Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic Chemistry 33 (2005) 264–273
BIOORGANIC CHEMISTRY
www.elsevier.com/locate/bioorg

Reactivity of Cdc25 phosphatase at low pH and with thiophosphorylated protein substrate

Johannes Rudolph *

Departments of Biochemistry and Chemistry, Duke University Medical Center, Durham, NC 27710, USA

Received 1 December 2004
Available online 22 February 2005

Abstract

Cdc25s, dual-specificity phosphatases that dephosphorylate and activate cyclin-dependent kinases, are important regulators of the eukaryotic cell cycle. Herein, we probe the protonation state of the phosphate on the protein substrate of Cdc25 by pH-dependent studies and thiosubstitution. We have extended the useable range of pH for this enzyme substrate pair by using high concentrations of glycerol under acidic conditions. Using the protein substrate, we find a slope of 2 for the acidic side of the bell-shaped pH-rate profile, as found with other protein tyrosine phosphatases. Using thiophosphorylated protein substrate, we find no change in the basic side of the pH-rate profile, despite a large reduction in activity as measured by k_{cat}/K_m (0.18%) or k_{cat} (0. 11%). In contrast, the acidic side of the profile changes shows a slope of 1, consistent with the 1.5 pH unit shift associated with thiosubstitution. Thus, Cdc25, like other protein phosphatases, uses a dianionic phosphorylated substrate.

Keywords: Thiophosphate; Phosphatase; Cdc25; pH-dependence; Cdk; Cyclin

1. Introduction

The enzymatic transfer of phosphate monoesters is a fundamental element of intracellular regulation and thus has been extensively studied in solution model sys-

* Fax: +1 919 613 8642.
E-mail address: rudolph@biochem.duke.edu.

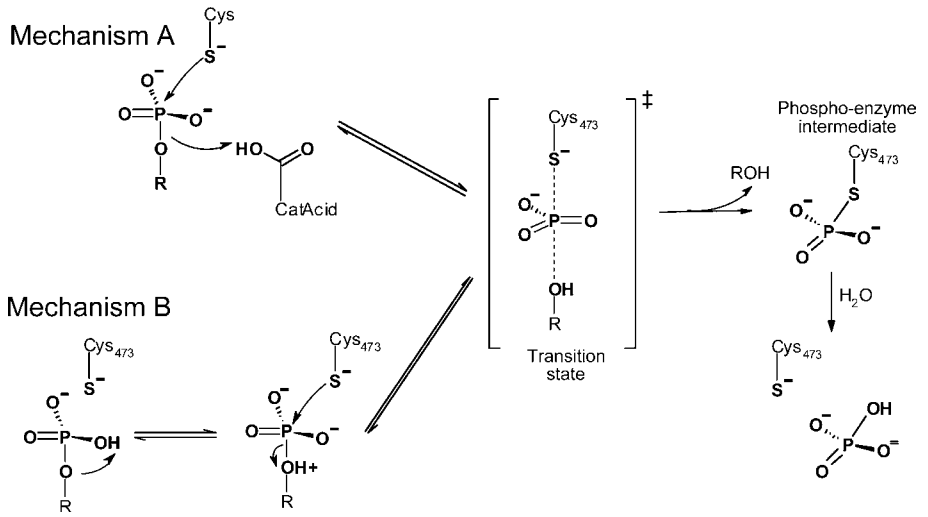
0045-2068/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bioorg.2005.01.004

tems and for many specific enzymatic examples [1]. Here, we consider the mechanism of phosphate monoester hydrolysis by Cdc25 phosphatase, a dual-specificity phosphatase (DSP)[1] of the protein tyrosine phosphatase (PTP) family [2]. Cdc25 phosphatases are regulators of the eukaryotic cell cycle during normal cell cycle progression and in response to DNA damage through their activity on the cyclin-dependent kinases. Because their overexpression is frequently associated with the rapid and aggressive cell growth that characterizes cancerous cells, the Cdc25 phosphatases have served as targets for anti-cancer development. Thus, the elucidation of their detailed enzymatic mechanism may contribute toward the design of novel and specific inhibitors.

Cdc25 phosphatases contain the CX_5R motif common to all PTPs, where C is the catalytic cysteine and the amide backbones of the five X residues form a phosphate binding loop along with the arginine R. The active site cysteine exists as a thiolate with a pK_a of 5.9 and forms a transient covalent intermediate consisting of a phospho-cysteine [3,4]. For the preferred bis-phosphorylated protein substrate Cdk2-pTpY/CycA ($k_{cat}/K_m = 10^6\ M^{-1}\,s^{-1}$), phospho-threonine is preferentially dephosphorylated, whereas phospho-tyrosine is preferred in the poorly utilized peptidic substrates ($k_{cat}/K_m = 1$–$10\ M^{-1}\,s^{-1}$) [5]. The protonation state of the phosphate on the protein substrate and its potential role as the proton donor to the leaving group oxygen are the subject of this investigation.

Phosphate monoesters have pK_as of 6–6.5 and therefore both the monoanionic and dianionic species are physiologically accessible. The relative reactivity of monoanionic and dianionic phosphate monoesters depends differently on the pK_a of the leaving group to which they are attached [6]. This is observed in the dependence of the log of the rate constant on the pK_a of the leaving group (Brønsted β_{lg}), which is -1.23 for dianions and only -0.2 for monoanions. Thus, for all phosphate monoesters except those with highly activated leaving groups (such as 2,4-dinitrophenyl phosphate), the monoanion is much more reactive than the dianion at physiological pHs. In fact, for physiologically relevant phospho-serine or phospho-threonine hydrolysis, the dianion has been shown to be $\sim 10^9$-fold harder to cleave than the monoanion [7]. With a half-life for the non-enzymatic reaction of 1.1×10^{12} years, the hydrolysis of dianionic phospho-monoesters is one of the harder reactions for which an enzyme has evolved a mechanism [8]. In the case of non-enzymatic hydrolysis of monoanionic phosphate monoesters, a pre-equilibrium protonation of the leaving group from the protonated phosphate (probably through one or more intermediate water molecules) is thought to precede the rate-determining step of P–O bond cleavage (Mechanism B, Scheme 1) [6]. Recent ^{18}O and solvent deuterium isotope effect studies using *m*-nitrobenzyl phosphate with a leaving group pK_a of 14.9 support this mechanism [9].

[1] *Abbreviations used:* DSP, dual-specificity phosphatase; PTP, protein tyrosine phosphatase; Cdk2-pTpY/CycA, bis-phosphorylated cyclin-dependent kinase 2 complexed with cyclin A; Cdk2-p_STp_SY/CycA, thio-bis-phosphorylated cyclin-dependent kinase 2 complexed with cyclin A; TMAO, trimethylamine *N*-oxide.



Scheme 1.

Despite the much more favorable reactivity of monoanionic phosphate monoesters, much experimental data solidly support the generally accepted enzymatic mechanism in which a dianionic phosphate undergoes nucleophilic attack with concerted departure of a protonated leaving group via a loose transition state (Mechanism A, Scheme 1) [1]. The k_{cat}/K_m pH-rate profiles for the substrate *p*-nitrophenyl phosphate for protein tyrosine phosphatase 1B [10] and the dual-specificity phosphatase VHR [11], for example, are bell-shaped, with an acid limb with a slope of 2 and a basic limb with a slope of 1. These three ionizations have been assigned to: (1) the deprotonated active site thiolate that performs the nucleophilic attack, (2) the dianionic phosphate of the substrate, and (3) the catalytic acid (an aspartic acid in the so-called WPD loop) that donates a proton to the oxygen of the leaving group. Consistent with this interpretation, the acidic limb in the profile for k_{cat} shows only the one ionization of the active site thiolate. Characteristically, mutagenesis of the catalytic acid in any of these phosphatases leads to a significant loss of activity at the optimal pH and concomitant loss of the basic limb in the pH-rate profile. ^{18}O kinetic isotope effects for PTPs and DSPs with specifically labeled phosphate in *p*- and, more recently, *m*-nitrobenzyl phosphate provide further strong evidence for the reaction of phosphate monoesters as dianions with loose, metaphosphate-like transition states [12–15].

In contrast, we have recently proposed a monoanion-mediated mechanism in the hydrolysis of phospho-threonine from the protein substrate Cdk2/CycA for the phosphatase Cdc25 [4]. We favored this mechanism based on the inherently more reactive monoanion, the inability to remove the basic limb from the pH-rate profile by mutagenesis of any possible catalytic acid suggested from the crystal structure [3], and our demonstration that the apparent protonation of the leaving group oxygen occurs from the protein substrate, not the enzyme. Contrary to our observations with protein substrate, pH-rate profiles with a variety of small molecule substrates,

Brønsted plots, and ^{18}O isotope effect studies with *p*- and *m*-nitrobenzyl phosphate provide evidence for a dianionic substrate with donation of a proton to the leaving group by the glutamate adjacent to the active site cysteine (Glu431 in Cdc25A and Glu474 in Cdc25B) [16,17].

Seeking further experimental data to provide insight into the mechanism of Cdc25 phosphatase, we have turned to stabilization of the enzyme at low pH and thiophosphorylation, two approaches that can directly address the protonation state of the phosphorylated substrate. Stabilization of Cdc25 phosphatase and its protein substrate by the addition of high concentrations of glycerol has allowed us to perform pH-dependent experiments below the previous limit of 5.25 and thus determine the slope of the acidic limb of the pH-rate profile for the physiologically relevant protein substrate. Use of thiophosphorylated substrate allows us to study two potential thiophosphate-specific effects, their intrinsically higher reactivity and their perturbed pK_a compared to regular phosphate. Given that for the Cdc25-catalyzed reaction the active site is readily accessible to the surface, the rate of reaction with the protein substrate Cdk2-pTpY/CycA is limited by chemistry, and there are no metals in the phosphatase, the thiophosphate substitution serves as an ideal minor chemical modification for probing the mechanism of Cdc25.

2. Materials and methods

Trimethylamine *N*-oxide (TMAO), unlabeled ATP, inorganic thiophosphate, and [γ-S]ATP were obtained from Sigma. Labeled [γ-^{35}S]ATP and [γ-^{32}P]ATP were obtained from Amersham. The catalytic domain of Cdc25B phosphatase was prepared as described previously [3]. Reactions using 3-*O*-methyl fluorescein phosphate (mFP) were followed by continuous UV–Vis spectroscopy at 477 nm ($\varepsilon = 27{,}200\ M^{-1}\ m^{-1}$) in a three-component (3-C) buffer (50 mM Tris, 50 mM Bis-Tris, and 100 mM Na acetate) at 25 °C. The IC-50 for inorganic thiophosphate at pH 6.5 in 3-C buffer was determined using the substrate mFP at 25 μM in triplicate using eight different concentrations of inhibitor (1–100 mM). Thiophosphorylated substrate Cdk2-p_STp_SY/CycA was prepared identically to the normal protein substrate Cdk2-pTpY/CycA except [γ-S]ATP was substituted for ATP and the reaction time was increased to 24 h [5]. Incorporation of thiophosphate into Cdk2/CycA was quantitated using the known specific activity of the [γ-^{35}S]ATP and by measuring protein concentration using the Bio-Rad protein assay solution. Direct comparisons of the dephosphorylation of radiolabeled Cdk2-pTpY/CycA and Cdk2-p_STp_SY/CycA as a function of pH were performed using 3-C buffer containing 1 mM DTT and 1 mg/ml bovine serum albumin at 25 °C. Reactions were quenched by addition of TCA to 12%, and following centrifugation to remove the precipitated substrate, the supernatant was subjected to scintillation counting. Reactions in 40% glycerol were prepared by pre-mixing buffer, glycerol, and protein substrate at 50% glycerol and initiating with enzyme by vigorous pipetting. All k_{cat}/K_m experiments were performed in duplicate or triplicate as a function of time, substrate concentration, and enzyme concentration with at least five time points. All single turnover experiments

included at least 20 time points and were fitted to a single exponential as previously described [4]. The pH-dependent activities were fitted to Eq. (1) using IGOR software (Wavemetrics) where C is the pH-independent value of k_{cat}/K_m, [H] is the proton concentration, and K_a, K_b, and K_c are three independent ionization constants

$$v = C/(1 + [H]/K_a)(1 + [H]/K_b + K_c/[H]). \quad (1)$$

3. Results and discussion

3.1. Stabilization of Cdc25 and Cdk2-pTpY/CycA at low pH

One of the limitations in the study of Cdc25 phosphatase, both Cdc25A and Cdc25B, has been the instability of the enzyme below pH 5.25 [3,4,16,17]. Thus, it has been impossible to determine whether the acidic limb of the pH-rate profile for artificial substrates or protein substrate has a slope of 1 or 2, making comparisons to other PTPs and definitive mechanistic conclusions impossible. We sought to stabilize Cdc25 by addition of reagents that might prevent its denaturation at low pH. Trimethylamine *N*-oxide (TMAO) is an osmolyte commonly used in protein refolding studies to stabilize the folded state by its unfavorable interaction with peptide backbone in the unfolded state [18]. Addition of up to 2 M TMAO at pH 6.5 did not affect the activity of Cdc25 using the small molecule substrate 3-*O*-methylfluorescein phosphate (mFP, data not shown). However, it did not prevent acid induced denaturation of Cdc25 below pH 5.2 (data not shown). Glycerol, a polyhydric alcohol known to stabilize protein conformation, is another commonly used additive, although by altering the solvent viscosity it is also known to affect the rate of free diffusion, particularly of proteins, and can also slow the rate of conformational changes. Addition of glycerol to 50% final concentration at pH 6.5 did not affect the activity of Cdc25 with mFP (data not shown). Control experiments that incubated enzyme in the pH range 4–6 in the presence of 40% glycerol followed by dilution and assay at pH 6.5 demonstrated the stability of Cdc25 under low pH conditions (Fig. 1). Similar experiments performed using pre-incubation of Cdk2-pTpY/CycA demonstrated the stability of protein substrate to low pH conditions as well (Fig. 1). Additionally, using the viscogen sucrose we have previously shown that the rate-determining step with the protein substrate Cdk2-pTpY/CycA is the actual chemical step, not free diffusion [4]. Therefore, we were not surprised to find the overall reaction rate to be reduced by only 50% at pH 6.5 in the presence of 40% glycerol (data not shown). Thus, the addition of high concentrations of glycerol enables the extension of the useable pH range to 4.25 in the study of the activity of Cdc25 phosphatase.

3.2. pH-dependence of Cdc25 at low pH with Cdk2-pTpY/CycA

We determined the k_{cat}/K_m using the protein substrate Cdk2-pTpY/CycA in the pH range 4.25–6.5 (Fig. 2). Good fitting of these data, along with previous data

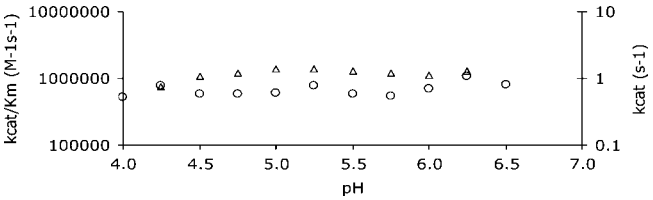


Fig. 1. The pH-dependent stability of Cdc25 and its protein substrate Cdk2-pTpY/CycA. Cdc25 (200 nM) or Cdk2-pTpY/CycA (300 nM) was incubated at varying pHs for 5 min in the presence of 40% glycerol and subsequently diluted at least 10-fold into buffer at pH 6.5. For Cdc25, measuring the activity with saturating concentrations of mFP (200 μM) to determine k_{cat} assessed the stability of the enzyme (triangles). For Cdk2-pTpY/CycA, measuring the k_{cat}/K_m with Cdc25 assessed the stability of protein substrate (circles).

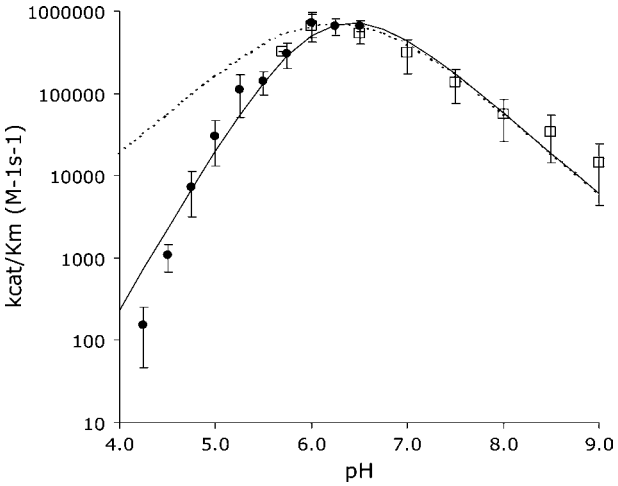


Fig. 2. The pH-dependent activity of Cdc25 with Cdk2-pTpY/CycA as fitted to Eq. (1) with two acidic pK_as and one basic pK_a. The new data from pH 4.25 to 6.25 (filled circles) are superimposed on the data from pH 5.7 to 9 taken from [3] (open squares) after correcting for the 50% reduced activity seen in 40% glycerol. The dotted line represents a hypothetical fitting to a bell-shaped curve with only one acidic pK_a and one basic pK_a.

for the pH range 5.7–9, to a bell-shaped pH-rate profile with an ascending slope of 2 and descending slope of 1 could be achieved using Eq. (1). The fitted values include a pK_a for the catalytic cysteine of 5.9 [3,4], a pK_a for the phospho-threonine substrate of pH 6.1 [19,20], and a pK_a for the catalytic acid of 6.4 [3,4], entirely consistent with previous results and expectations for phosphorylated substrate (Table 1). Thus, the pH-dependent k_{cat}/K_m rate profile for Cdc25 phosphatase is identical to those determined previously for other PTPs such as the protein tyrosine phosphatase 1B [10] and the dual-specificity phosphatase VHR [11], and most consistent with Mechanism A in Scheme 1. Due to limitations in preparation of protein substrate, we are unable to obtain saturation curves to determine the pH-dependence of k_{cat}. Single turnover conditions under rapid quench conditions, a kinetic tool we have used previously to

Table 1
Summary of kinetic parameters for the reaction of Cdc25 with phosphorylated and thiophosphorylated protein substrate

Kinetic parameter	Cdk2-pTpY/CycA	Cdk2-p$_S$Tp$_S$Y/CycA
k_{cat}/K_m ($M^{-1}s^{-1}$)	$(2.4 \pm 0.5) \times 10^6$	4400 ± 320
k_{cat} (s^{-1})	1.2 ± 0.2	$(1.3 \pm 0.4) \times 10^{-3}$
pK_{a1}	5.9 ± 0.2	5.9 ± 0.2
pK_{a2}	6.1 ± 0.2	4.5 ± 0.2
pK_{a3}	6.4 ± 0.2	6.5 ± 0.2

measure k_{cat} for Cdk2-pTpY/CycA [4], were also not amenable due to mixing difficulties arising from the high macro-viscosity of the samples at 40% glycerol. However, the k_{cat}/K_m data alone suggest that Cdc25 uses a dianionic protein substrate.

3.3. Thiophosphorylation of Cdk2/CycA

We set out to provide further evidence for the protonation state of the phosphate using thiophosphorylated protein substrate. Use of thiophosphorylated substrate allows us to study two potential thiophosphate-specific effects, namely their intrinsically higher chemical reactivity and their perturbed pK_as compared to regular phosphate. The key to studying Cdc25 phosphatase using thiosubstituted protein substrate was the preparation of Cdk2-p$_S$Tp$_S$Y/CycA. Many protein kinases are able to utilize [γ-S]ATP, albeit at significantly slower rates. As our protocol for quantitative substrate preparation with normal ATP uses a ratio of Myt1 kinase to Cdk2/CycA substrate of 0.5 [5], we did not anticipate much success for the reaction with [γ-S]ATP by simply increasing the amount of Myt1 kinase. However, extending the reaction time to 20–24 h yielded phosphorylation levels of 1.8–2.2 thiophosphates per Cdk2/CycA, which is comparable to the yields using [γ-P]ATP.

3.4. Activity of Cdc25 with Cdk2-p$_S$Tp$_S$Y/CycA

The pH-independent activity of Cdk2-p$_S$Tp$_S$Y/CycA was only 0.18% (k_{cat}/K_m) of that with Cdk2-pTpY/CycA (Table 1). We wished to determine whether the significantly lower activity with the thiophosphorylated substrate was attributable to k_{cat}, K_m, or a combination of the two. Because saturation concentrations of protein substrate are not readily attainable, we derived the apparent rate-determining chemistry on the enzyme under single turnover conditions (k_{cat}), as previously described [4]. The k_{cat} we determined by fitting to the single exponential at pH 6.5 with thiophosphorylated substrate is only 0.11% of the k_{cat} with regular phosphate at pH 6.5 (Table 1). Therefore, the observed difference in k_{cat}/K_m can be attributed to k_{cat}, within experimental error. The similar affinity for thiophosphorylated vs. normally phosphorylated substrate is not surprising given the surface proximity of the phosphate binding pocket of the active site. To confirm the unchanged affinity with thiosubstitution, we found that the IC-50 for inorganic thiophosphate was

17.9 ± 1.2 mM at pH 6.5, consistent with the previously reported pH-independent IC-50 of 18.7 ± 3.5 mM for inorganic phosphate [4].

The lower reactivity (in k_{cat}) with the thiosubstitution in the protein substrate was not necessarily expected. Sulfur has a lower electronegativity and greater polarizability compared to oxygen, leading to easier access to the dissociative metathiophosphate transition state or intermediate implicated in the hydrolysis of thiophosphate *O*-monoesters. That is, the non-enzymatic hydrolysis of thiophosphate monoesters proceeds 10–100-fold faster than for the corresponding phosphate monoesters [21]. In support of these data and this interpretation, evidence for the existence of a true metathiophosphate intermediate (as opposed to transition state) has been obtained from stereochemical studies and pressure-dependent rate measurements [22–24]. On an enzyme such as Cdc25, of course, additional effects such as the size of the sulfur substitution, the length of the sulfur–phosphorus bond, and the ability to hydrogen bond could account for the slower reactivity of the thiosubstituted substrate. The van der Waals radius of sulfur is 0.45 Å larger than that of oxygen and the P═S distance is 1.94 Å, compared with a P═O distance of 1.57 Å [25,26]. Similar observations and concerns have been raised for studies of slowly reacting thiophosphorylated substrates (0.3–20% compared to the regular substrate) with other enzymes by Herschlag, Breslow, van Etten, and others [21,27–33].

3.5. pH-dependence of Cdc25 with Cdk2-p_STp_SY/CycA

As the pK_as of thiophosphate *O*-monoesters are lower than regular phosphate monoesters by 1.2–1.6 U, a thiophosphorylation-dependent perturbation in the pH-rate profile is a powerful mechanistic probe, despite the low activity caused by this modification [34–36]. If the phosphoryl group in the substrate for Cdc25 is monoanionic and responsible for the basic limb in the bell-shaped pH rate profile [4], the basic limb of the pH-rate profile should shift left by ~1.5 U. On the other

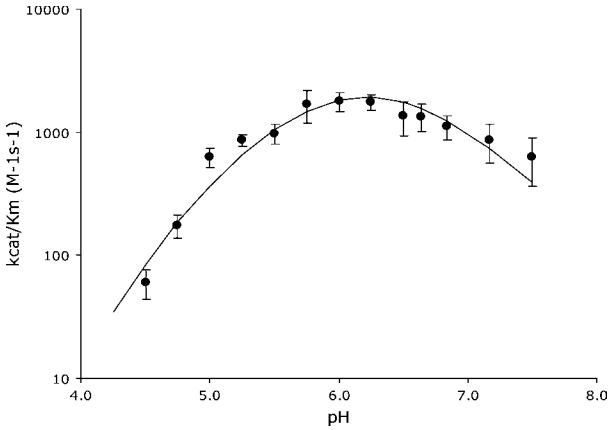


Fig. 3. The pH-dependent activity of Cdc25 with Cdk2-p_STp_SY/CycA as fitted to Eq. (1) with two acidic pK_as and one basic pK_a.

hand, if the substrate is dianionic, as supported by the evidence above, then the acidic limb should change from an apparent slope of 2 to a slope of 1 because the pK_a of the substrate drops below the measured range in pH. Using glycerol stabilization, we determined the k_{cat}/K_m using the thiophosphorylated protein substrate Cdk2-p$_S$Tp$_S$Y/CycA in the pH range 4.5–7.5 (Fig. 2). Good fitting of these data to Eq. (1) was obtained using a pK_a for the catalytic cysteine of 5.9, a pK_a for the thiophospho-threonine substrate of pH 4.5, and a pK_a for the catalytic acid of 6.5. This fitting is consistent with the results in Fig. 2 and expectations for the thio-induced shift in the pK_a of the substrate (Table 1). Thus, these data also support the dianionic phosphate in Mechanism A of Scheme 1, in accord with other well-studied protein phosphatases (Fig. 3).

4. Conclusion

The data presented herein clearly demonstrate that Cdc25, like other PTPs, utilizes a classic dianionic phosphate in the reaction with its native protein substrate. Thus, despite the lower intrinsic reactivity of the dianionic substrate, nature appears to always have evolved a mechanism that utilizes the physiologically more prevalent dianionic form and provides a general acid for protonation of the leaving group. In the case of Cdc25, however the identity of this apparent catalytic acid remains unidentified. Mutagenesis of all reasonable residues on the enzyme yielded unchanged pH-rate profiles with native protein substrate [3]. Also, single turnover experiments suggest that the proton is derived from the Cdk2/CycA protein substrate [4]. Mutagenesis of several acidic residues near pThr14 and pTyr15 has not yet revealed a potential catalytic acid. As we have recently shown that the docking site for Cdc25 on the Cdk/CycA substrate is 20–30 Å away from the active site [37], the search for the protonated residue responsible for the basic limb of the pH-rate profile is perhaps more complex than simple proximity based on the known crystal structure of the protein substrate. Computational docking and further site-directed mutagenesis studies are underway to elucidate the details involved in recognition of the protein substrate.

Acknowledgment

This work was supported by NIH Grant R01 GM61822.

References

[1] A.C. Hengge (Ed.), Transfer of the PO_3^{2-} Group, Comprehensive Biological Catalysis: A Mechanistic Reference, Academic Press, San Diego, 1998.
[2] K. Kristjánsdóttir, J. Rudolph, Chem. Biol. 11 (2004) 1043.
[3] W. Chen, M. Wilborn, J. Rudolph, Biochemistry 39 (2000) 10781.

[4] J. Rudolph, Biochemistry 41 (2002) 14613.
[5] J. Rudolph, D.M. Epstein, L. Parker, J. Eckstein, Anal. Biochem. 289 (2001) 43.
[6] A.J. Kirby, A.G. Varvoglis, J. Am. Chem. Soc. 89 (1967) 415.
[7] C. Lad, N.H. Williams, R. Wolfenden, Proc. Natl. Acad. Sci. USA 100 (2003) 5607.
[8] R. Wolfenden, M.J. Snider, Acc. Chem. Res. 34 (2001) 938.
[9] P.K. Grzyska, P.G. Czyryca, J. Purcell, A.C. Hengge, J. Am. Chem. Soc. 125 (2003) 13106.
[10] Z.-Y. Zhang, J. Biol. Chem. 270 (1995) 11199.
[11] J.M. Denu, J.E. Dixon, Proc. Natl. Acad. Sci. USA 92 (1995) 5910.
[12] A.C. Hengge, G.A. Sowa, L. Wu, Z.-Y. Zhang, Biochemistry 34 (1995) 13982.
[13] A.C. Hengge, J.M. Denu, J.E. Dixon, Biochemistry 35 (1996) 7084.
[14] J.D. Rigas, R.H. Hoff, A.E. Rice, A.C. Hengge, J.M. Denu, Biochemistry 40 (2001) 4398.
[15] P.K. Grzyska, Y. Kim, M.D. Jackson, A.C. Hengge, J.M. Denu, Biochemistry 43 (2004) 8807.
[16] D.F. McCain, I.E. Catrina, A.C. Hengge, Z.-Y. Zhang, J. Biol. Chem. 277 (2002) 11190.
[17] D.F. McCain, P.K. Grzyska, L. Wu, A.C. Hengge, Z.-Y. Zhang, Biochemistry 43 (2004) 8256.
[18] A. Wang, D.W. Bolen, Biochemistry 36 (1997) 9101.
[19] P.S. Charifson, L.M. Shewchuk, W. Rocque, C.W. Hummel, S.R. Jordan, C. Mohr, G.J. Pacofsky, M.R. Peel, M. Rodriguez, D.D. Sternbach, T.G. Consler, Biochemistry 36 (1997) 6283.
[20] M. Schutkowski, A. Bernhardt, X.Z. Zhou, M. Shen, U. Reimer, J.-U. Rahfeld, K.P. Lu, G. Fischer, Biochemistry 37 (1998) 5566.
[21] R. Breslow, I. Katz, J. Am. Chem. Soc. 90 (1968) 7376.
[22] P.M. Cullis, R. Misra, D.J. Wilkins, J. Am. Chem. Soc. 109 (1987) 1594.
[23] S.P. Harnett, G. Lowe, J. Chem. Soc. Chem. Commun. (1987) 1416.
[24] J. Burgess, N. Blundell, P.M. Cullis, C.D. Hubbard, R. Misra, J. Am. Chem. Soc. 110 (1988) 7900.
[25] W. Saenger, F. Eckstein, J. Am. Chem. Soc. 92 (1970) 4712.
[26] S.J. Benkovic, K.J. SchrayThe Enzymes, vol. VIII, Academic Press, New York, 1978, p. 201.
[27] P. Mushak, J.E. Coleman, Biochemistry 11 (1972) 201.
[28] J.F. Chlebowski, J.E. Coleman, J. Biol. Chem. 249 (1974) 7192.
[29] R.L. Van Etten, M.S. Saini, Arch. Biochem. Biophys. 219 (1982) 155.
[30] D. Herschlag, J.A. Piccirilli, T.R. Cech, Biochemistry 30 (1991) 4844.
[31] D. Herschlag, J. Am. Chem. Soc. 116 (1994) 11631.
[32] F. Hollfelder, D. Herschlag, Biochemistry 34 (1995) 12255.
[33] R. Breslow, W.H.J. Chapman, Proc. Natl. Acad. Sci. USA 93 (1996) 10018.
[34] E.K. Jaffe, M. Cohn, Biochemistry 17 (1978) 652.
[35] G.W. Smithers, W.J. O'Sullivan, Biochemistry 23 (1984) 4773.
[36] P.A. Frey, R.D. Sammons, Science 228 (1985) 541.
[37] J. Sohn, K. Kristjánsdóttir, A. Safi, B. Parker, B. Kiburz, J. Rudolph, Proc. Natl. Acad. Sci. USA 47 (2004) 16437.