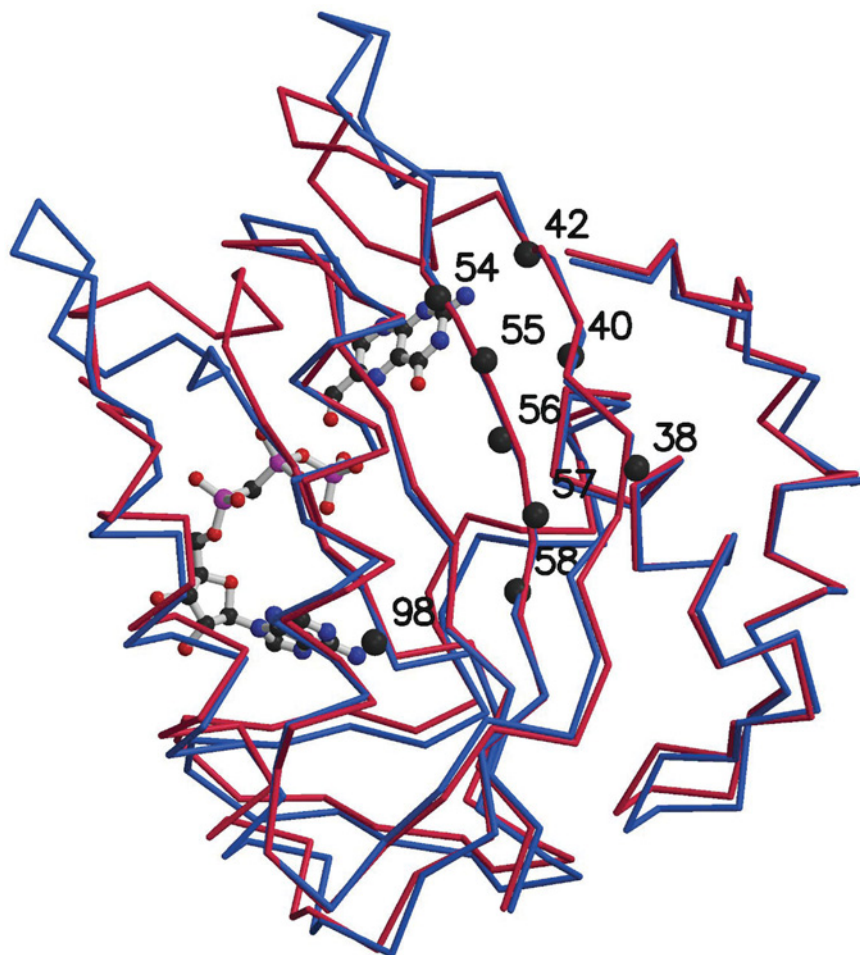


Figure 5 View of HPPK backbone structures with location of residues perturbed by DHPPP binding

HPPK apoenzyme structure is shown in blue (Protein Database code 1HKA) and the HPPK-AMPCPP-HMDP ternary complex in red (Protein Data Bank code 1EQO), with both ligands shown. The positions of amide N atoms of residues that specifically broaden in the two-dimensional HSQC $^1\text{H}/^{15}\text{N}$ spectrum on binding of DHPPP are marked, superimposed on the apoenzyme structure. The figure was generated using MOLSCRIPT [14].

‘product-like’ complex. The rate of this transition would appear to be slower than the rate of product formation but faster than the k_{cat} for the reaction.

The NMR experiments provided further information about the structure and dynamics of the HPPK-DHPPP binary complex. Titration of DHPPP into HPPK apoprotein produced a complex in which a number of cross peaks in the HSQC spectrum have selectively broadened or disappeared altogether. The most likely explanation is a specific exchange-broadening effect, caused by alterations in the local dynamics of particular segments of the polypeptide chain. It was not possible to assign all cross peaks that were perturbed but it was clear that the HPPK-DHPPP binary complex was more dynamic than the apoenzyme. Some of the perturbed residues mapped in a cluster to one end of the pterin binding site. We presume that the presence of the pyrophosphate moiety in DHPPP contributes to the much higher affinity of DHPPP for HPPK compared with HMDP.

The observation that DHPPP can bind to HPPK with high affinity has important ramifications for the order of departure of products from the enzyme. A random order of product departure needs to be considered; the kinetic model for HPPK proposed by Li et al. [9] subsumed the dissociation of the products from the

enzyme into a single rate constant, k_4 , with a measured value of 1.8 s^{-1} . One or more of these steps appears to be rate-limiting for the reaction and the high affinity of the enzyme for DHPPP suggests that departure of this product could be the rate-limiting step.

The results reported here suggest that the HPPK-DHPPP binary complex differs in several important respects from the substrate ternary complex. This observation has implications for the design of inhibitors of the enzyme: an alternative approach could target inhibitor design against the product-bound form of the enzyme, rather than its substrate-bound forms. In particular, the results suggest that the binding site for the pterin moiety is substantially altered, opening up the possibility that other ligands could be designed to bind to this site.

We thank the Medical Research Council and PanTherix Ltd for financial support, and Dr Alexander Golovanov for assistance in collection of NMR data.

REFERENCES

- Bermingham, A. and Derrick, J. P. (2002) The folic acid biosynthesis pathway in bacteria: evaluation of potential for antibacterial drug discovery. *Bioessays* **24**, 637–648

- 2 Hennig, M., Dale, G. E., D'Arcy, A., Danel, F., Fischer, S., Gray, C. P., Jolidon, S., Muller, F., Page, M. G. P., Pattison, P. and Oefner, C. (1999) The structure and function of the 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase from *Haemophilus influenzae*. *J. Mol. Biol.* **287**, 211–219
- 3 Stammers, D. K., Achari, A., Somers, D. O., Bryant, P. K., Rosemond, J., Scott, D. L. and Champness, J. N. (1999) 2.0 Å X-ray structure of the ternary complex of 7,8-dihydro-6-hydroxymethylpterinpyrophosphokinase from *Escherichia coli* with ATP and a substrate analogue. *FEBS Lett.* **456**, 49–53
- 4 Xiao, B., Shi, G. B., Chen, X., Yan, H. G. and Ji, X. H. (1999) Crystal structure of 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase, a potential target for the development of novel antimicrobial agents. *Structure* **7**, 489–496
- 5 Blaszczyk, J., Shi, G., Yan, H. and Ji, X. (2000) Catalytic center assembly of HPPK as revealed by the crystal structure of a ternary complex at 1.25 Å resolution. *Structure* **8**, 1049–1058
- 6 Blaszczyk, J., Li, Y., Shi, G. B., Yan, H. G. and Ji, X. H. (2003) Dynamic roles of arginine residues 82 and 92 of *Escherichia coli* 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase: crystallographic studies. *Biochemistry* **42**, 1573–1580
- 7 Li, Y., Wu, Y., Blaszczyk, J., Ji, X. H. and Yan, H. G. (2003) Catalytic roles of arginine residues 82 and 92 of *Escherichia coli* 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase: site-directed mutagenesis and biochemical studies. *Biochemistry* **42**, 1581–1588
- 8 Bermingham, A., Bottomley, J. R., Primrose, W. U. and Derrick, J. P. (2000) Equilibrium and kinetic studies of substrate binding to 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase from *Escherichia coli*. *J. Biol. Chem.* **275**, 17962–17967
- 9 Li, Y., Gong, Y. C., Shi, G. B., Blaszczyk, J., Ji, X. H. and Yan, H. G. (2002) Chemical transformation is not rate-limiting in the reaction catalyzed by *Escherichia coli* 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase. *Biochemistry* **41**, 8777–8783
- 10 Kuzmic, P. (1996) Program DYNFIT for the analysis of enzyme kinetic data: application to HIV proteinase. *Anal. Biochem.* **237**, 260–273
- 11 Shi, G. B., Gao, J. H. and Yan, H. G. (1999) H-1, C-13 and N-15 resonance assignments of *Escherichia coli* 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase and its complex with MgAMPPCP. *J. Biomol. NMR* **14**, 189–190
- 12 Xiao, B., Shi, G. B., Gao, J. H., Blaszczyk, J., Liu, Q., Ji, X. H. and Yan, H. G. (2001) Unusual conformational changes in 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase as revealed by X-ray crystallography and NMR. *J. Biol. Chem.* **276**, 40274–40281
- 13 Yan, H. G., Blaszczyk, J., Xiao, B., Shi, G. B. and Ji, X. H. (2001) Structure and dynamics of 6-hydroxymethyl-6,8-dihydropterin pyrophosphokinase. *J. Mol. Graph.* **19**, 70–77
- 14 Kraulis, P. J. (1991) MOLSCRIPT: a program to produce both detailed and schematic plots of protein structures. *J. Appl. Crystallog.* **24**, 946–950

Received 2 December 2003/2 March 2004; accepted 12 March 2004

Published as BJ Immediate Publication 12 March 2004, DOI 10.1042/BJ20031850