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Minireview

The catalytic mechanism of protein tyrosine phosphatases revisited

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Abstract Experimental and theoretical studies of the catalytic mechanism in protein tyrosine phosphatases and dual specific phosphatases are reviewed. The structural properties of these enzymes contributing to the efficient rate enhancement of phosphate monoester hydrolysis have been established during the last decade. There are, however, uncertainties in the interpretation of available experimental data that make the commonly assumed reaction mechanism somewhat doubtful. Theoretical calculations as well as analysis of crystal structures point towards an alternative interpretation of the ionisation state in the reactive complex.

Key words: Protein tyrosine phosphatase; Catalytic mechanism; pH rate profile

1. Introduction

Phosphorylation of tyrosine residues in proteins is an abundant mechanism that regulates fundamental biochemical processes in the cell such as cell growth, differentiation and proliferation [1–3]. The protein tyrosine kinases (PTKs) phosphorylate specific tyrosine residues in proteins using ATP as the phosphate source. The counteracting protein tyrosine phosphatases (PTPs) hydrolyse the phosphotyrosines yielding the restored amino acid residue and inorganic phosphate as products. The opposing activity of these enzyme families ensures a correct level of protein phosphorylation in the cell. Due to their role in cellular signalling and potential as new pharmaceutical targets the PTPs have been the focus of much research during the last decade.

The PTPs can be classified into four families: (i) the main group of tyrosine specific phosphatases, (ii) the VH1 related dual specific phosphatases (DSP), (iii) the low molecular weight PTPs (LMPTPs) and (iv) the dual specific cdc25 phosphatases. The main group of about 100 tyrosine specific phosphatases includes both membrane bound receptors with intracellular catalytic domains as well as cytosolic PTPs. The most studied enzymes of this family are the human PTP1B and *Yersinia* PTP and their three-dimensional structures were determined at an early stage [4,5]. VHR, a human *vaccinia* H1-related DSP is a phosphatase that can hydrolyse pSer and pThr in addition to pTyr. VHR is structurally related to the PTPs [6]. The LMPTPs are not homologous to the tyrosine specific PTPs, but share the same active site structure and catalytic mechanism [7,8]. In this family both mammalian and yeast LMPTPs are well characterised. The dual specific cdc25 phosphatases have yet another origin but possess many of the characteristic features of the PTPs [9,10]. Although sequentially and topologically different, all enzymes in the PTP and DSP families share a common active site structure, which indicates that they use the same mechanism for catalysis.

The identification of important catalytic residues and their function has been established from a wealth of experiments including enzyme kinetics, site directed mutagenesis and structure determination. Hence, the basis for catalysis is well known, but there are some uncertainties regarding the interpretation of the available data and several contradictions are found in the literature. In particular the pK_as and ionisation states of the catalytic groups are not well established. These issues have also been the subject of computational studies aimed at a more detailed picture of phosphate hydrolysis by the PTPs.

2. Structural basis for catalysis

The characteristic sequence (H/V)CX_5R(S/T) is the common motif found in all PTPs. (One exception is the cdc25 phosphatases lacking the hydroxyl containing residue just after the arginine.) The sequence constitutes the phosphate binding loop, or so-called P-loop, where the main chain nitrogens and the guanidinium group of the arginine residue are oriented as to coordinate the equatorial oxygens of the phosphate group during substrate binding and catalysis (Fig. 1). The geometry of the P-loop provides a perfect complementary structure to the two transition states of the reaction and the bidentate interaction provided by the arginine side chain is essential for catalysis [11,12].

The PTPs utilise a two step reaction for phosphate monoester hydrolysis (Scheme 1). The first step is initiated by a nucleophilic attack of the active site cysteine on the phosphorus atom of the bound substrate. At the same time as the ester bond is cleaved, a well positioned general acid residue donates its proton to the leaving group oxygen. This first substitution reaction leaves the phosphate group covalently attached to the nucleophile via a thioester linkage [13,14]. The phosphoenzyme intermediate is hydrolysed by a water molecule in a second displacement reaction, yielding the restored enzyme and inorganic phosphate. The water molecule is activated by

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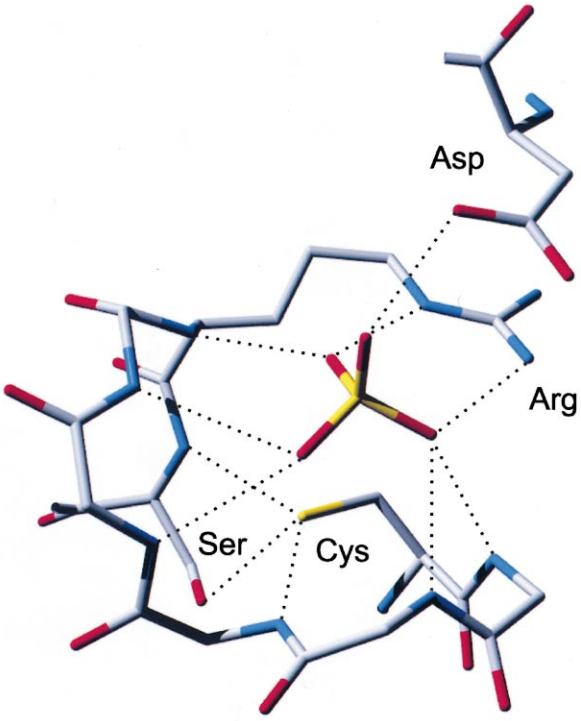


Fig. 1. The structure of the characteristic sequence motif C–X_5–R–(S/T) and the general acid in a typical PTP (bovine LMPTP in complex with sulfate [7]). The side chains of the five residues between Cys and Arg are omitted for clarity. Hydrogen bonds stabilising the nucleophile and the anion are indicated by dotted lines.

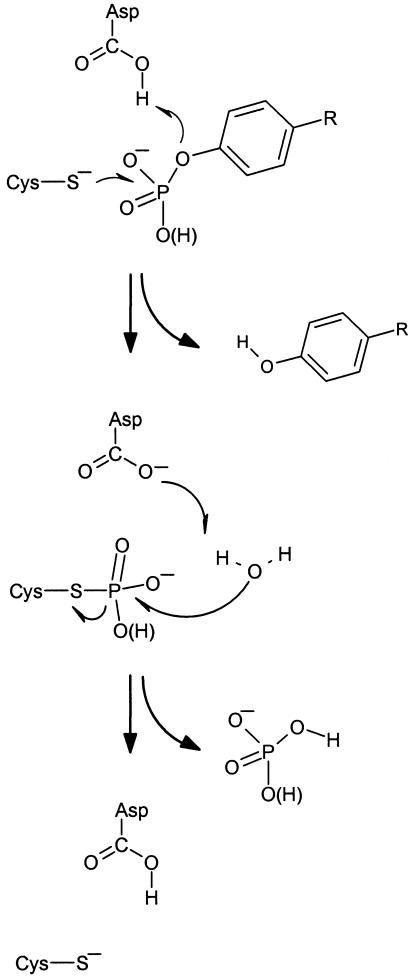
a general base, which is the same residue that acted as a general acid in the first step.

The normal pK_a of 8.3 makes cysteine residues relatively good nucleophiles. In PTPs it is found that among other interactions the hydroxyl group of the serine or threonine residue in the signature motif is important for stabilising the thiolate form of the active site cysteine [15,16] resulting in an even lower pK_a. The fact that the P-loop is found at the N-terminus of an α-helix should also contribute to thiolate stabilisation. The histidine residue just before the nucleophile in sequence is not in close contact with the sulfur, but still has a considerable effect on the pK_a as found by site directed mutagenesis [17]. The valine residue found instead of the histidine in the LMPTPs may explain the higher pK_a of the nucleophile in these enzymes.

The general acid, usually an aspartic acid, is important for catalysis and mutation of this residue leads to impaired turnover rate [18–22]. The general acid is positioned on a more or less flexible loop that folds over the active site as the Michaelis complex is formed. The aspartic acid is believed to be protonated in the complex since it is found at hydrogen bond distance to the apical oxygen of the ligands in the crystal structures. This is consistent with its role as a proton donor in the reaction and requires an increased pK_a compared to a free aspartic acid. An increased pK_a is also of advantage in the second and rate limiting reaction step where it acts as a general base.

The cdc25 phosphatases do not seem to have a corresponding general acid residue well positioned for donating a proton to the leaving group. There are nevertheless two glutamic acids in the phosphate binding loop, but these can be mutated without dramatic decrease in activity [23,24]. Chen et al. showed that cdc25 has a much higher turnover rate for its natural substrate than for the small arylphosphates often used in the assays and proposed that the general acid is instead provided by the substrate protein, cdk2-pTpY/CycA [24]. Using the natural substrate the k_{cat}/K_M pH rate profiles also showed a typical slope of −1 on the basic side, often assigned to general acid catalysis. It is also noteworthy that the necessary conformational change for catalysis in cdc25 was correctly predicted by MD simulations [25] as judged from a recent crystal structure [26].

In the second step of the reaction, which involves hydrolysis of the phosphoenzyme, a water molecule acts as the nucleophile. The aspartate activates the water by abstracting one of its hydrogens. The water molecule needs to be perfectly positioned in the active site for efficient hydrolysis. In PTP1B and *Yersinia* PTP a glutamine residue is found to be important for coordinating the hydrolytic water [14,27]. Mutation of this residue leads to accumulation of the phosphoenzyme intermediate and the Q262A mutant of PTP1B even made it possible to isolate the intermediate and to solve its crystal structure [14]. In LMPTP the corresponding water coordinating residue is proposed to be the cysteine adjacent to the arginine in the active site motif [28,29].



Scheme 1. The reaction mechanism catalysed by the PTPs. The two proposed ionisation states of the substrate are indicated.

3. pK_a of the nucleophilic cysteine

A network of hydrogen bonds stabilises negative charges in the active site. The resulting Cys pK_a has been measured by different techniques in some of the most studied PTPs. The most straightforward method is to measure the inactivation of the enzymes by iodoacetate or iodacetamide at different pH. The reported pK_as from such experiments are 4.67 in *Yersinia* PTP [17], 5.57 in PTP1 [20], 6.75–7.52 in bovine liver LMPTP [30] and 8.3 in yeast LMPTP [31]. Thus, the catalytic cysteines of the tyrosine specific PTPs are more acidic than a free cysteine and are therefore proposed to be ionised in the free enzymes at physiological pH (or even at the pH optimum of 5–6). However, the corresponding residue of the LMPTPs appears to be less acidic.

Another often employed method to estimate the pK_a of important ionisable groups is the use of pH rate profiles. The pH dependence of k_{cat}/K_M describes the ionisation of the free enzyme and substrate, whereas k_{cat} vs pH usually (and for PTPs) refers to the Michaelis complex. In one case (PTP1) the pK_a value assigned to the cysteine obtained from pH rate profiles agrees well with that from inactivation experiments [20]. However, in the case of bovine LMPTP the interpretations of pH rate profiles do not agree with the inactivation experiments. Evans et al. [15] assigned a pK_a below 4 to Cys12, which is more than three pK_a units lower than the value obtained from inactivation by iodoacetate.

The pK_a of the catalytic cysteine has also been addressed in several computational studies [16,32,33], e.g. by Poission–Boltzmann calculations [32,33]. One drawback with this type of method is that the results depend on the macroscopic dielectric constant, which is difficult to estimate for a protein interior (protein relaxation is usually also neglected). Peters et al. reported calculated pK_as for the catalytic cysteines in a number of different unliganded PTP structures [32]. They reproduced the experimental values using a relatively high dielectric constant of 20. For LMPTP the calculated value agreed well with the value obtained from inactivation experiments, but contradicted the extremely low value (<4) suggested by Evans et al. [15]. A less successful attempt to calculate the pK_a of the cysteine and the general base was reported by Dillet et al. [33]. Using a low protein dielectric constant of 4 they obtained calculated pK_as below -4 for the cysteine in most cases and suggested that this provided clear evidence for the presence of an ionised nucleophile. Hence, in the case of PTP1B and *Yersinia* the errors in the calculations are clearly on the order of 10 pK_a units, which makes them highly unreliable. Also in the case of LMPTP the extremely low pK_a of -2.4 reported in [33] disagrees significantly with the experimental value of around 7, as well as with the calculations in [16,32].

In summary, it seems well established both by experiments and computational methods that the nucleophilic cysteines in the tyrosine specific phosphatases have relatively low pK_as in the free enzyme. In LMPTP most data suggest that the pK_a is somewhat higher, but still below that of a free cysteine. However, it should be noted that the PTPs have optimal activity at low pH (5–6) and it is in this pH region where most experiments are done and mechanistic interpretations made. The fact that several active site groups (including the substrate) are likely to ionise near the pH optimum makes it more difficult to evaluate the ionisation state of the Michaelis complex where the cysteine pK_a also will be affected by the presence of a negatively charged substrate.

4. Ionisation state of the substrate

Recent papers in the PTP field usually emphasise the importance of the ionised state of the catalytic cysteine. However, the ionisation state of the bound substrate is just as important for the reaction mechanism. Given an ionised nucleophile, the substrate could bind either with a completely ionised dianionic phosphate group or with the phosphate in its protonated monoanionic form. The possible existence of a proton in the enzyme–substrate complex is subject to some debate. A general opinion seems to be that the substrate is fully deprotonated in the reaction despite the low pH optimum. This model is based on interpretations of k_{cat}/K_M vs pH rate profiles and means that the negatively charged cysteine would attack the dianionic phosphate group of the substrate. Hansson et al. [16] challenged this model using the basic electrostatic argument that a fully deprotonated substrate does not like to interact with an ionised nucleophile. They suggested that if the nucleophile is ionised the substrate will bind as the monoanion. On the other hand, if the nucleophile is in its neutral form the substrate can bind as a dianion. The nucleophile could then be activated by proton transfer to the substrate. Calculations showed that such an activation is catalysed by the protein environment [16].

The improbability of binding a dianion, as opposed to a monoanion, in the active site carrying an ionised nucleophile was further shown by Kolmodin et al. using binding free energy calculations [34]. They also reproduced the energetics of the whole reaction pathway for both the wild type and D128A mutant LMPTP using a monoanionic substrate [29]. Furthermore, the effect of the C17S mutation on the rate limiting step was correctly predicted in this study. Molecular dynamics simulations were also used to examine structural differences between the complexes formed with monoanionic and dianionic substrates in LMPTP with an ionised cysteine. It was then found that the agreement with crystallographic data was significantly better with a protonated substrate [34]. Alhambra et al. [35] performed QM/MM simulations of the first reaction step in LMPTP using both a monoanionic and a dianionic substrate. They obtained activation barriers of approximately equal height for both mechanisms. However, the energetics of substrate binding was not considered in that work. The empirical valence bond calculations of [34] also yielded very similar barriers, but a significant destabilisation of the enzyme–substrate complex for the fully ionised case. Hart et al. have also questioned the fully deprotonated enzyme–substrate complex based on quantum chemical calculations [36].

5. Interpretation of pH rate profiles

The pH dependence of enzyme activity can yield useful information on ionisation processes of enzyme groups that are necessary for catalysis. However, in the case of PTPs there are considerable ambiguities in the interpretation of such pH rate profiles [15,21,37,38] and a unique solution does not appear obvious. A typical pH rate profile obtained for the tyrosine specific phosphatases is bell shaped with a pH optimum at pH 5–6. Plotting k_{cat}/K_M against pH an ascending slope of

+2 is observed on the acidic side of the pH optimum. A descending slope of −1 is seen on the alkaline side. This indicates that two ionisable groups need to be deprotonated and one group needs to be protonated in the free enzyme for optimal activity (binding and catalysis). The corresponding curves for k_{cat} vs pH usually show an ascending slope of +1 and a descending slope of −1, suggesting that one group should be deprotonated and another one protonated in the enzyme–substrate complex.

It should be emphasised that identification of the three possible ionisable groups on the enzyme and substrate is not so straightforward. One reason for this is that both the substrate, the nucleophile and the general acid seem to have their pK_as in a rather narrow range. In addition, structurally important amino acids not directly involved in catalysis affect the slopes of the profiles [21]. Thus, there is more than one possible assignment of the ionisation state in the free enzyme and in the Michaelis complex. The fact that the reaction is a two step reaction may also complicate the situation. In earlier work the descending slope on the alkaline side was said to result from the deprotonation of the substrate, since the pK_a given by the negative slope precisely coincided with the pK_a of different substrates [37,38]. On the other hand, this ionisation has also been interpreted as the deprotonation of the general acid residue [20–22]. Since k_{cat}/K_M refers to the free enzyme and substrate and the rate limiting step has been reported as the breakdown of the phosphoenzyme intermediate [38], it is difficult to see how the proposed ionisation of the general acid (in the free enzyme) could show a substrate dependence.

Mutant PTPs with the general acid residue replaced by alanine show a pH independent k_{cat} above pH 5 [20–22], which suggests that the descending slope, at least in the k_{cat} profile, originates from the general acid. However, if such a mutation leads to changes of the overall energetics, k_{cat} of the mutant and the wild type may not be directly comparable. For example, mutation of the general acid in LMPTP results in accumulation of the intermediate [18], which means that the energy of this state becomes lower than that of the enzyme–substrate complex. k_{cat} will then be measured relative to the intermediate where the catalytic groups may have different protonation states than in the Michaelis complex. Consequently, one should be careful when drawing conclusions about the wild type mechanism from mutant enzymes.

The origin of the ascending slope is not entirely obvious either. A common interpretation of the +2 slope for k_{cat}/K_M is that both the nucleophile and the substrate need to be fully ionised. However, as shown for *Yersinia* PTP other residues than those directly involved in the reaction may affect the ascending slope. Mutation of E290 in *Yersinia* PTP made the slope vanish in the k_{cat} vs pH curve, which was initially interpreted such that E290 would function as a general base [21]. When the structure of the enzyme was solved this proposed function could be ruled out. Instead, E290 forms an ion pair with the arginine in the P-loop, a structural motif found in all PTPs and DSPs. How mutations of the corresponding residues in other PTPs and DSPs affect the pH dependence is not well established, but the ion pair with the P-loop arginine is found to be important for substrate binding in LMPTP [39]. The other ionisation showing up on the acidic side of k_{cat}/K_M pH rate profiles seems likely to reflect deprotonation of the cysteine, but it has also been ascribed to the substrate. Regarding the k_{cat} vs pH profile in *Yersinia* an alternative interpretation consistent with experiments would be the following: E290 is the residue responsible for the acidic limb while the general acid is that visible on the basic side, implying that neither of the ionisations of the Cys–substrate complex show up in the k_{cat} profile. This would be in line with the general expectation that the two pK_as of the Cys–substrate moiety are likely to be shifted in opposite directions due to the electrostatic interaction. This is hence the regular effect observed on ionisation equilibria due to electrostatic interactions [40] which is, e.g. reflected in aspartic proteases by the proton shared between the two catalytic Asps [41]. At any rate, the notion that the pK_a of the catalytic cysteine would

Table 1
Summary of crystallised PTP–ligand complexes and the possible number of negative charges on the residue in position of the nucleophile and the functional group bound in the active site

Enzyme	Residue in position of the nucleophile	P-loop binding group of the ligand	Possible number of negative charges			Reference
LMPTP	Cys	SO_4		2	3	[7]
	Cys	SO_3-R	1	2		[42,46]
	Cys	PO_4	1	2	3	[47]
	Ala	PO_4-R	1	2		[46]
	Cys	-VO_4		2		[43]
PTP1B	Cys	PO_3-R	1	2	3	[48]
	Cys	malonate		2	3	[48]
	Cys	oxalate	1	2		[49]
	Cys	WO_4		2	3	[4]
	Cys	-VO_4		2		[14]
	Ser	PO_4-R	1	2		[50]
	Ala	PO_4-R	1	2		[51]
Yersinia	Cys	NO_3	1	2		[52]
	Cys	WO_4		2	3	[5,52]
	Ser	SO_4		2		[53]
VHR	Cys	SO_3-R	1	2		[6]
	Cys	SO_4		2	3	[6]
	Ser	PO_4-R	1	2		[54]
cdc25	Cys	SO_4		2	3	[26]
	Cys	WO_4		2	3	[26]

The cysteine is considered to be either ionised or neutral. Different ionisation states of the ligands (in the relevant pH range) are also taken into account.

be unaffected by the insertion of a doubly negatively charged phosphate group at van der Waals distance, or vice versa, is highly counterintuitive.

The pH rate profiles of LMPTP differ from the tyrosine specific PTPs by showing pH independence below pH 5 [15,37]. This has been interpreted as an ionised cysteine with such a low pK_a (<4) that it is not visible in the plots [15]. It should also be noted that the ascending slope often assigned to the ionisation of the substrate is not visible at all in the pH rate profiles for LMPTP [15,37]. Since no ionisation on the acidic side of the k_{cat}/K_M profile is observed near the substrate pK_a in this case the conclusion that the substrate needs to be deprotonated for catalysis is not supported by the experiments [15].

The k_{cat} profile is perhaps more informative for the catalytic mechanism since it directly refers to the ionisation state of the Michaelis complex. Ionisation in the k_{cat}/K_M profile may reflect, e.g. groups that are involved in conformational changes required for substrate binding. At any rate, it is obvious that the pH rate profiles alone do not provide sufficient information to allow unique assignment of the protonation states of the active site groups in this case, in particular since there are at least four possible ionisable groups that may be involved. To determine the protonation state of the reacting groups we need to study data from other types of experiments.

6. Structures of substrate and transition state analogues

There are a large number of crystal structures of inhibited PTPs available today. They are either inactive mutants in complex with substrates or wild type enzymes in complex with various phosphate-like inhibitors. X-ray crystallography does not allow us to directly determine the number of protons in the enzyme–ligand complex, but the side chain at the position of the nucleophile typically shows van der Waals contact distance to the ligand oxygens. In addition, the PTPs have close to identical binding conformations and the active site structures can be almost perfectly superimposed. Therefore, it seems reasonable to expect that they share the same overall charge state on the active site groups. The only possibility to accomplish this is a common charge state of -2 (Table 1). For example, in the structures inhibited by HEPES (containing a sulfonate group) [6,42] a total charge of -3 is not possible. Yet, these structures are referred to as substrate analogues in studies advocating the fully ionised (-3) type of mechanism [33]

In this context it is also interesting to note the reported crystal structures of PTPs in complex with vanadate mimicking the transition state [14,43]. These structures show a typical trigonal bipyramidal conformation with the vanadate covalently linked to the catalytic cysteine. Since vanadate at the given concentration exists as $H_2VO_4^-$ in solution between pH 4 and 8.3 [44], it is most likely that the observed complex corresponds to (Cys)S–$VO_4H_2^{2-}$. Thus, the P-loop appears to be optimally designed for stabilisation of two negative charges and there is no indication of an altered charge state in the active Michaelis complex. It seems reasonable to conclude that the various PTP crystallographic complexes are good models of enzyme–substrate complexes and transition state, not only in terms of structure but also with respect to the overall ionisation state.

It has sometimes been argued that ^{18}O kinetic isotope effects (KIEs) for the non-bridge phosphate oxygens show that the reacting groups are fully ionised [45]. However, the reported isotope effects were already corrected according to the assumption that the reacting groups are indeed ionised and the small resulting value of the KIE cannot be used to validate the assumption. As noted in [34], if the observed KIE had instead been corrected according to a mechanism with a protonated substrate the resulting value of the KIE would be very similar to that observed for hydrolysis of monoanionic *p*-nitrophenyl phosphate in solution. It is also well known that protonated phosphate monoesters hydrolyse much faster than the unprotonated species in absence of catalysts. In fact, hydrolysis of dianionic phosphates by a negative nucleophile is never observed in solution. In this context it is interesting to note that the inhibition studies of [8] showed that inorganic phosphate binds three times better to LMPTP at pH 5 than at 7.5, which exactly correlates with the respective fractions of the monoanion.

7. Conclusions

The reaction mechanism used by the PTPs and DSPs has been re-examined. We have found that the general model of the mechanism with an ionised nucleophile and a dianionic phosphate group on the substrate may be questioned on the basis of available data. The model is mainly based on pH rate profiles, which do not have a unique interpretation. It is neither supported by structural data, since available crystal structures indicate that only two negative charges are preferred in the active site. Furthermore, none of the recent theoretical studies of the PTPs have supplied evidence for this ionisation state. An alternative model would be the case where one proton is present on the nucleophile and the substrate in the Michaelis complex. Depending on the relative pK_as of these groups the hydrogen would either be bound to the sulfur or to one of the equatorial oxygens of the phosphate group when the complex is formed. The electrostatic interaction would be expected to cause the pK_a associated with loss of the single proton from the complex to go up, while that corresponding to uptake of an additional proton would be significantly shifted downwards. In fact, both theoretical studies as well as analysis of crystal structures support such a mechanistic model without being in conflict with enzymological data.

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References

[1] Hunter, T. (1989) Cell 58, 1013–1016.
[2] Fischer, E.H., Charbonneau, H. and Tonks, N.K. (1991) Science 253, 401–406.
[3] Barford, D., Jia, Z. and Tonks, N.K. (1995) Nat. Struct. Biol. 2, 1043–1053.
[4] Barford, D., Flint, A.J. and Tonks, N.K. (1994) Science 263, 1397–1404.
[5] Stuckey, J.A., Schubert, H.L., Fauman, E.B., Zhang, Z.-Y. and Dixon, J.E. (1994) Nature 370, 571–575.
[6] Yuvaniyama, J., Denu, J.M., Dixon, J.E. and Saper, M.A. (1996) Science 272, 1328–1331.
[7] Su, X.-D., Taddei, N., Stefani, M., Ramponi, G. and Nordlund, P. (1994) Nature 370, 575–578.
[8] Zhang, M., Van Etten, R.L. and Stauffacher, C.V. (1994) Biochemistry 33, 11087–11096.

[9] Fauman, E.B., Cogswell, J.P., Lovejoy, B., Rocque, W.J., Holmes, W., Montana, V.G., Piwnica-Worms, H., Rink, M.J. and Saper, M.A. (1998) Cell 93, 617–625.
[10] Reynolds, R.A., Yem, A.W., Wolfe, C.L., Deibel, M.R., Chidester, C.G. and Watenpaugh, K.D. (1999) J. Mol. Biol. 293, 559–568.
[11] Cirri, P., Chiarugi, P., Camici, G., Manao, G., Raugei, G., Cappugi, G. and Ramponi, G. (1993) Eur. J. Biochem. 214, 647–657.
[12] Hoff, R.H., Wu, L., Zhou, B., Zhang, Z.-Y. and Hengge, A.C. (1999) J. Am. Chem. Soc. 121, 9514–9521.
[13] Guan, K.-L. and Dixon, J.E. (1991) J. Biol. Chem. 266, 17026–17030.
[14] Pannifer, A.D.B., Flint, A.J., Tonks, N.K. and Barford, D. (1998) J. Biol. Chem. 273, 10454–10462.
[15] Evans, B., Tischmack, P.A., Pokalsky, C., Zhang, M. and Van Etten, R.L. (1996) Biochemistry 35, 13609–13617.
[16] Hansson, T., Nordlund, P. and Åqvist, J. (1997) J. Mol. Biol. 265, 118–127.
[17] Zhang, Z.-Y. and Dixon, J.E. (1993) Biochemistry 32, 9340–9345.
[18] Taddei, N., Chiarugi, P., Cirri, P., Fiaschi, T., Stefani, M., Camici, G., Raugei, G. and Ramponi, G. (1994) FEBS Lett. 350, 328–332.
[19] Wu, L. and Zhang, Z.-Y. (1996) Biochemistry 35, 5426–5434.
[20] Lohse, D.L., Denu, J.M., Santoro, N. and Dixon, J.E. (1997) Biochemistry 36, 4568–6575.
[21] Zhang, Z.-Y., Wang, Y. and Dixon, J.E. (1994) Proc. Natl. Acad. Sci. USA 91, 1624–1627.
[22] Denu, J.M., Zhou, G., Guo, Y. and Dixon, J.E. (1995) Biochemistry 34, 3396–3403.
[23] Xu, X. and Plisinski Burk, S. (1996) J. Biol. Chem. 271, 5118–5124.
[24] Chen, W., Wilborn, M. and Rudolph, J. (2000) Biochemistry 39, 10781–10789.
[25] Kolmodin, K. and Åqvist, J. (2000) FEBS Lett. 465, 8–11.
[26] Reynolds, R.A., Yem, A.W., Wolfe, C.L., Deibel Jr., M.R., Chidester, C.G. and Watenpaugh, K.D. (1999) J. Mol. Biol. 293, 559–568.
[27] Zhao, Y., Wu, L., Noh, S.J., Guan, K.-L. and Zhang, Z.-Y. (1998) J. Biol. Chem. 273, 5484–5492.
[28] Cirri, P., Chiarugi, P., Camici, G., Manao, G., Raugei, G., Cappugi, G. and Ramponi, G. (1993) Eur. J. Biochem. 214, 647–657.
[29] Kolmodin, K. and Åqvist, J. (1999) FEBS Lett. 456, 301–305.
[30] Zhang, Z.-Y., Davis, J.P. and Van Etten, R.L. (1992) Biochemistry 31, 1701–1711.
[31] Wu, L. and Zhang, Z.-Y. (1996) Biochemistry 35, 5426–5434.
[32] Peters, G.H., Frimurer, T.M. and Olsen, O.H. (1998) Biochemistry 37, 5383–5393.
[33] Dillet, V., Van Etten, R.L. and Bashford, D. (2000) J. Chem. Phys. B 104, 11321–11333.
[34] Kolmodin, K., Nordlund, P. and Åqvist, J. (1999) Proteins 36, 370–379.
[35] Alhambra, C., Wu, L., Zhang, Z.-Y. and Gao, J. (1998) J. Am. Chem. Soc. 120, 3858–3866.
[36] Hart, J.C., Hillier, I.H., Burton, N.A. and Sheppard, D.W. (1998) J. Am. Chem. Soc. 120, 13535–13536.
[37] Taga, E.M. and Van Etten, R.L. (1982) Arch. Biochem. Biophys. 214, 505–515.
[38] Zhang, Z.-Y., Malachowski, W.P., Van Etten, R.L. and Dixon, J.E. (1994) J. Biol. Chem. 269, 8140–8145.
[39] Zhang, Z., Harms, E. and Van Etten, R.L. (1994) J. Biol. Chem. 269, 25947–25950.
[40] Perrin, D.D., Dempsey, B., Serjeant, E.P. (1981) p*K*a Prediction for Organic Acids and Bases, Chapman and Hall, London.
[41] Hyland, L.J., Tomaszek Jr., T.A. and Meek, T.D. (1991) Biochemistry 30, 8454–8463.
[42] Zhang, M., Stauffacher, C.V., Lin, D. and Van Etten, R.L. (1998) J. Biol. Chem. 273, 21714–21720.
[43] Zhang, M., Zhou, M., Van Etten, R.L. and Stauffacher, C.V. (1997) Biochemistry 36, 15–23.
[44] Pope, M.T. and Dale, B.W. (1968) Q. Rev. Chem. Soc. 22, 527–548.
[45] Hengge, A.C., Zhao, Y., Wu, L. and Zhang, Z.-Y. (1997) Biochemistry 36, 7928–7936.
[46] Wang, S., Tabernero, L., Zhang, M., Harms, E., Van Etten, R.L. and Stauffacher, C.V. (2000) Biochemistry 39, 1903–1914.
[47] Tabernero, L., Evans, B.N., Tishmack, P.A., Van Etten, R.L. and Stauffacher, C.V. (1999) Biochemistry 38, 11651–11658.
[48] Groves, M.R., Yao, Z.-J., Roller, P.P., Burke Jr., T.R. and Barford, D. (1998) Biochemistry 37, 17773–17783.
[49] Iversen, L.F., Andersen, H.S., Branner, S., Mortensen, S.B., Peters, G.H., Norris, K., Olsen, O.H., Jeppesen, C.B., Lundt, B.F., Ripka, W., Møller, K.B. and Møller, N.P.H. (2000) J. Biol. Chem. 275, 10300–10307.
[50] Jia, A., Barford, D., Flint, A.J. and Tonks, N. (1995) Science 268, 1754–1758.
[51] Salmeen, A., Anderson, J.N., Myers, M.P., Tonks, N.K. and Barford, D. (2000) Mol. Cell 6, 1401–1412.
[52] Fauman, E.B., Yuvaniyama, C., Schubert, H.L., Stuckey, J.A. and Saper, M.A. (1996) J. Biol. Chem. 271, 18780–18788.
[53] Schubert, H.L., Stuckey, J.A., Fauman, E.B., Dixon, J.E. and Saper, M.A. (1995) Protein Sci. 4, 1904–1913.
[54] Schumacher, M.A., Denu, J.M., Tanner, K.G., Todd, J.L., The Structural Basis for Recognition of Bisphosphor Substrates by Vhr (PDB code 1F5D), unpublished.