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Allosteric Inhibition of NAD$^+$-Specific Isocitrate Dehydrogenase by a Mitochondrial mRNA†

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ABSTRACT: NAD$^+$-specific isocitrate dehydrogenase (IDH) has been reported to bind sequences in 5′-untranslated regions of yeast mitochondrial mRNAs. In the current study, an RNA transcript containing the 5′-untranslated region of the mRNA from the yeast mitochondrial *COX2* gene is shown to be an allosteric inhibitor of the affinity-purified yeast enzyme. At 0.1 μM concentrations of the transcript, velocity of the IDH reaction is reduced to 20% of the value obtained in the absence of the RNA transcript. This inhibition is due to a 2.5-fold increase in the $S_{0.5}$ value for isocitrate. Significant inhibition of IDH activity is also obtained with a transcript containing a portion of the 5′-untranslated region of the yeast mitochondrial *ATP9* gene and with an antisense form of the *COX2* transcript, both of which contain potential stem-loop secondary structures implicated in binding of IDH. In contrast, much higher concentrations of yeast tRNA or poly(A)mRNA, respectively, 33- and 60-fold greater than that required for the *COX2* transcript, are required to produce a 50% decrease in velocity. These results suggest that inhibition of activity is relatively specific for the 5′-untranslated regions of mitochondrial mRNAs. All measurable inhibition of IDH activity by RNA is eliminated by addition of 100 μM concentrations of the allosteric activator AMP. At equivalent concentrations, dAMP is less efficient than AMP as an allosteric activator of IDH and is proportionally less effective in protecting against inhibition of activity by the *COX2* transcript. Other nucleotides that are not allosteric activators fail to protect IDH activity from inhibitory effects of RNA. Thus, alleviation of catalytic inhibition of IDH by mitochondrial mRNA correlates with the property of allosteric activation.

The oxidative decarboxylation reaction catalyzed by mitochondrial NAD$^+$-specific isocitrate dehydrogenase (IDH)[1] is considered to be a rate-limiting step in the tricarboxylic acid cycle. Sensitive kinetic response to the positive allosteric regulator ADP by the mammalian enzyme (*1*) and to AMP by the yeast enzyme (*2*) is the basis for the hypothesis that rates of respiration are finely controlled at the level of this reaction by cellular energy charge (*3*).

IDH enzymes are structurally complex. The mammalian enzyme is composed of three subunits in an α_2:β:γ ratio (*4*−*6*). The yeast (*Saccharomyces cerevisiae*) enzyme is an octamer composed of equimolar amounts of two subunits designated IDH1 and IDH2 (*7*). IDH1 and IDH2 share a residue sequence identity of 42%, and the mature polypeptides are similar in size with respective molecular weights of 38 000 and 37 800. Both subunits are essential for catalytic activity (*8*, *9*). However, mutagenesis studies have shown that IDH2 is the primary contributor to catalytic function and IDH1 is the primary contributor of regulatory properties to the holoenzyme (*10*, *11*).

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[1] Abbreviations: IDH, isocitrate dehydrogenase; PCR, polymerase chain reaction; UTR, untranslated region.

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In addition to kinetic regulation of energy metabolism, yeast IDH has been found to be an RNA-binding protein (*12*). The holoenzyme binds sequences near potentially conserved secondary structures within the 5′ untranslated regions of all eight major yeast mitochondrial mRNA transcripts (*13*). Both IDH subunits are necessary for this RNA binding (*12*). The specificity of this interaction was established by gel shift binding and competition experiments that demonstrated no significant binding of IDH to mitochondrial RNAs lacking the 5′-untranslated regions or to a variety of other nonspecific RNAs (*14*). The significance of IDH/mitochondrial mRNA interactions is unclear at present. However, in analogy to iron-responsive control of mRNA stability and/or translation imparted by binding of cytosolic aconitase (also called iron-responsive protein) in mammalian cells (*15*), Elzinga et al. (*12*) have suggested that the IDH/mRNA interaction might regulate similar aspects of expression in response to energy charge. Reported phenotypes for yeast mutants containing gene disruptions in *IDH1* and/or *IDH2* loci include an inability to grow with acetate as a carbon source (*8*, *9*), a phenotype shared with several other yeast tricarboxylic acid cycle mutants (*16*, *17*), and an increase in frequency of petite offspring, a phenotype shared with yeast mutants with defects in mitochondrial translation (*18*). These phenotypes suggest that IDH may be a multifunctional enzyme.

RNA−protein interactions are commonly investigated to examine consequences on RNA function or stability. As reported here, we have taken the opposite approach in investigating the effect of RNA on kinetic properties of yeast IDH. These studies were conducted with an RNA transcript essentially identical to the RNA transcript originally used to identify and investigate IDH−RNA interactions with gel shift assays (*14*). The transcript contains the 54-nucleotide 5′-untranslated region of the mRNA from the yeast *COX2* gene, one of three mitochondrial genes encoding subunits of cytochrome oxidase. The *COX2* leader sequence is the shortest of the 5′-untranslated regions of the eight yeast mitochondrial mRNAs and has been extensively analyzed for its role in efficient translation of COX2 (*19*−*21*). We have found that the RNA transcript containing the *COX2* 5′-untranslated region has a remarkable inhibitory effect on IDH catalysis by reducing apparent affinity for isocitrate and that this allosteric inhibition by RNA is alleviated in the presence of AMP.

EXPERIMENTAL PROCEDURES

Yeast Strains and Growth Conditions. Yeast strain D273-10B (*MATα mal [rho$^+$] CAN^S*, American Type Culture Collection no. 24657) was used as a source of genomic DNA for polymerase chain reaction (PCR). The yeast Δ*IDH1*Δ*IDH2* gene disruption mutant used for expression of histidine-tagged enzymes was previously constructed using deletion/disruption methods (*9*) with strain S173−6B (*MATa leu2-3,112 his3-1 ura3-52 trp1-289*, *22*). Minimal YNB medium (0.17% yeast nitrogen base, 0.5% ammonium sulfate, pH 6.5) with 2% glucose and 20 μg/mL of appropriate supplements to satisfy auxotrophic requirements was used in agar plates for storage and in liquid form for precultures of disruption mutant transformants. For preparation of enzyme, precultures were diluted into 500 mL of YP medium (1% yeast extract, 2% Bacto-peptone) containing 2% glycerol and 2% lactate and cells harvested by centrifugation upon reaching culture densities of $A_{600nm} = 1.6-1.9$.

Preparation of RNA Transcripts. Genomic DNA from yeast strain D273-10B was used for PCR to generate a 133 bp DNA fragment containing the 5′-untranslated sequence and a portion of the coding sequence from the mitochondrial *COX2* gene. Oligonucleotides used for amplification were a 44-mer containing a *Bam*HI restriction site and sequences from the 5′ end of the untranslated sequence (5′-GCGGGATT-CTAATTAAAAGTAGTATTAACATATTATAAATAGAC) and a 38-mer containing sequences complementary to the 3′ end of the coding sequence and a *Hin*dIII restriction site (5′-GGGGAAGCTTCATCATTCATAATGAATGTTGTTA-ATTG). The resulting PCR fragment was subcloned into the polylinker downstream of a T7 promoter in plasmid pBS$^+$ (Stratagene) and sequenced. For some experiments, runoff transcription assays were conducted using as the template the pBS*COX2* plasmid linearized with *Hin*dIII; resulting transcripts contain sequences from the plasmid multicloning sites. For most experiments, transcripts lacking the multicloning sequences were generated by in vitro transcription of PCR fragments. A PCR fragment containing the *COX2* sequence was amplified using the *Bam*HI/*Hin*dIII fragment from pBS*COX2* as template with a 58-mer oligonucleotide containing a T7 promoter sequence adjacent to the *COX2* 5′-untranslated sequence (5′-TAATACGACTCACTATAGGG-AGGTAATTAAAAGTAGTATTAACATATTATAAATAGAC) and the 38-mer oligonucleotide described above. For some experiments, the 38-mer oligonucleotide was replaced with a 28-mer primer (5′-CATCATTCATAATGAATGTTGT-TAATTG) to generate a PCR fragment also lacking the 3′ *Hin*dIII restriction site. For synthesis of antisense transcript, a PCR fragment was amplified using the same template with a 51-mer oligonucleotide containing the T7 promoter sequence adjacent to the complementary 3′ end of the *COX2* coding sequence (5′-TAATACGACTCACTATAGGGAG-GCATCATTCATAATGAATGTTGTTAATTG) and the 44-mer oligonucleotide described above. Another PCR product generated with genomic DNA as the template was a fragment encoding a 130 nucleotide portion of the 5′-untranslated region of the mitochondrial *ATP9* mRNA (oligos: 5′-TAATACGACTCACTATAGGGAGGAATATATATAGTTT-ATCATA and 5′-TTATTTTATTTAATAGATGTTC).

In vitro transcription was conducted using a T7-MEGA-shortscript kit (Ambion) following protocols provided by the supplier. Transcription reactions conducted in 20 μL volumes were incubated at 37 °C for 4 h and for an additional 15 min following addition of 2 units of DNase I to each reaction. Control reactions containing 1 μg of a 3.0 kbp plasmid were examined by gel electrophoresis at this point to ascertain complete digestion of template DNAs. The transcription reactions were passed through Probe Quant G-50 Microcolumns (Amersham Pharmacia Biotech) to remove NTPs and unincorporated dNTPs. The RNA transcripts were stored in aliquots at −70 °C. Product yields were ∼60 μg of RNA transcript/4.5 μg of linearized DNA plasmid in runoff transcription assays and ∼30 μg of transcript/150−200 ng of PCR DNA template.

Yeast tRNA (Sigma) and yeast poly(A)mRNA (Clontech) were resuspended in sterile water and the tRNA subjected to gel filtration as described above. Samples of these RNAs were examined by gel electrophoresis to examine size and

integrity. The tRNA migrated as a relatively uniform band ~90 nucleotides in size in agreement with reported sizes for yeast tRNAs (*23*). The poly(A)mRNA migrated as a diffuse band with an average size of 1300 nucleotides. For experimental manipulations using RNA, water for solutions was treated with diethyl-pyrocarbonate (*24*) and all glassware and cuvettes were treated with RNase ZAP (Ambion).

Enzyme Expression and Purification. To achieve multicopy expression of IDH in yeast, a 4.1 kbp *Hin*dIII/*Spe*I DNA fragment containing both a histidine-tagged form of *IDH1* and the *IDH2* gene was subcloned from a previously described plasmid (p*IDH1*^{His}*/IDH2*, *11*) into pRS426 (Stratagene), a 2 μm-based expression vector. The recombinant pRS plasmid was transformed into the yeast Δ*IDH1*Δ*IDH2* disruption strain using a version (Clontech Protocol no. PT1030-1) of the lithium acetate method (*25*) and transformants cultivated as described above.

Enzyme purification using Ni^{2+}-nitrilotriacetic acid superflow resin (Ni^{2+}-NTA, QIAGEN Inc.) was conducted as previously described (*11*). Fractions from the final eluate were assayed for IDH activity, and those containing the highest levels of activity were pooled and stored on ice at 4 °C prior to kinetic analyses. Enzyme purity of >95% was confirmed by electrophoresis. Concentrations of purified IDH were determined by Bradford assays (*26*) and by estimates of molar extinction coefficient using the method of Pace et al. (*27*).

Kinetic Analyses. IDH activity was measured as NADH production at A_{340nm} in assays containing 40 mM Tris-HCl (pH 7.4), 4 mM $MgCl_2$, and 0.25 mM NAD^+. Affinity-purified enzyme was added at a concentration of 5 μg/mL. Reactions were initiated by addition of DL-isocitrate. Concentrations of the substrate D-isocitrate were calculated as 50% of the total isocitrate. With saturating concentrations of isocitrate, the maximum velocities (μmol NADH produced/min/mg enzyme) measured for different enzyme preparations ranged from 16 to 22.

Assay volumes of 100 μL were used for reactions with RNA. An RNase inhibitor (ANTI-RNase, Ambion) was added at concentrations of 30 units/100 μL to all assays using RNA. All nucleotides (AMP, dAMP, ADP, ATP, and GMP) used in kinetic assays were purchased from Sigma and purities of >99% were confirmed by HPLC analyses.

RESULTS

Effect of RNA on IDH Activity. For kinetic analyses, yeast IDH was affinity purified using a penta-histidine tag on the IDH1 subunit. As previously described (*11*), the addition of histidine residues to the carboxyl terminus of either IDH1 or IDH2 can be used for purification of holoenzyme and has no apparent effect on kinetic behavior of the purified enzyme. To increase yield for the current studies, *IDH1*^{His} and *IDH2* genes were subcloned into a multicopy pRS plasmid and the plasmid transformed into a yeast strain containing disruptions of chromosomal *IDH1* and *IDH2* loci (*9*). Use of the multicopy plasmid results in an ~20-fold increase in total cellular IDH specific activity. Yields of ~200 μg of enzyme/g cells were obtained using affinity purification conducted as described in Experimental Procedures. Following each enzyme preparation, purity was examined by gel electrophoresis and kinetic properties were examined with enzyme assays. Average values measured for $S_{0.5}$ for D-isocitrate were 0.69 mM in the absence of AMP and 0.08 mM in the presence of 100 μM AMP. These values and respective Hill coefficients of 3.9 and 3.2 are similar to previously reported values (*11*).

The RNA transcript used in most kinetic analyses was synthesized using in vitro transcription methods with a template DNA fragment generated by PCR as described in Experimental Procedures. The 130-nucleotide RNA transcript (Figure 1), which contains the 54-nucleotide 5′-untranslated region and an adjacent 46-nucleotide segment of the coding region of the yeast mitochondrial *COX2* mRNA is similar to that previously used for analyses of IDH/mRNA interactions (*14*). Sequences within the 5′-untranslated region (underlined in Figure 1) are found in this region of several other yeast mitochondrial mRNAs and are proposed to form a base-paired helical secondary structure near single-stranded regions identified as sites for IDH binding (*13*). Following in vitro transcription, DNA templates were removed by DNase treatment and nucleotides removed by gel filtration as described in Experimental Procedures. Gel electrophoresis was used to verify integrity of the RNA transcripts prior to and following kinetic assays.

In initial kinetic analyses, increasing concentrations of the *COX2* UTR transcript were added to IDH assays containing saturating concentrations of Mg^{2+} and NAD^+ and 1 mM D-isocitrate, a concentration producing velocities of ~85% V_{max}. This substrate concentration was chosen because it provides an easily measurable rate and permits detection of allosteric effects. As illustrated in Figure 2A, addition of the RNA transcript dramatically reduces IDH activity under these assay conditions. With 1 μM concentrations of transcript, IDH activity is ~7% of that observed in the absence of RNA. At equimolar concentrations of transcript and holoenzyme, ~16 nM in these assays, IDH activity is reduced by ~50%, suggesting that the transcript is a potent inhibitor of IDH.

Similar assays conducted in the presence of 100 μM concentrations of the allosteric activator AMP (Figure 2A) show a complete protection from inhibition of IDH activity by RNA.

Enzymatic assays with the *COX2* UTR transcript were conducted in 100 μL volumes to conserve that reagent and in the presence of an RNase inhibitor to ensure integrity of the transcript. We determined that the inhibitor and the buffers used for preparation of the RNA transcript have no independent effect on IDH activity. We also tested variants of the *COX2* UTR transcript including a transcript generated from a PCR template that lacks the 3′ *Hin*dIII recognition sequence shown in Figure 1 as well as a transcript generated by runoff transcription from a plasmid containing *COX2* sequences. The latter transcript contains an additional 28-nucleotide extension at the 5′ end due to multicloning sequences. These variants of the *COX2* UTR transcript were found to produce kinetic effects essentially identical to those shown in Figure 2A.

As a final control, we repeated the assays illustrated in Figure 2A using an affinity-purified form of IDH containing the histidine-tag on the other subunit (IDH1/IDH2^{His}, *11*). Again, essentially identical results were obtained, suggesting that the observed inhibition of activity by the *COX2* transcript and the protection from this inhibition by AMP are not a function of the location of the affinity tag.

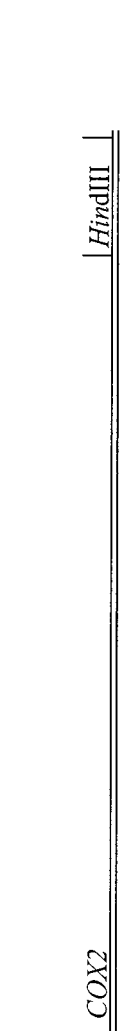

FIGURE 1: Synthesis and sequence of RNA transcripts. A DNA template containing a T7 promoter sequence and portions of the yeast mitochondrial *COX2* gene was constructed by PCR and used for in vitro transcription as described under Experimental Procedures. The resulting RNA transcript contains 17 nucleotides preceding the authentic transcription initiation site, the full 5′-untranslated region of the mRNA, a portion of the *COX2* coding sequence, and nucleotides at the 3′ end from a *Hin*dIII restriction site used in cloning. Shown are the sequences of the *COX2* UTR transcript, of an antisense form of the transcript, and of a transcript from the 5′-untranslated region of the mRNA from the yeast mitochondrial *ATP9* gene. Underlined regions have been proposed to form imperfectly based-paired helical secondary structures (*13*).

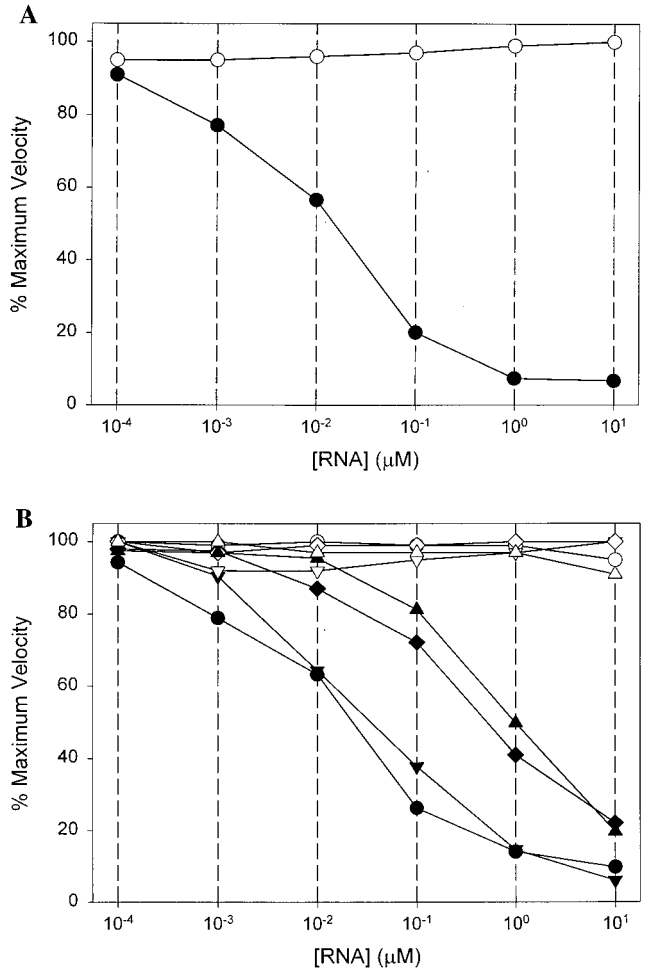

FIGURE 2: Effect of RNA on IDH activity. IDH assays were conducted with 1.0 mM D-isocitrate as described under Experimental Procedures. Values for velocity represent the average of at least two independent experimental determinations and are presented as percent of the activity measured in the absence of RNA. (A) Increasing concentrations of the *COX2* UTR mRNA transcript were added to the IDH assays conducted in the absence (●) or presence (○) of 100 μM AMP. (B) IDH activity was measured in the presence of increasing concentrations of the *ATP9* UTR transcript (●), of the antisense *COX2* transcript (▼), of yeast tRNA (◆), and of yeast poly(A)mRNA (▲). Open symbols indicate assays conducted in the presence of corresponding RNAs and 100 μM AMP.

Specificity of the RNA Effect. To further examine the specificity of the inhibitory effect of *COX2* UTR RNA on IDH activity, we used in vitro transcription to generate transcripts of the same size (130 nucleotides) corresponding to a portion of the 5′-untranslated region of the mRNA from the mitochondrial *ATP9* gene containing IDH-binding sequences (*13*) and to the antisense form of the *COX2* UTR RNA (Figure 1). As illustrated in Figure 2B, IDH activity is significantly inhibited by the *ATP9* UTR transcript at concentrations similar to those observed for the *COX2* UTR transcript. The *COX2* antisense transcript also produces a significant reduction in IDH activity. The concentration of antisense transcript required to reduce activity by 50% is ~2.2-fold greater than that required for similar reduction with the *COX2* UTR transcript. Assuming that inhibition may be related to secondary structure, it is noted that the complementary base pairing proposed for the *COX2* UTR and *ATP9* UTR sequences (*13*) could also occur in the antisense transcript (Figure 1). Papadopoulou et al. (*14*) also reported

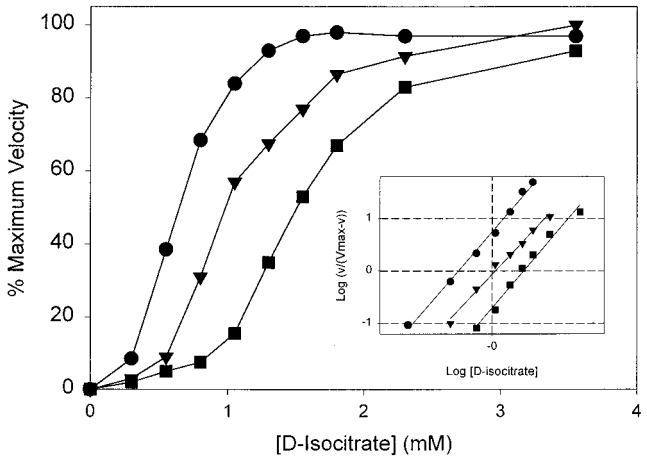

FIGURE 3: Effect of RNA on the $S_{0.5}$ value for isocitrate. IDH activity was measured as a function of the concentration of D-isocitrate in the absence of RNA (●) or in the presence of 0.01 μM (▼) or 0.1 μM (■) concentrations of the *COX2* UTR mRNA transcript. Values for velocity represent the average of two independent experimental determinations. Inset: Hill plot.

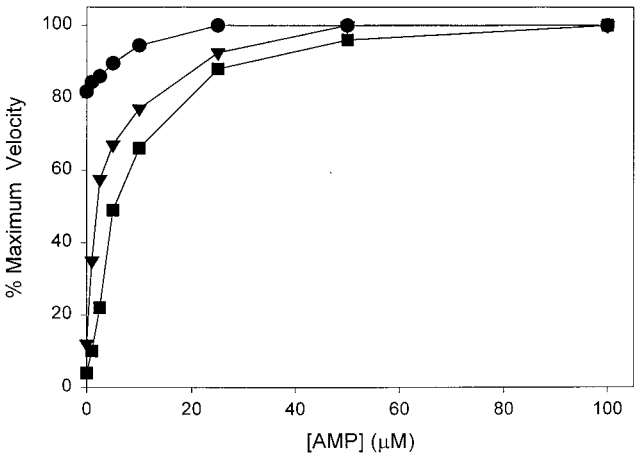

FIGURE 4: RNA inhibition of IDH as a function of AMP concentration. IDH activity was measured using 1.0 mM D-isocitrate. Activity was measured at increasing concentrations of AMP in the absence of RNA (●) and in the presence of 0.1 μM (▼) or 1.0 μM (■) concentrations of the *COX2* UTR transcript.

that an antisense *COX2* UTR transcript binds IDH in competitive gel shift assays with an affinity ~10-fold less than the sense strand transcript.

To test the extent that RNA with unrelated sequence might inhibit IDH activity, kinetic assays were conducted with comparable concentrations of yeast tRNA and yeast poly(A)-mRNA. As illustrated in Figure 2B, both RNAs inhibit IDH activity. However, an ~33-fold higher concentration of tRNA and an ~60-fold higher concentration of poly(A)mRNA are required to produce the 50% inhibition of activity observed with the *COX2* UTR transcript. These results suggest that IDH activity is particularly sensitive to sequences in and/or secondary structures attained by the 5′-untranslated region of the *COX2* (and *ATP9*) transcript.

Reduction of IDH activity by ≥80% can be obtained with 10 μM concentrations of all of the RNAs tested. However, all inhibitory effects, even at 10 μM RNA concentrations, are prevented by the presence of 100 μM AMP (Figure 2).

To further examine the effect of RNA on IDH activity, catalytic rates were measured as a function of isocitrate concentration. As shown in Figure 3, the primary effect of the presence of 0.01 μM or of 0.1 μM *COX2* UTR transcript is an increase in the $S_{0.5}$ value for isocitrate to, respectively, 1.05 and 1.57 mM relative to a value of 0.62 mM measured for the uninhibited enzyme in parallel assays. The transcript has no effect on values obtained for V_{max} and little effect on cooperativity. Hill coefficients calculated for these curves are 3.5 in the absence of RNA, 3.2 in the presence of 0.01 μM transcript, and 3.6 in the presence of 0.1 μM transcript. Thus, the *COX2* UTR transcript behaves as an allosteric inhibitor with an effect opposite to that of AMP, which reduces the $S_{0.5}$ value for isocitrate.

The concentrations of AMP required to reverse inhibition of IDH activity by the *COX2* UTR transcript were examined using assays containing 0.1 or 1.0 μM RNA concentrations (Figure 4). Concentrations of 10 μM AMP were found to be sufficient to restore, respectively, 77 and 65% of the activity measured in the absence of the transcript.

Specificity of the AMP Effect. AMP presumably protects against inhibitory effects of the *COX2* UTR transcript by allosteric activation of IDH. To test this presumption, we examined IDH activity in the presence of different concentrations of the transcript and in the presence of 100 μM concentrations of other nucleotides including ADP, ATP, dAMP, and GMP. As illustrated in Figure 5A, dAMP was found to partially prevent the inhibition observed with 0.01, 0.1, and 1.0 μM concentrations of the *COX2* transcript. At 1.0 μM RNA concentrations, for example, IDH activity with dAMP is 42% of the value measured with AMP. In contrast, ADP, ATP, and GMP were found to have no effect on levels of IDH activity measured in the presence of RNA (data not shown), i.e., inhibition by RNA is not attenuated by the presence of these nucleotides.

We further examined IDH activity in the presence of 100 μM dAMP as a function of isocitrate concentration. As illustrated in Figure 5B, dAMP reduces the $S_{0.5}$ value for isocitrate to 0.45 mM. $S_{0.5}$ values of 0.70 in the absence of nucleotide and of 0.08 mM in the presence of 100 μM AMP were measured in parallel experiments. dAMP produces a slight apparent increase in cooperativity with a Hill coefficient of 5.5 relative to 3.9 measured in the absence of nucleotide and 3.2 measured with AMP. Thus, under these experimental conditions, dAMP is an allosteric activator of IDH but, at similar concentrations, is less effective than AMP. In contrast, similar assays conducted in the presence of 100 μM ADP, ATP, or GMP produced no evidence for effects on catalytic activity (data not shown). Therefore, there is a close correlation between the relative effects (or the absence of effects) of these nucleotides as allosteric activators with their relative effects (or absence of effects) in reducing inhibition of IDH activity by the *COX2* UTR transcript.

DISCUSSION

The RNA-binding properties of yeast NAD^+-specific isocitrate dehydrogenase were originally reported by Papadopoulou et al. (*14*) who partially purified an abundant nuclear-encoded mitochondrial protein (p40) based on gel shift assays using a 140-nucleotide transcript from *COX2* similar to that illustrated in Figure 1. p40 was subsequently shown to be IDH based on partial amino acid sequence analysis and on studies using *IDH* gene disruption mutants (*12*). The *COX2* RNA transcript and IDH were found to form

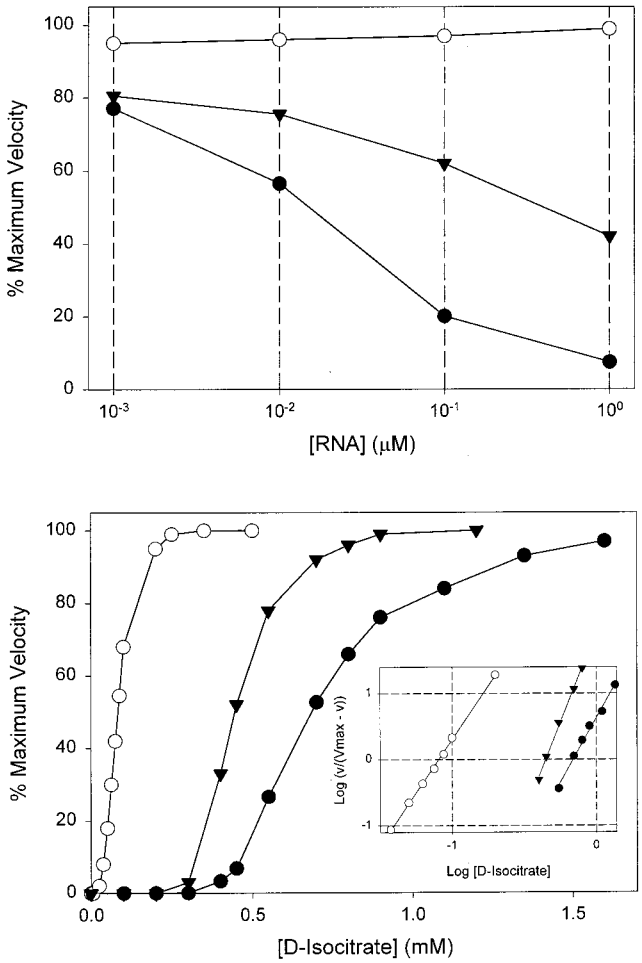

FIGURE 5: Protection from RNA inhibition and allosteric activation of IDH by adenine nucleotides. IDH assays were conducted with 1.0 mM D-isocitrate. (A) Increasing concentrations of the *COX2* UTR transcript were added to IDH assays conducted in the presence of 100 μM dAMP (▼). Values for velocity represent the average of two independent experimental determinations and are presented as the percent of activity measured in the absence of RNA. Values for activity measured in the absence of nucleotide (●) or in the presence of 100 μM AMP (○) are from Figure 2A. (B) IDH activity was measured as a function of the concentration of D-isocitrate in the absence of nucleotide (●) or in the presence of 100 μM AMP (○) or 100 μM dAMP (▼). Inset: Hill plot.

a stable complex with a dissociation half-life of 22 min (*14*). IDH was additionally shown to bind 5′-untranslated regions of all eight yeast mitochondrial mRNAs although the location of binding sequences relative to translation initiation codons varies (*13*). Mapping of IDH binding sequences in several transcripts indicated that the binding sequences themselves are not conserved but appear to be single-stranded regions bounded by relatively conserved sequences that may form a base-paired helical structure.

The sizes of yeast mitochondrial mRNA 5′-untranslated sequences vary in size from 54 nucleotides for *COX2* to 954 nucleotides for *COB* (reviewed in refs *17* and and *31*). The 5′-untranslated regions contain consensus sequences similar to procaryotic Shine Delgarno sequences at various distances from initiator methionine codons (*32*) and a consensus 8-nucleotide sequence that is proposed to be a generic ribosome recognition site (*19*). In addition, there is growing genetic evidence that the 5′-untranslated regions of several of the yeast mRNAs contain sequences important for binding mRNA-specific nuclear-encoded translational activators (reviewed in refs *17*, *21*, and *31*). The specific translational activators, including PET111 for *COX2* (*33*), are hydrophobic proteins and are proposed, through membrane association, to couple translation with efficient membrane insertion of the hydrophobic components of electron transport and ATPase complexes encoded by seven of the eight mitochondrial mRNAs. The eighth mRNA encodes VAR1, a hydrophilic component of mitochondrial ribosomes. In the compact 5′-untranslated region of the *COX2* transcript (Figure 1), the IDH binding site (nucleotides −21 to −12, ref *13*) overlaps the Shine Delgarno-like sequence (nucleotides −15 to −9, ref *32*), and the upstream sequence that may be part of a base-paired structure (nucleotides −42 to −36, ref *32*) overlaps the putative ribosome recognition site (nucleotides −41 to −34, ref *19*). Thus, Dekker et al. (*13*) have suggested that binding of this soluble matrix enzyme might repress translation until an appropriate association between the mRNA and hydrophobic elements in mitochondrial membranes is attained.

Results presented here suggest that interaction with mRNA would also have dramatic effects on IDH activity. We find that the 130-nucleotide *COX2* transcript containing the 5′-untranslated region is a potent allosteric inhibitor of IDH and that this inhibition is relieved by the presence of the allosteric activator AMP. The effect of the mRNA appears to be relatively specific since similar levels of inhibition are obtained with an *ATP9* UTR transcript and slightly reduced levels are obtained with the antisense form of the *COX2* transcript. The sequences suggest that all three transcripts may adopt similar secondary structures, and it is reported that the specific IDH-binding sequences in different mitochondrial mRNAs are not conserved (*13*). That much higher concentrations of tRNA and poly(A)mRNA are required to inhibit IDH activity does suggest the importance of specific elements of the secondary structure attained by the *COX2* and *ATP9* UTR transcripts.

The potential physiological significance of kinetic effects measured in vitro is sometimes supported by a consideration of stoichiometry. Mueller and Getz (*34*) calculated that a yeast cell cultivated under derepressing conditions contains 100−500 copies of each mitochondrial mRNA, and Dekker et al. (*35*) estimated that there are 25 000 p40 subunits of IDH/cell under similar conditions. Since there are eight mitochondrial mRNAs in yeast and since the IDH holoenzyme is an octamer, the physiological ratio of mRNA to holoenzyme could range from ∼1:4 to ∼1:1. In kinetic assays, these ratios of the *COX2* UTR transcript to IDH holoenzyme produce significant inhibition of activity (Figure 2A). Thus, the kinetic effects we observe occur within the limits of physiological conditions and suggest that mitochondrial IDH could exist in an equilibrium between active and inactive pools depending on RNA association.

With respect to substrate, Gadde and McCammon (*36*) have measured cellular concentrations of 2.5 μmol/g cellular protein for isocitrate in yeast cells grown under derepressing conditions. On the basis of a commonly used value of 2 μL cell volume/mg dry weight (*37*, *38*) and on a reported conversion factor of ∼0.4 μg of protein/μg of dry weight (*38*, *39*), a cellular concentration of 500 μM can be calculated for isocitrate. This concentration is similar to the IDH $S_{0.5}$ value for the substrate measured in the absence of AMP.

This suggests that any kinetic effects of RNA on IDH in vivo would not be masked by saturation with isocitrate (Figure 3). However, cellular concentrations of AMP in yeast of 50−300 μM have been reported (*37*, *40*). In kinetic assays, these concentrations are sufficient to prevent the inhibitory effect of the *COX2* transcript (Figure 4). These estimates of cellular concentrations are clearly biased by a number of assumptions and do not consider free or localized levels of metabolites. They do, however, suggest that RNA inhibition of IDH activity, and perhaps any physical interaction of IDH with RNA in vivo, would be highly dependent on actual cellular levels and compartmentation of AMP.

With regard to the AMP effect, we find that dAMP also reduces inhibition of IDH activity by the *COX2* transcript, although less efficiently than AMP, and that this property appears to correlate with efficiency as allosteric activators. The coordinate absence of any allosteric activation and of any alteration in RNA effects by ADP, ATP, and GMP also suggests that the AMP effects are relatively specific.

In terms of the physiological significance of IDH−mRNA interactions, a complex of these macromolecules could be a mechanism to sequester IDH in an inactive form and RNA in a form that may not be amenable to translation. Release from the complex could depend on proper delivery of the mRNA to membrane-bound translational activators and/or on elevated mitochondrial levels of AMP. Thus, the interaction could coordinate, on a very fine level, relative rates of oxidative metabolism and of functional expression of mitochondrial genes. This will be tested by expression in vivo of mutant forms of IDH that exhibit reduced kinetic inhibition by mitochondrial mRNA 5′-untranslated regions in in vitro assays.

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