Subunit Interactions of Yeast NAD⁺-specific Isocitrate Dehydrogenase*

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Yeast mitochondrial NAD⁺-specific isocitrate dehydrogenase is an octamer composed of four each of two nonidentical but related subunits designated IDH1 and IDH2. IDH2 was previously shown to contain the catalytic site, whereas IDH1 contributes regulatory properties including cooperativity with respect to isocitrate and allosteric activation by AMP. In this study, interactions between IDH1 and IDH2 were detected using the yeast two-hybrid system, but interactions between identical subunit polypeptides were not detected with this or other methods. A model for heterodimeric interactions between the subunits is therefore proposed for this enzyme. A corollary of this model, based on the three-dimensional structure of the homologous enzyme from Escherichia coli, is that some interactions between subunits occur at isocitrate binding sites. Based on this model, two residues (Lys-183 and Asp-217) in the regulatory IDH1 subunit were predicted to be important in the catalytic site of IDH2. We found that individually replacing these residues with alanine results in mutant enzymes that exhibit a drastic reduction in catalysis both in vitro and in vivo. Also based on this model, the two analogous residues (Lys-189 and Asp-222) of the catalytic IDH2 subunit were predicted to contribute to the regulatory site of IDH1. A K189A substitution in IDH2 was found to produce a decrease in activation of the enzyme by AMP and a loss of cooperativity with respect to isocitrate. A D222A substitution in IDH2 produces similar regulatory defects and a substantial reduction in Vmax in the absence of AMP. Collectively, these results suggest that the basic structural/functional unit of yeast isocitrate dehydrogenase is a heterodimer of IDH1 and IDH2 subunits and that each subunit contributes to the isocitrate binding site of the other.

NAD⁺-specific isocitrate dehydrogenase is thought to be important for regulatory control of mitochondrial energy metabolism primarily because of kinetic responses to adenine nucleotides in in vitro assays. For example, catalytic activation of this tricarboxylic acid cycle enzyme from Saccharomyces cerevisiae by AMP (i.e. a response to low levels of ATP) was described several decades ago (1). A correlate of allosteric regulation is multisubunit structure, and, based on sedimentation velocity and gel filtration experiments, an octameric structure was proposed for yeast isocitrate dehydrogenase (2, 3). However, a nonequivalence of subunits was suggested by equilibrium binding studies that demonstrated twice the number of isocitrate binding sites relative to binding sites for Mg²⁺ or NAD⁺ (4).

In more recent studies, the yeast enzyme was shown to be composed of two nonidentical subunits, IDH1 (M₉ = 38,001) and IDH2 (Mₑ = 37,755), with both being equally represented in the holoenzyme (5). The two subunits share 42% identity at the level of amino acid sequence. Both subunits were shown to be essential for holoenzyme activity, since disruption of either or both genes encoding the subunits results in yeast strains that exhibit no detectable NAD⁺-specific isocitrate dehydrogenase activity and that are unable to grow with acetate as a carbon source (6, 7). The acetate growth phenotype is shared with yeast mutants containing disruptions in genes encoding other tricarboxylic acid cycle enzymes including malate dehydrogenase (8) and citrate synthase (9).

The yeast IDH1 and IDH2 subunits also share 32% sequence identity with Escherichia coli isocitrate dehydrogenase, which requires NADP⁺ as cofactor. The bacterial enzyme is a homodimer and contains two identical isocitrate-Mg²⁺ and NADP⁺ binding sites per dimer (12). The E. coli enzyme exhibits no allosteric regulation but is instead regulated by phosphorylation. This covalent modification of a specific serine residue located in the isocitrate binding pocket inactivates the enzyme under physiological conditions requiring reduced flux through the tricarboxylic acid cycle (11, 12). The target serine residue of the E. coli enzyme is conserved in both IDH1 and IDH2, and mutagenesis was previously performed to determine the function of the corresponding residues in each yeast subunit (13). An S98A substitution in IDH2 produced a mutant enzyme that exhibited a 60-fold decrease in Vmax but retained AMP activation and cooperativity. In contrast, an S92A substitution in IDH1 produced a mutant enzyme with primary kinetic defects in regulatory properties of the enzyme, including loss of both AMP activation and cooperativity with respect to isocitrate. Based on these results, different functions were assigned to the subunits, with IDH2 being primarily responsible for catalysis and with IDH1 playing the primary role in regulation. In support of these assignments, residues comprising the catalytic site of the bacterial enzyme are more highly conserved in IDH2 than in IDH1 (7). We therefore postulate that both subunits contain isocitrate binding sites, but while the “active” site in IDH2 is catalytic, the active site in IDH1 has evolved to bind isocitrate for the purpose of cooperative control rather than catalysis.

Some subsequent mutagenesis studies have supported these designations of subunit function (14). In these studies, specific adjacent residues thought to be important for cofactor specificity were altered. These changes in IDH2 (D286A plus I287A) resulted in a dramatic decrease in activity due to a reduction in affinity for NAD⁺. However, parallel residue replacements in IDH1 (D279A plus I280A) eliminated AMP activation of the
enzyme. These results supported the designations of IDH2 as the catalytic subunit and of IDH1 as the regulatory subunit, and we postulate that homologous adenine nucleotide binding domains have evolved for binding of the cofactor by IDH2 and for binding of the allosteric activator by IDH1.

In contrast with results described above, other recent experiments have revealed that catalytic and regulatory functions are not strictly confined to distinct subunits. Based on a residue in pig heart isocitrate dehydrogenase that was previously implicated in the binding of an adenine nucleotide analog (15), the homologous conserved aspartate residues in IDH1 and IDH2 were altered. Mutation of the conserved aspartate residue (D197A) in IDH2 reduced AMP activation, cooperativity of substrate binding, and \( V_{\text{max}} \). Altering the homologous residue in IDH1 (D191A) produced an inactive enzyme (14).

To understand the functions of IDH subunits in more detail, the current study further examines the interactions and specific functions of subunits in yeast isocitrate dehydrogenase. Based on previous results showing that both IDH1 and IDH2 subunits copurify when only one is affinity-tagged (14) and that each residual subunit appears to be monomeric in the absence of the other subunit (6, 7), we have investigated the possibility that the basic structural/functional unit of the enzyme is a heterodimer of an IDH1 and an IDH2 subunit. As a corollary, based on the bacterial enzyme model for homodimeric subunit interactions, we have examined the potential contribution of a few residues from each yeast subunit to the isocitrate binding site of the other subunit. In light of results presented in this paper, we now interpret the kinetic effects of the D197A replacement in IDH2 and of the D191A replacement in IDH1 as indicative of contributions of each residue to the active site of the other subunit in the heterodimer.

EXPERIMENTAL PROCEDURES

Yeast Strains and Growth Conditions—In expression studies, the yeast haploid strain S173-6B (MATa, leu2-3, 112, his3-1, ura3-52, trp1-289, Ref. 16) was the parental wild-type control. Strains containing gene disruptions (ΔIDH1::LEU2, ΔIDH2::HIS3, or ΔIDH1::ΔLEU2/ΔIDH2::HIS3) were constructed using the parental strain as described previously (6, 7). Yeast strains used in two-hybrid analyses were Y190 (MATa, ura3-52, his3-200, lys2-801, ade2-101, trp1-901, leu2-3, 112, gal4Δ, gal80Δ, cyb52, LYS2::GAL1::HIS3, URA3::GAL1::lacZ), provided by CLONTECH Laboratories, Inc., and PJ69–4 (MATa, trp1-901, leu2-3, 112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL2::HIS3, GAL2::ADE2, met2::GAL1::lacz), provided by Dr. Philip James (17). Strains were grown in YP medium (1% yeast extract, 2% Bactopeptone) or in YNB medium (0.17% yeast nitrogen base, 0.5% ammonium sulfate, pH 5.8) containing 2% final concentrations of various carbon sources. YNB medium contained the appropriate amino acids or nucleotides required for growth.

Yeast Two-hybrid Constructs and Analyses—Polymerase chain reaction (PCR)1 was used to synthesize IDH1 and IDH2 gene fragments that lacked codons for the mitochondrial targeting sequences. PCR primers also introduced BamHI restriction sites at 5′ and 3′ of the coding sequences for the mature forms. The two gene fragments were subcloned into vectors pGBT9 and pGAD424 (CLONTECH Laboratories, Inc.), and the inserts were sequenced by the Center for Advanced DNA Technologies, San Antonio, TX, to ensure that no errors were introduced by PCR. The BamHI fragments were then subcloned into two other sets of two-hybrid vectors: pAS2–1 and pACT2 (CLONTECH Laboratories, Inc.) and pGBD-C2 and pGAD-C2 (17). The fusion constructs were then transformed singly and in all possible binding/activation domain pairs into the appropriate host strains: the CLONTECH vector constructs into strain Y190 and the pGBD-C2 and pGAD-C2 fusion constructs into PJ69–4. Transformants were selected for the presence of plasmids by growth on YNB glucose plates lacking appropriate nutrients. After streaking onto similar plates, transformants were tested for their ability to activate the reporter gene(s). Y190 transformants were analyzed using a β-galactosidase colony lift filter assay, which was performed using CLONTECH Laboratories Protocol PT1030–1. A control strain contained a plasmid encoding the wild type GAL4 protein. PJ69–4 transformants were tested for transformants to grow on plates in the absence of adenine or histidine after incubation from 3 to 5 days at 30 °C.

Affinity Purification Tests for Identical Subunit Interactions—A 2.3-kilobase pair Xba/HindIII fragment containing the IDH2 gene with codons for a C-terminal histidine tag was subcloned from pIDH1/IDH2Δhis into pRS316. The resulting construct (pRS316 IDH2Δhis) was transformed into the ΔIDH1 host strain. Immunoblot analysis was used to verify that this strain expressed two versions of IDH2, one lacking and one histidine-tagged. Similarly, a 4.0-kilobase pair XbaI fragment containing the IDH1 gene with codons for a histidine tag was subcloned from pIDH1/IDH2Δhis into pRS316. The resulting construct (pRS316 IDH1Δhis) was transformed into the ΔIDH2 strain. This transformant contained two types of IDH1, one histidine-tagged and one native. The two strains were grown in 500-ml cultures of YP medium with glycerol/lactate as the carbon source to induce expression of the isocitrate dehydrogenase subunits (15). The cells were harvested, and extracts were used for affinity purification as described previously (14) with the following modifications. Buffer A contained only 20 mM imidazole, and the column absorption/elution procedure was not repeated. Column flow-through, wash, and eluant fractions were combined with equal volumes of loading buffer and electrophoresed on a 15% polyacrylamide/SDS gel. The gel was transferred to a polyvinylidene difluoride membrane and blocked in 5% bovine serum albumin over-night. For analysis of fractions from the ΔIDH1 transformant expressing IDH2Δhis, the membrane was incubated in a 1:500 dilution of IDH antiserum (5), and protein was detected by autoradiography after incubation of the membrane with \( ^{125} \text{T}-labeled \) protein A (19). Analysis of fractions from the ΔIDH2 transformant was conducted using enhanced chemiluminescence (Amersham Pharmacia Biotech ECL Kit and protocol).

Construction and Purification of Mutant Enzymes—Mutagenesis was performed using the Transform Site-Directed Mutagenesis Kit from CLONTECH Laboratories and the following primers to introduce planned substitutions: IDH1 K183A (5′-CAGCTTGTCGATCCGCAATTAATCATTACG), IDH1 D217A (5′-ATGCGTCTACATGCTTATCCAGGAC), and IDH2 D222A (5′-GAACTGTAACATTATGCGGCGCAGTTAAAG). Another primer was used to simultaneously eliminate a unique BamHI site in the vector for selection of the mutant plasmids. All modifications were confirmed by sequencing. A 1.5-kilobase pair pHis/EcoRI fragment containing each mutation in IDH1 was subcloned into pRS316 (14). Similarly, a 1.5-kilobase pair pHis/HindIII fragment containing each mutation in IDH2 was subcloned into pIDH1/IDH2Δhis (14). Each of the four resulting plasmids and the two wild-type plasmids (pIDH1Δhis/IDH2Δhis and pIDH1/IDH2Δhis) were transformed into a ΔIDH1/ΔIDH2 host strain. Transformant strains were grown in 0.5–1.0 liters of YP medium with glycerol/lactate as the carbon source.

Affinity-tagged enzymes were purified by chromatography using Ni2+–NTA resin (Qiagen) as described previously (14). Concentrations of purified proteins were determined by the method of Lowry (20). To ensure that holoenzyme was purified from all strains, samples of \( 15 \mu \text{g} \) of each purified enzyme were electrophoresed on a 10% polyacrylamide/SDS gel and stained with Coomassie Blue (19).

Kinetic Analyses of Purified Enzymes—Isocitrate dehydrogenase activity was measured as described previously (5, 14). Values for \( V_{\text{max}} \) and \( K_m \) were determined from Hanes analysis (21) of initial velocity data. One unit of isocitrate dehydrogenase activity was defined as production of 1.0 pmol of NADH/min. All assays were performed at 24 °C and initiated by the addition of isocitrate. Data shown in plots are from a single representative experiment, whereas tabulated data represent results from three independent experiments. Hill plots include data points between 10 and 90% of \( V_{\text{max}} \).

RESULTS

Yeast Two-hybrid Analyses—For analysis of subunit interactions in yeast isocitrate dehydrogenase using the yeast two-hybrid system, PCR was used to amplify IDH1 and IDH2 coding regions lacking mitochondrial targeting sequences. The amplified genes were sequenced to ensure that no errors were introduced by PCR and ligated into three different sets of yeast two-hybrid plasmids. Two vector pairs, pGWB9 and pGAD424 and pAS2–1 with pACT2, were utilized. The first in each pair contains the nucleotide sequence for the GAL4 DNA-binding

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1 The abbreviations used are: PCR, polymerase chain reaction; NTA, nitrilotriacetic acid.
IDH1 and IDH2 were isolated, and all possible combinations of GAL4 domain fusions with sequence. Transformants of Y190 containing plasmids encoding was also used to test isocitrate dehydrogenase subunit interactions. With this assay, as results are defined as the absence of detectable expressed with any of these vector pairs (Table I). Negative tests for growth without adenine. Again, no interaction between IDH1 subunits, with the bulk of the native subunit and a small portion of the affinity-tagged species appearing in the column flow-through fraction (Fig. 1b, lane 1). Again, only the affinity-tagged form of IDH1 is observed in the fraction eluted with high concentrations of imidazole (Fig. 1b, lanes 3–5).

Thus, results from both two-hybrid and affinity purification methods demonstrate that subunit polypeptides show no substantial homomeric interactions, at least in the absence of the other subunit, and suggest that the basic structural unit of the enzyme is a heterodimer of an IDH1 and an IDH2 subunit.

**Mutagenesis and Kinetic Analyses**—Our current structural model for the yeast enzyme is based on information from the x-ray crystallographic structure of E. coli NADP⁺-dependent isocitrate dehydrogenase (22). In the homodimeric bacterial enzyme, residues comprising the isocitrate binding site, as illustrated in Fig. 2, are predominantly located in one subunit. However, two residues designated by prime symbols, Lys-230 and Asp-283′, are contributed by the other subunit (Fig. 2). Lys-230′ has been shown to be essential for the decarboxylation step in catalysis (23), and Asp-283′ contributes to coordination of Mg²⁺ in the active site of the other subunit (22). These two residues are conserved in similar relative positions in both IDH1 and IDH2 (Fig. 3). Based on our model for heterodimeric interaction, we propose that the conserved residues in IDH1, Lys-183 and Asp-217, might be important in the catalytic sub-

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**Table I**

<table>
<thead>
<tr>
<th>Heterodimer tests</th>
<th>Homodimer tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Construct pair a</td>
<td>Reporter gene assay b</td>
</tr>
<tr>
<td>pGBT9-IDH1 + pGAD424-IDH2</td>
<td>β-gal +</td>
</tr>
<tr>
<td>pGBT9-IDH2 + pGAD424-IDH1</td>
<td>β-gal -</td>
</tr>
<tr>
<td>pAS2–1-IDH1 + pACT2-IDH2</td>
<td>β-gal -</td>
</tr>
<tr>
<td>pAS2–1-IDH1 + pACT2-IDH2</td>
<td>β-gal -</td>
</tr>
<tr>
<td>pGBT9-IDH1 + pACT2-IDH2</td>
<td>β-gal +</td>
</tr>
<tr>
<td>pGBT9-IDH2 + pACT2-IDH1</td>
<td>β-gal +</td>
</tr>
<tr>
<td>pAS2–1-IDH2 + pGAD424-IDH2</td>
<td>β-gal +</td>
</tr>
<tr>
<td>pGBD-C2-IDH1 + pGAD-C2-IDH2</td>
<td>β-gal -</td>
</tr>
<tr>
<td>pGBD-C2-IDH1 + pGAD-C2-IDH2</td>
<td>β-gal -</td>
</tr>
<tr>
<td>pGBD-C2-IDH2 + pGAD-C2-IDH1</td>
<td>β-gal /His +</td>
</tr>
</tbody>
</table>

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a The first plasmid in each pair contains an IDH gene fusion with the GAL4 DNA binding sequence, and the second contains an IDH gene fusion with the GAL4 transcription activation domain sequence.

b Reporter gene activity was determined as outlined under “Experimental Procedures.” β-gal is β-galactosidase activity indicated by filter lift assays; Ade and His refer to growth on plates with minimal medium in the absence of adenine or histidine.
alle of the other subunit in a \( \Delta IDH1 \Delta IDH2 \) yeast strain. Mutant enzymes were purified by Ni\(^{2+}\)-NTA affinity chromatography as described under “Experimental Procedures.” Samples of all purified enzymes were electrophoresed on a denaturing gel and stained with Coomassie Blue. As shown in Fig. 4, both subunits copurify during affinity chromatography of the mutant enzymes. Differences in electrophoretic mobility are due to the histidine tags (e.g., compare mobility of the more slowly migrating histidine-tagged form of IDH1 in lanes 1–3 versus that for the native IDH1 subunit in lanes 4 and 5) and presumably to amino acid substitutions in mutant subunits as also previously observed (14). Differences in mobility are observed for both subunits containing substitutions for aspartate residues, D222A in IDH2 (Fig. 4, lane 2) and D217A in IDH1 (Fig. 4, lane 5). None of the residue substitutions appears to have any gross conformational effect on holoenzyme structure, since both subunits copurify when only one subunit is affinity-tagged.

Measurements of \( V_{\text{max}} \) were conducted using affinity-purified enzymes. \( S_{0.5} \) values for isocitrate were measured in the presence or absence of 100 \( \mu M \) AMP, and corresponding Hill coefficients were calculated. \( S_{0.5} \) values and Hill coefficients for NAD\(^+\) were determined in the presence of AMP. Results of the kinetic studies are illustrated in Fig. 5 and summarized in Table II. As previously reported (14), the two histidine-tagged wild-type enzymes share similar kinetic properties. \( S_{0.5} \) values for isocitrate are similar and reduced 5–6-fold in the presence of 100 \( \mu M \) AMP. For both enzymes, Hill coefficients calculated in the presence or absence of AMP range from 3.2 to 3.9.

As predicted by the model described above, mutant enzymes containing IDH1 K183A or D217A substitutions (with IDH2 histidine-tagged) exhibit severe defects in catalytic activity. For these mutant enzymes, using as much as 19 \( \mu g \) of enzyme per 1-ml reaction produces a barely measurable change in absorbance unit per min. Thus, the full range of kinetic parameters for these enzymes could not be determined. However, based on the minimally measurable activities, the substitution of alanine for Asp-217 produces a greater catalytic defect than the same replacement for Lys-183. Respective velocities of 6.7 \( \times 10^{-3} \) units/mg and 3.2 \( \times 10^{-2} \) units/mg are more than 1000- and 300-fold lower than the \( V_{\text{max}} \) value of the corresponding wild-type enzyme.

As also predicted by the model, we find that the cooperativity with respect to isocitrate is lost in the mutant IDH2 enzymes containing K189A or D222A substitutions; i.e., Hill coefficients for isocitrate are reduced to values slightly greater than 1 (Fig. 5 and Table II). In addition, allosteric activation of catalytic activity by AMP is also eliminated by the substitutions for Lys-189 and Asp-222; i.e., \( S_{0.5} \) values for isocitrate are largely unaffected by the presence of AMP. Thus, while \( S_{0.5} \) values for isocitrate measured in the absence of this activator are similar for mutant and wild-type enzymes, the \( S_{0.5} \) values measured in the presence of 100 \( \mu M \) AMP are, respectively, 7- and 9-fold greater for the K189A and D222A enzymes than that measured for the corresponding wild-type enzyme. However, interestingly, AMP does appear to activate the D222A mutant enzyme by increasing \( V_{\text{max}} \) approximately 4-fold over values measured in the absence of AMP. In the absence of AMP, the \( V_{\text{max}} \) for the D222A mutant enzyme is 18-fold lower, whereas the \( V_{\text{max}} \) for the IDH2 K189A enzyme is only 2-fold lower than that of the wild-type control. In fact, for these assays examining kinetic parameters, it was necessary to use a concentration of the IDH2 D222A enzyme 10-fold greater than that of the IDH2 K189A enzyme. Thus, while expected effects on regulatory properties were obtained with both substitutions, a more dramatic effect on catalysis is obtained with the D222A substitu-
Partial amino acid sequence alignments of IDH1 and IDH2 with *E. coli* isocitrate dehydrogenase. Boxed are bacterial residues Lys-230’ and Asp-283’ that contribute to the isocitrate binding site of the other subunit. Conserved residues are shown in boldface type.

**Fig. 3.** Partial amino acid sequence alignments of IDH1 and IDH2 with *E. coli* isocitrate dehydrogenase. Boxed are bacterial residues Lys-230’ and Asp-283’ that contribute to the isocitrate binding site of the other subunit. Conserved residues are shown in boldface type.

**Fig. 4.** Electrophoretic analysis of affinity-purified wild-type and mutant forms of yeast isocitrate dehydrogenases. Histidine-tagged enzymes were expressed and purified as described under “Experimental Procedures.” Samples containing ~15 μg of each purified enzyme were utilized for electrophoresis and Coomassie Blue staining. The purified enzymes included IDH1His/IDH2 (lane 1), IDH1His/IDH2D222A (lane 2), IDH1His/IDH2K189A (lane 3), IDH1/IDH2His (lane 4), IDH1D217A/IDH2His (lane 5), and IDH1K183A/IDH2His (lane 6). In each lane, the slower migrating band is IDH1, and the faster migrating band is IDH2.
whereas identical subunit polypeptides show no substantial interactions with these tests. This experimental evidence suggests that the basic structural/functional unit of this enzyme is a heterodimer, although other interactions clearly occur within the octameric holoenzyme. Furthermore, based on the structure of the E. coli isocitrate dehydrogenase homodimer, which shows that specific residues, Lys-230' and Asp-238' (Fig. 2), from one subunit contribute to the isocitrate binding site of the other, results of this study suggest that the same is true for the IDH2. This residue is located near IDH1 Lys-183', and it corresponds to the bacterial residue Asp-238 located near residues 234–236 that are known to be involved in intersubunit contacts (24).

For mutant yeast enzymes with substitutions in residues with putative functions in regulatory binding of isocitrate (Table III, IDH1 Ser-92 (13) and IDH2 Lys-183' or Asp-238' (this study)), both cooperativity and reduction of $S_{0.5}$ values by AMP are defective. Adjacent substitutions within the putative AMP binding region of IDH1 (Asp-279/Ile-280 (14)) produce a loss of activation but no effect on cooperativity. We also include in this regulatory category a previous mutant enzyme with a substitution for IDH2 Asp-197', the reciprocal of IDH1 Asp-191. This residue is located near IDH2 Lys-189', and the IDH2 D197A enzyme exhibits kinetic defects consistent with a contribution to the regulatory isocitrate binding site of IDH1.

Of particular interest are the effects of substitutions for “regulatory” residues on $V_{\text{max}}$ (Table III). None of these effects are as striking as the defects produced with substitutions for “catalytic” residues, but deleterious effects range from 2- to 18-fold decreases relative to $V_{\text{max}}$ values for corresponding wild-type enzymes. That we always see some effect on catalysis indicates that full catalytic activity may require regulatory functions. It was in fact predicted over 30 years ago (1) that the regulatory isocitrate binding site or the AMP binding site must be occupied to obtain maximal catalytic activity. However, because effects on $V_{\text{max}}$ vary significantly for mutant enzymes that exhibit similar defects in cooperativity, it may be that some residues have dual functions or that portions of the polypeptide chains containing these residues may be located in proximity of both subunit active sites. IDH2 Asp-222' is particularly interesting in this respect. This residue appears to be.

Table II
Kinetic properties of affinity-purified enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$V_{\text{max}}$</th>
<th>Isocitrate</th>
<th>NAD</th>
<th>Hill coefficient</th>
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</thead>
<tbody>
<tr>
<td>$V_{\text{max}}$</td>
<td>$S_{0.5}$</td>
<td>$-\text{AMP}+/\text{AMP}$</td>
<td>Hill coefficient</td>
<td>$-\text{AMP}+/\text{AMP}$</td>
</tr>
<tr>
<td>IDH1/IDH2$^{2	ext{nd}}_{\text{His}}$</td>
<td>10.43 ± 1.30</td>
<td>0.80/0.13 ± 0.08/0.03</td>
<td>3.9/3.2 ± 0.1/0.3</td>
<td>0.15 ± 0.05</td>
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<tr>
<td>IDH1$^{1	ext{st}}<em>{\text{His}}$/IDH2$^{2	ext{nd}}</em>{\text{His}}$</td>
<td>-a</td>
<td>-a</td>
<td>-a</td>
<td>-a</td>
</tr>
<tr>
<td>IDH1$^{1	ext{st}}<em>{\text{His}}$/IDH2$^{2	ext{nd}}</em>{\text{His}}$</td>
<td>10.89 ± 1.49</td>
<td>0.81/0.17 ± 0.08/0.04</td>
<td>3.6/3.2 ± 0.2/0.1</td>
<td>0.16 ± 0.02</td>
</tr>
<tr>
<td>IDH1$^{1	ext{st}}<em>{\text{His}}$/IDH2$^{2	ext{nd}}</em>{\text{His}}$</td>
<td>5.11 ± 0.72</td>
<td>1.38/1.16 ± 0.32/0.18</td>
<td>1.5/1.6 ± 0.1/0.1</td>
<td>0.45 ± 0.29</td>
</tr>
<tr>
<td>IDH1Asp$^{1	ext{st}}$/IDH2Asp$^{2	ext{nd}}$</td>
<td>-AMP 0.62/AMP 2.72</td>
<td>1.14/1.47 ± 0.29/0.37</td>
<td>1.3/1.6 ± 0.3/0.1</td>
<td>0.84 ± 0.52</td>
</tr>
</tbody>
</table>

a, --; these mutant enzymes are not amenable to kinetic characterization due to minimal levels of activity detected with optimal assay conditions.

Table III
Summary of kinetic effects of reciprocal residue replacements in IDH subunits

<table>
<thead>
<tr>
<th>Enzyme(a)</th>
<th>$V_{\text{max}}$</th>
<th>Cooperative binding (isocitrate)</th>
<th>Activation(BAMP)</th>
<th>Enzyme(a)</th>
<th>$V_{\text{max}}$</th>
<th>Cooperative binding (isocitrate)</th>
<th>Activation(BAMP)</th>
</tr>
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<td>$V_{\text{max}}$</td>
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<tr>
<td>IDH1$^{1	ext{st}}$/IDH2$^{2	ext{nd}}$</td>
<td>↓ 60</td>
<td>+</td>
<td>+</td>
<td>IDH1$^{2	ext{nd}}$/IDH2$^{1	ext{st}}$</td>
<td>↓ 6</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>IDH1$^{1	ext{st}}$/IDH2$^{2	ext{nd}}$</td>
<td>↓ 180</td>
<td>ND</td>
<td>ND</td>
<td>IDH1$^{2	ext{nd}}$/IDH2$^{1	ext{st}}$</td>
<td>↓ 2</td>
<td>+</td>
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<td>IDH1$^{1	ext{st}}$/IDH2$^{2	ext{nd}}$</td>
<td>↓ 1000</td>
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<td>ND</td>
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<td>↓ 18</td>
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<td>–</td>
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<tr>
<td>IDH1$^{1	ext{st}}$/IDH2$^{2	ext{nd}}$</td>
<td>↓ 300</td>
<td>ND</td>
<td>ND</td>
<td>IDH1$^{2	ext{nd}}$/IDH2$^{1	ext{st}}$</td>
<td>↓ 13</td>
<td>–</td>
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- fold in $V_{\text{max}}$ values relative to those for corresponding histidine-tagged wild-type enzymes; ND, not determined due to low or undetectable catalytic activity.
- Values based on Hill coefficients determined for isocitrate. +, values of 3–4; –, values of 1–2; ND, not determined due to low catalytic activity.
- Values based on relative decrease in $S_{0.5}$ values for isocitrate when measured in the presence of 100 μM AMP. +, 7–10-fold decrease; –, 0–2-fold decrease; ND, not determined due to low catalytic activity.
- For this mutant enzyme, $V_{\text{max}}$ when measured in the presence of 100 μM AMP, is reduced 4-fold relative to wild type.

Subunit Interactions of IDH
important for regulatory properties presumably through contributions to the IDH1 active site. However, its replacement also has a deleterious effect on $V_{\text{max}}$, more so in the absence of AMP (a 4-fold decrease). One possibility is that IDH2 Asp-222 is important for functional communication between the active sites of the IDH1 and IDH2 subunits, particularly in the absence of allosteric activator.

Overall, it appears that the yeast IDH1 and IDH2 subunits have independently evolved for different functions but that physical interactions between the subunits, including residues that contribute to reciprocal subunit function, have been maintained. It is also clear that, while the bacterial and yeast isocitrate dehydrogenases differ substantially in terms of subunit composition, cofactor specificity, and regulation, significant structural motifs have been conserved. We predict that information obtained from the $E. \text{coli}$ enzyme will continue to aid in functional analysis of the more complex $S. \text{cerevisiae}$ homologue. Ongoing crystallographic analysis of the yeast enzyme should also clarify, by comparison with the bacterial structure, the basis and evolution of its allosteric properties.

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REFERENCES