Isocitrate dehydrogenase (IDH) of *Escherichia coli* is regulated by a bifunctional protein, IDH kinase/phosphatase. In this paper, we demonstrate that the effectors controlling these activities belong to two distinct classes that differ in mechanism and in the locations of their binding sites. NADPH and isocitrate are representative members of one of these effector classes. NADPH inhibits both IDH kinase and IDH phosphatase, whereas isocitrate inhibits only IDH kinase. Isocitrate can "activate" IDH phosphatase by reversing product inhibition by dephospho-IDH. Mutations in *icd*, which encodes IDH, had parallel effects on the binding of these ligands to the IDH active site and on their effects on IDH kinase and phosphatase, indicating that these ligands regulate IDH kinase/phosphatase through the IDH active site. Kinetic analyses suggested that isocitrate and NADPH prevent formation of the complex between IDH kinase/phosphatase and its protein substrate. AMP, 3-phosphoglycerate, and pyruvate represent a class of regulatory ligands that is distinct from that which includes isocitrate and NADPH. These ligands bind directly to IDH kinase/phosphatase, a conclusion which is supported by the observation that they inhibit the IDH-independent ATPase activity of this enzyme. These effector classes can also be distinguished by the observation that mutant derivatives of IDH kinase/phosphatase expressed from aceK3 and aceK4 exhibited dramatic changes in their responses to AMP, 3-phosphoglycerate, and pyruvate but not to NADPH and isocitrate.

In *Escherichia coli*, the Krebs cycle enzyme isocitrate dehydrogenase (IDH) is regulated by reversible phosphorylation (1, 2). Phosphorylation of IDH controls the partitioning of isocitrate between this cycle and the glyoxylate bypass. The flux of isocitrate through the bypass is essential for growth on acetate because it prevents the quantitative loss of the acetate carbons as CO₂ in the Krebs cycle (3, 4). During growth on acetate, ~70% of IDH is maintained in the inactive, phosphorylated form, forcing isocitrate through the bypass (5–9).

Crystallography demonstrated that the site of phosphorylation, Ser-113, lies within the active site cleft. Ser-113 forms a hydrogen bond with isocitrate in the active, dephosphorylated form of IDH. Phosphorylation of Ser-113 inactivates IDH by blocking isocitrate binding through a combination of electrostatic and steric effects. In contrast, NADP⁺ and NADPH bind to both the phosphorylated and dephosphorylated forms for IDH (10–13).

The IDH phosphorylation cycle is catalyzed by IDH kinase/phosphatase, a bifunctional protein encoded by *aceK* (14, 15). The phosphatase activity has an absolute requirement for ATP or ADP (14). This protein also has an intrinsic ATPase activity (16). The results of affinity labeling and analyses of the effects of site-directed and random mutagenesis of aceK suggest that all three activities are catalyzed by the same active site (17–20).

The IDH phosphorylation cycle is controlled by a variety of metabolites. For example, AMP, pyruvate, and 3-phosphoglycerate activate IDH phosphatase and inhibit IDH kinase. Others, such as NADPH, inhibit both activities. Isocitrate inhibits IDH kinase but its effect on IDH phosphatase is controversial; some studies found no effect, whereas others report activation of this activity (reviewed in Ref. 18). The mechanisms by which these ligands elicit such a diversity of regulatory effects remain a mystery. Here, we demonstrate that there are at least two mechanisms by which the IDH kinase/phosphatase is regulated. Members of one of these effector classes bind directly to IDH kinase/phosphatase, whereas members of the other act through the active site of IDH.

**Experimental Procedures**

*Materials—*γ-[32P]ATP was purchased from NEN Life Science Products. It was then subjected to further purification by the method of Axelson et al. (21). All reagents were of the purest grades available.

*Protein Purification—*Wild-type IDH was overexpressed from plasmids pTK509 in *E. coli* strain Y1090 (6). Mutant IDHs were engineered in plasmid pTK513 and overexpressed from their respective plasmids in *E. coli* strain SL4, which carries a deletion of *icd* (22, 23). Wild-type and mutant IDHs were then purified by a modification of the method of Garnak and Reeves (24). All preparations were at least 95% pure, as judged by Coomassie Blue staining following SDS-polyacrylamide gel electrophoresis (25).

Phospho-IDH was prepared in vitro using purified IDH and wild-type IDH kinase/phosphatase. IDH kinase/phosphatase and residual dephospho-IDH were then removed by chromatography on an Affi-Gel Blue column (9).

Wild-type IDH kinase/phosphatase was overexpressed from plasmid pEKL1 in strain ST2010R. The mutant AceK3 and AceK4 proteins were overexpressed from plasmids pEKL5 and pEKL3, respectively, in strain SL1R (*aceK-Kon*) (17, 19, 26). Cultures were grown at 37 °C in L broth (1% tryptone, 1% NaCl, 0.5% yeast extract) with 40 μg/ml tetracycline in a 3.5-liter high-density fermentor. Expression of the *aceK* genes was induced with 3 mM isopropyl-b-D-thiogalactopyranoside during the mid-log phase of growth. The phosphorylation of IDH kinase/phosphatase has been described previously (14). Protein concentrations were determined by the method of Lowry et al. (27) using bovine serum albumin as the standard.

**Enzyme Activity Determinations—**IDH phosphatase was assayed by measuring the release of [32P]phosphate from [32P]phospho-IDH at 37 °C, as described previously (9). The standard reaction (50 μl con-
Regulation of IDH Kinase/Phosphatase

The amount of [32P]phosphate released was determined following abatement of the unreacted ATP to activated charcoal. The amount of [32P]phosphate released was determined following absorption of the unreacted ATP to activated charcoal.

Data Analyses—The velocities of the enzyme reactions in the presence of effectors can be described by Equation 1,

\[ v_e = v_0 + (1 - \frac{v_0}{K_v}) \]

where \( v_e \) is the velocity at a given concentration of effector, \( f \) is the fraction of the effector sites which are occupied, \( v_0 \) is the velocity at saturating effector, and \( v_i \) is the velocity in the absence of effector. Assuming that IDH kinase/phosphatase exhibits simple, hyperbolic binding of the effector, this equation can be expressed as,

\[ V_e = \frac{[e]}{[e] + K} (v_e - v_i) + v_i \]

where \([e] \) is the effector concentration and \( K \) is the effective dissociation constant for that effector. Nonlinear regression employing Equation 2 was used to analyze the activation of IDH kinase/phosphatase. A linearized form of this equation was used to analyze the inhibition of IDH kinase and the ATPase.

\[ \frac{1}{(v_e - v_i)} = \frac{K}{(v_i - v_i)} \left( \frac{1}{[e]} \right) + \frac{1}{(v_i - v_i)} \]

RESULTS

Location of the Regulatory Site for NADPH—A number of small molecules, such as isocitrate and NADPH, play multiple roles for IDH. In addition to acting as substrates for this enzyme, they also regulate IDH kinase and IDH phosphatase. This multiplicity of roles raised the possibility that these ligands act through a single binding site, the active site of IDH. However, other authors have suggested that distinct regulatory sites for these ligands were present on IDH kinase/phosphatase (29, 30).

We began by testing whether occupation of the coenzyme site of IDH could inhibit IDH kinase/phosphatase. This question was addressed using a derivative of IDH that has an inverted regulatory site for these ligands. Isocitrate is known to inhibit IDH kinase, although its effect on IDH phosphatase is controversial (see below). We suspected that isocitrate, like NADPH, affects IDH kinase by binding to the active site of IDH. We tested this possibility using a derivative of IDH, IDHN115L, in place of wild-type dephospho-IDH. This derivative includes an amino acid substitution two residues away from the phosphorylation site, Ser-113. This mutation causes a conformational change in the active site that dramatically reduces affinity for isocitrate (23). Although IDHN115L retained the ability to act as a substrate for IDH kinase (Table I), the effective \( K \) for isocitrate was increased almost 20-fold (Fig. 2, Table II). This observation indicates that isocitrate also acts through the active site of IDH.

Activation of IDH Phosphatase by Isocitrate—Reports that isocitrate activated IDH phosphatase have been used to support the conclusion that this ligand bound to IDH kinase/phosphatase (29, 30). However, we found that isocitrate had no effect on IDH phosphatase activity in the absence of dephospho-IDH (Fig. 3). We suspected that the “activation” observed by previous authors had resulted from isocitrate binding to the active site of dephospho-IDH, preventing this protein from acting as a product inhibitor of IDH phosphatase. Consistent with this idea, we found that IDH phosphatase was strongly inhibited by dephospho-IDH and isocitrate was able to reverse this inhibition (Fig. 3). IDHN115L retained the ability to inhibit IDH phosphatase. IDH phosphatase activity was reduced by a factor of 6 by 5 \( \mu M \) wild-type dephospho-IDH or 1 \( \mu M \) dephospho-IDHN115L (Fig. 3), consistent with the difference in \( K_m \) values exhibited by IDH kinase for these proteins (Table I). However, isocitrate was much less effective in reversing inhibition by dephospho-IDHN115L (Fig. 3), indicating that isocitrate activated IDH phosphatase by binding to the active site of dephospho-IDH, thereby reversing product inhibition.

NADPH and Isocitrate Are Competitive Inhibitors—The ability of isocitrate to reverse the inhibition of IDH phosphatase by dephospho-IDH suggested that this ligand acted by preventing the formation of the complex between these proteins. A similar mechanism appears to be responsible for the inhibition of IDH kinase by isocitrate, because this ligand increased the \( K_m \) for dephospho-IDH without affecting the \( V_{max} \) (Fig. 4).
NADPH may also act by preventing the formation of complexes between IDH kinase/phosphatase and its protein substrates. This ligand increased the $K_m$ of IDH kinase for dephospho-IDH and that of IDH phosphatase for phospho-IDH. NADPH did not affect the $V_{max}$ of either of these activities (Fig. 5).

Location of the Regulatory Sites for AMP, 3-Phosphoglycerate, and Pyruvate—The conclusion that the effector sites for isocitrate and NADPH were located on IDH raised the question of whether the sites for other effectors were also located on this protein. We addressed this question by examining the IDH-independent ATPase activity of IDH kinase/phosphatase (16).

### Table II

| IDH  | Effective $K_i$ | IDH Kinase | IDH Phosphatase |
|------|----------------|------------|-----------------|
|      |                | NADPH      | NADH            | NADPH | NADH |
| Wild-type | 0.016 | 0.08 | 8.1 | 0.29 | 5.0 |
| IDH$^{115L}$ | 0.97 | 1.7 | 0.5 | 5.2 | 0.82 |

NADPH may also act by preventing the formation of complexes between IDH kinase/phosphatase and its protein substrates. This ligand increased the $K_m$ of IDH kinase for dephospho-IDH and that of IDH phosphatase for phospho-IDH. NADPH did not affect the $V_{max}$ of either of these activities (Fig. 5).

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### Figure 1

**Inversion of coenzyme specificity for IDH kinase and IDH phosphatase.** The assays contained the indicated concentrations of NADPH, (○, □) or NADH (■, △) (see “Experimental Procedures”). Standard errors were less then 5%. **Left panel,** IDH kinase assays included 0.5 μM wild-type dephospho-IDH and 8 ng of IDH kinase/phosphatase (□, □) or 0.5 μM dephospho-IDH$^{115L}$ and 110 ng of IDH kinase/phosphatase (○, ■). **Right panel,** IDH phosphatase assays included 0.2 μM wild-type phospho-IDH and 25 ng of IDH kinase/phosphatase (□, □) or 0.2 μM phospho-IDH$^{115L}$ and 2.5 μg of IDH kinase/phosphatase (○, ■). ATP was present at 50 μM in all assays.

### Figure 2

**An amino acid substitution in IDH alters inhibition of IDH kinase by isocitrate.** The assays contained 50 μM Mg$^{2+}$[$^{32}$P]ATP, the indicated concentrations of Mg$^{2+}$-isocitrate, and either 0.5 μM wild-type dephospho-IDH and 5 ng of IDH kinase/phosphatase (○) or 0.5 μM dephospho-IDH$^{115L}$ and 14 ng of IDH kinase/phosphatase (□) (see “Experimental Procedures”). Standard errors were less then 6%.

### Figure 3

**Activation of IDH phosphatase by isocitrate.** IDH kinase/phosphatase (13 ng) was assayed in the presence of 20 μM ATP, 0.1 μM [$^{32}$P]phospho-IDH, and the indicated concentrations of Mg$^{2+}$-isocitrate (see “Experimental Procedures”). Assays contained no dephospho-IDH (□), 5.0 μM wild-type dephospho-IDH (○), or 1.0 μM dephospho-IDH$^{115L}$ (■). Data are presented as relative velocities standardized to that observed in the absence of isocitrate. In the absence of isocitrate, the observed velocities were 19 fmol/min, no dephospho-IDH; 3.5 fmol/min, wild-type dephospho-IDH; and 2.4 fmol/min, dephospho-IDH$^{115L}$. Standard errors were less then 6%.

### Figure 4

**Inhibition of IDH kinase by isocitrate.** The assays contained 5 ng of IDH kinase/phosphatase, 50 μM Mg$^{2+}$[$^{32}$P]ATP and the indicated concentrations of dephospho-IDH (see “Experimental Procedures”). Mg$^{2+}$-D-Isocitrate was present at 0 (●), 25 (□), 50 (○), or 100 μM (△). Standard errors were less then 5%.

In the absence of IDH, any effector capable of modulating this ATPase activity must bind directly to IDH kinase/phosphatase.

Isocitrate and NADPH had no effect on the intrinsic ATPase.
The responses of IDH kinase, IDH phosphatase, and the ATPase activity of the wild-type and mutant proteins to AMP, 3-phosphoglycerate, pyruvate, isocitrate, or NADPH were determined as described under “Experimental Procedures” and in the figure legends. The values shown represent the averages of three independent experiments. Typical experiments are shown in Figs. 1, 2, and 6–8. Standard errors of the mean were less than 20%. $K_{\text{act}}$, effective activation constant; $K_i$, effective inhibition constant; $v_o/v_o^\text{*}$, the ratio of the calculated velocity predicted at saturating concentrations of the effector ($v_o$) to the velocity observed in the absence of the effector ($v_o^\text{*}$); WT, wild-type.

| Effector     | Protein | $K_{\text{act}}$ | $v_o/v_o^\text{*}$ | $K_i$ | $v_o/v_o^\text{*}$ | ATPase |
|--------------|---------|------------------|------------------|-------|------------------|--------|
| AMP ($\mu$M) | WT      | 7                | 2                | 8     | 0.3              | 10     |
|              | AceK3   | 900              | 80               | 170   | 0.3              | 1      |
|              | AceK4   | 60               | 30               | 20    | 0.3              | 1      |
|              | AceK3   | 1                | 1                | 1     | 0.1              | 1.0    |
|              | AceK4   | 30               | 10               | 20    | 0.5              | 0.1    |
|              | AceK3   | 10               | 10               | 4     | 0.2              | 3.0    |
|              | AceK4   | 30               | 100              | 1     | 0.2              | 0.2    |
| Pyruvate (mM)| WT      | 0.2              | 2                | 0.2   | 0.3              | 1      |
|              | AceK3   | 80               | 70               | 4     | 0.4              | 4      |
|              | AceK4   | 10               | 70               | 1     | 0.2              | 0.2    |
| Isocitrate ($\mu$M)| WT | 1                | 16               | 0     | 1                | 1      |
|              | AceK3   | 1                | 15               | 0     | 0                | 0      |
|              | AceK4   | 1                | 11               | 0     | 0                | 0      |

The $K_i$ values describing the inhibition of the ATPase activity by AMP and 3-phosphoglycerate are in good agreement with the constants describing the activation of IDH phosphatase and inhibition of IDH kinase by these effectors. The $K_i$ for inhibition of the ATPase by pyruvate was significantly greater than the $K_i$ for the kinase and the $K_{\text{act}}$ for the phosphatase. The reason for this discrepancy is unclear, but it may reflect differences in the assay conditions (e.g. the presence of IDH in the kinase and phosphatase assays but not in the ATPase assay).

The abilities of AMP, 3-phosphoglycerate, and pyruvate to inhibit the IDH-independent ATPase activity indicate that these effectors bind to IDH kinase/phosphatase. Because isocitrate and NADPH act through the active site of IDH, it appears that effectors that regulate the IDH phosphorylation cycle represent at least two distinct classes that can be distinguished by their site of action.

**Mutations in aceK Affect Regulation of IDH Kinase/Phosphatase**—As a further test of our conclusion that the effectors of IDH kinase/phosphatase fall into two distinct classes, we examined the effects of mutations in aceK, the gene encoding this protein. We focused our efforts on derivatives of IDH kinase/phosphatase having marked reductions in IDH phosphatase activity but retaining a near wild-type level of IDH kinase activity, AceK3 and AceK4. Each protein includes a single amino acid substitution: Q373R in AceK3 and Y414C in AceK4 (17, 26, 31).

AMP, 3-phosphoglycerate, and pyruvate produce modest activations of wild-type IDH phosphatase and partial inhibitions of wild-type IDH kinase (Figs. 6–8, Table III). For each of these effectors, the $K_{\text{act}}$ for activation of the phosphatase was virtually identical to the $K_i$ for inhibition of IDH kinase. The relative activations of the residual AceK3 and AceK4 IDH phosphatase activities by these effectors were dramatically increased compared with the wild-type protein (by 3- to 40-fold). We also observed striking increases in the $K_i$ values for IDH phosphatase (10- to 400-fold). Although the extent of inhibition of the mutant kinases was similar to wild-type, the $K_i$ values were...
substantially increased (2.5- to 20-fold), an effect which was similar to that which we had observed for the $K_{\text{act}}$ values for IDH phosphatase. One result of the greater relative activations of the mutant IDH phosphatases is that the differences between the wild-type and mutant activities were greatly reduced. The specific activities of wild-type and AceK3 IDH phosphatase differed by 125-fold in the absence of effector but by only 3-fold in the presence of saturating levels of AMP (Table IV). Similarly, there was a 250-fold difference in the specific activities of wild-type and AceK4 IDH phosphatase. Saturating concentrations of 3-phosphoglycerate reduced this difference to 8-fold. Although this certainly does not represent an exhaustive analysis of these effects, it appears that changes in effector responses play a significant role in the selective loss of IDH phosphatase activities by AceK3 and AceK4.

In contrast with the results obtained with AMP, 3-phosphoglycerate, and pyruvate, the mutations in aceK3 and aceK4 had no effect on the responses of their products to isocitrate and NADPH (Table III). These results are consistent with the suggestion that these effectors fall into two distinct classes.

**DISCUSSION**

The IDH phosphorylation cycle of *E. coli* is catalyzed by a single, bifunctional protein, IDH kinase/phosphatase. Our results indicate that effectors regulating this protein belong to one of two classes, depending on their effects on IDH kinase and IDH phosphatase, the influence of mutations in *aceK* and *icd* on these responses and their abilities to inhibit the intrinsic ATPase activity. These two classes differ in the locations of the binding sites and in their mechanisms of action.

AMP, 3-phosphoglycerate, and pyruvate belong to one class of effectors. They activate IDH phosphatase and inhibit both IDH kinase and the intrinsic ATPase activities. Their ability to inhibit the ATPase activity in the absence of IDH indicates that they bind directly to IDH kinase/phosphatase. Mutations in *aceK3* and *aceK4* dramatically increase the fold activation and $K_{\text{act}}$ values for IDH phosphatase and the $K_i$ values for IDH
Finally, isocitrate and NADPH had no effect on the regulation of both IDH kinase and IDH phosphatase by these effectors. Both ligands inhibit IDH kinase, and NADPH also inhibits IDH phosphatase (18). These ligands differ from the class defined by AMP, 3-phosphoglycerate, and pyruvate in several respects: (i) they do not activate IDH phosphatase, (ii) they bind to IDH rather than to IDH kinase/phosphatase, (iii) they do not inhibit the IDH-independent ATPase activity, and (iv) the responses to these ligands are not affected by the mutations in aceK3 and aceK4.

Several lines of evidence support the conclusion that occupation of the active site of IDH by the substrates of this enzyme inhibits IDH kinase and phosphatase. A mutation in ica, which reduced the affinity of IDH for isocitrate, had parallel effects on the responses of both IDH kinase and IDH phosphatase to this ligand. Similarly, mutations that converted the preference of IDH from NADP(H) to NAD(H) had parallel effects on the regulation of both IDH kinase and IDH phosphatase by these ligands. Finally, isocitrate and NADPH had no effect on the intrinsic ATPase activity of IDH kinase/phosphatase, in contrast with AMP, pyruvate, and 3-phosphoglycerate, effectors which appear to act by binding to IDH kinase/phosphatase.

Peptide substrates have proven to be very valuable tools for studying protein kinases (32) and might have provided additional insights into the locations of the effector sites for IDH kinase/phosphatase. Unfortunately, that approach was not possible in this case because IDH kinase/phosphatase does not appear to recognize peptide substrates, a tentative conclusion based on our observation that neither tryptic nor chymotryptic peptides derived from either phospho- or dephospho-IDH are substrates for this protein.2 Our suspicion is that unlike many other protein kinases and phosphatase, IDH kinase/phosphatase does not simply recognize a short primary amino acid sequence.

Our conclusion that NADPH regulates IDH kinase/phosphatase through the IDH active site contradicts earlier reports that suggested that the site for this ligand was on IDH kinase/phosphatase (29). Indirect evidence (failure of phospho-IDH to enhance the fluorescence of NADPH) had suggested to the previous authors (29) that NADPH could not bind to phospho-

| Table IV |

Maximal activations of IDH phosphatase

| IDH Phosphatase | Basal | AMP | 3-phosphoglycerate | Pyruvate |
|------------------|-------|-----|-------------------|---------|
| Wild-type        | 25    | 50  | 75                | 50      |
| AceK3            | 0.21  | 17  | 2                 | 15      |
| AceK4            | 0.09  | 3   | 9                 | 6       |

*Figure 8.* Regulation of IDH kinase and IDH phosphatase by pyruvate. IDH phosphatase and IDH kinase were assayed as described under “Experimental Procedures” in the presence of varying concentrations of pyruvate. IDH kinase/phosphatase was wild-type (●), AceK3 (○), or AceK4 (■). Standard errors of the mean were less than 7% of the measured activities. Left panel, IDH phosphatase reactions included 0.3 μM [32P]phospho-IDH, 1.0 mM Mg-ATP, and 0.7 ng of wild-type (WT), 42 ng of AceK3, or 180 ng of AceK4 IDH kinase/phosphatase. Activation was calculated by dividing the activity observed in the presence of pyruvate by that observed without effector. Specific activities observed without effector were 28.17, and 0.06 nmol/mg/min for the wild-type, AceK3, and AceK4 IDH phosphatase, respectively. Right panel, IDH kinase reactions included 5.0 μM dephospho-IDH, 1.0 mM Mg-ATP, and 22 ng of wild-type, 3.8 ng of AceK3, or 6.4 ng of AceK4 IDH kinase/phosphatase. Data were plotted using Equation 3.

*Figure 9.* Model for the regulation of the IDH phosphorylation cycle. This model proposes that IDH kinase/phosphatase can exist in two conformations, one favoring the kinase reaction (Kin) and the other favoring the phosphatase (Pase). This conformational equilibrium is proposed to be controlled by metabolites such as AMP, which bind preferentially to the phosphatase form. IDH (I) can exist in open and closed conformations depending on whether the substrates isocitrate (i) or NADPH (N) are bound. The open conformation is a substrate for IDH kinase/phosphatase, whereas the closed conformation is not. Isocitrate and NADPH might also inhibit IDH kinase/phosphatase by directly interfering with complex formation. The site of phosphorylation, Ser-113, is indicated with a tick mark for dephospho-IDH. Phosphoserine 113 is indicated by a circled P.
IDH even though this ligand inhibited IDH phosphatase. However, it was subsequently demonstrated that NADPH does, indeed, bind to phospho-IDH (11), consistent with our conclusion that this is the site of action for this ligand.

The observation that isocitrate prevented product inhibition of IDH phosphatase by dephospho-IDH suggested that this ligand inhibited the formation of the IDH kinase/phosphatase-IDH complex. Additional support for this conclusion was provided by the observation that inhibition of IDH kinase by isocitrate appeared to be competitive with dephospho-IDH. Similar observations were obtained for NADPH; this ligand was competitive with dephospho-IDH for IDH kinase and with phospho-IDH for IDH phosphatase. Thus, it appears that occupation of either the coenzyme or organic acid binding sites of IDH can prevent complex formation with IDH kinase/phosphatase. We have not directly determined whether a similar mechanism is responsible for inhibition of IDH kinase/phosphatase by other ligands which can bind to the IDH active site (e.g. NADP\(^+\), \(\alpha\)-ketoglutarate), but it seems likely that this is the case.

Previous reports on the effect of isocitrate on IDH phosphatase activity have been contradictory. Whereas some studies found no effect, others reported that this ligand activated the phosphatase even in the absence of added dephospho-IDH (29). Our results suggest that this activation of IDH phosphatase by isocitrate was probably an artifact resulting from the presence of dephospho-IDH as a contaminant of the phospho-IDH.

Our results suggest that occupation of either the coenzyme or organic acid subsite of IDH inhibit complex formation with IDH kinase/phosphatase (see above and Fig. 9). The phosphorylation site, Ser-113, lies within the IDH active site forming a hydrogen bond to isocitrate in the catalytically active, dephospho-form of IDH. It seems very likely that formation of the complex between IDH and IDH kinase/phosphatase includes a number of other residues that also lie within this active site. This suggestion is supported by our observation that mutations within the adenine pocket of the NADPH binding site substantially increased the \(K_m\) of IDH kinase for IDH. Ligands such as isocitrate and NADPH may prevent complex formation by directly interfering with contacts between IDH and IDH kinase/phosphatase. They might also act by inducing the closed conformation of IDH, a form that has been suggested to prevent access to the site of phosphorylation (33). NADPH inhibits both IDH kinase and IDH phosphatase because it binds to both substrate proteins, dephospho- and phospho-IDH. In contrast, isocitrate inhibits only IDH kinase because it binds to the substrate, dephospho-IDH, of this enzyme but does not bind to phospho-IDH, the substrate of IDH phosphatase (11).

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