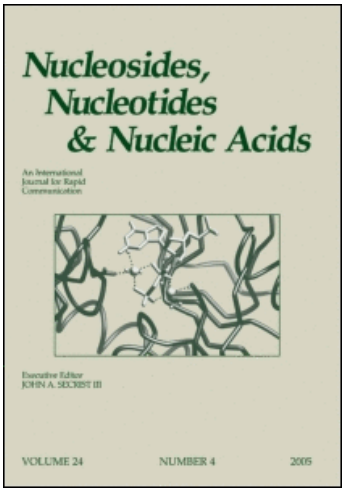

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Cardiac Muscle AMP-Deaminase from a 10-Year-Old Male Heterozygous for the *AMPD1* C34T Mutation

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CARDIAC MUSCLE AMP-DEAMINASE FROM A 10-YEAR-OLD MALE HETEROZYGOUS FOR THE *AMPD1* C34T MUTATION

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□ *A C34T mutation in the* AMPD1 *gene is proposed to cause local or systemic augmentations in blood adenosine level and improvement of prognoses in heart diseases like congestive heart failure or heart ischemic disease. This study examines some physico-chemical properties of AMP-deaminase isolated from cardiac muscle of a 10-year-old boy heterozygote for this mutation.*

Keywords AMP deaminase; heart; C34T mutation; kinetic properties

INTRODUCTION

AMP-deaminase (AMPD) catalyzes the hydrolytic deamination of adenylic acid (AMP) and plays an important role in energy metabolism of mammalian cells.[1] In humans, AMP-deaminase activity results from tissue-specific expression of a multigene family (*AMPD1*, *AMPD2,* and *AMPD3*) producing three main isoforms.[2] In human skeletal muscle, AMP-deaminase activity exceeds manifold that found in other tissues and organs.[3] This results not only from the abundant and exclusive expression of the *AMPD1* gene in this tissue, but also from high catalytic activity of its gene product.[2,4] In human heart muscle, *AMPD2* gene expression seems to prevail, whereas *AMPD1* gene expression is minor.[5] The kinetic and regulatory profile of human heart AMP-deaminase is characterized by strongly sigmoidal kinetics of substrate binding and potent activation by ATP, which converts the kinetic

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curve to a hyperbolic and reduces the half-saturation constant ($S_{0.5}$) value from 7–8 mM (measured in the absence of ATP), to about 2 mM. ADP is a less potent activator of the human heart enzyme, while orthophosphate inhibits it activity by accentuating the sigmoidal profile observed in the absence of activators.[6] Thirty years ago, a near complete lack of AMP-deaminase activity in human skeletal muscle was detected in patients with post-exercise muscle cramps and myalgias.[7] This was subsequently shown to be due to a nonsense (C34T) mutation in exon 2 of the *AMPD1* gene, encoding an inactive, truncated enzyme.[8] This *AMPD1* mutant allele exists in homozygous (2–5% of the normal AMPD activity in the muscle) and heterozygous (30–50% of the normal AMPD activity in the muscle) forms and is recognized as the most common skeletal muscle enzyme defect in man.[9] C34T homozygotes release enhanced amounts of adenosine from skeletal muscle as the result of a competing 5′-nucleotidase, which may be beneficial in the cardiovascular system. A correlation between the presence of this mutation and survival in patients with congestive heart failure has been described.[10] In this study, kinetic properties of AMP-deaminase purified from cardiac muscle of a 10-year-old male heterozygote for the *AMPD1* C34T mutation are described.

MATERIALS AND METHODS

Human heart was taken during autopsy from previously healthy 10-year old boy approximately 16 hours after death. Fat and main vessels were removed, the muscle was homogenized in 3 vol. (v/w) of extraction buffer (0.18 M KCl, 0.054 M KH_2PO_4, 0.035 M K_2HPO_4, and 1 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml trypsin inhibitor), centrifuged, and AMP deaminase was subsequently isolated by phosphocellulose chromatography, as described previously.[6] The most active fractions from the two peaks containing enzyme activity were pooled and used for kinetic studies. AMP deaminase activity was estimated according to the phenol-hypochlorite method of Chaney and Marbach.[11] The incubation medium, in a final volume of 0.5 ml, contained either 0.05 M succinate-KOH buffer, pH 6.5, with the addition of substrate and effector. Incubations were carried out for 15 minutes, and the velocity of the reaction was determined from the mean amount of ammonia liberated in three parallel samples. The kinetic parameters were calculated with the aid of a Sigmaplot computer software.

RESULTS AND DISCUSSION

The specific activity of AMP-deaminase in the homogenate prepared from cardiac muscle of the male heterozygote for the *AMPD1* C34T mutation was approximately 0.04 μmoles/min/mg of protein, about 50% of

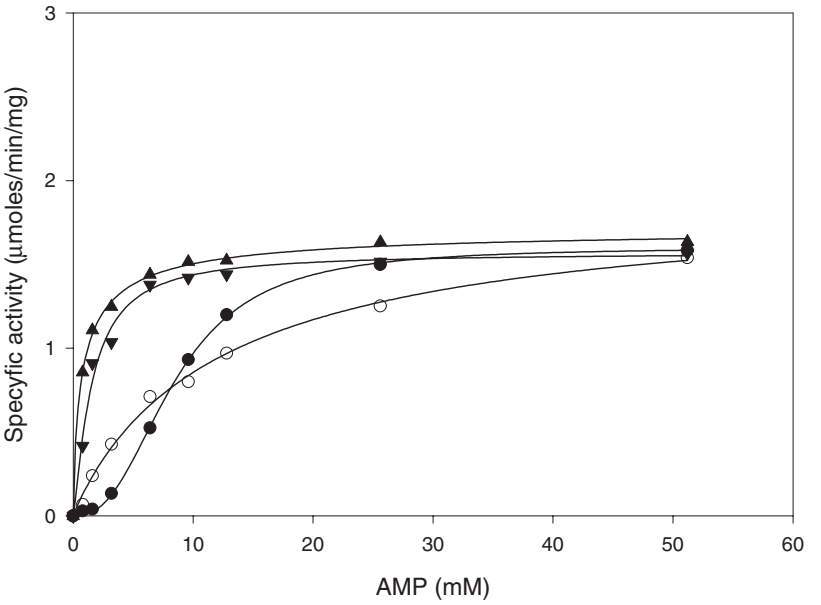

FIGURE 1 Kinetic and regulatory properties of human heart AMP-deaminase-heterozygous for C34T mutation. The reaction was measured in the absence of allosteric effectors (○) or in presence 1mM of ATP (▲), 1mM of ADP (▼), or 2.5 mM orthophosphate (●).

what we normally see in hearts of non-affected, adult individuals (0.07 ± 0.02 μmoles/min/mg of protein, unpublished data). Figure 1 presents a set of plots characterizing kinetic and regulatory properties of AMP-deaminase purified from the *AMPD1* C34T heterozygote. In the absence of allosteric effectors, substrate saturation of AMP-deaminase followed irregular, hyperbolic kinetics with an $S_{0.5}$ value nearly twice as high (12.5 mM) as that previously calculated for the enzyme isolated from the heart of non-affected, adult individuals (7.3 mM, unpublished data). In the presence of allosteric effectors (ATP, ADP, and orthophosphate), kinetic profiles generated from AMP-deaminase isolated from normal and C34T heterozygote hearts became more homogenous. For example, the $S_{0.5}$ values calculated for enzyme isolated from the *AMPD1* C34T heterozygote and from non-affected, adult individuals, were approximately 1, 2, and 10 mM for the reactions measured in the presence of 1mM ATP, 1mM ADP, and 2.5 mM orthophosphate, respectively. The results of this study show that the *AMPD1* C34T mutation also affects activity and kinetic properties of AMP-deaminase in the juvenile heart. Previous data generated in our laboratory indicate that the isozymic pattern of AMP-deaminase in human skeletal muscle changes during ontogenesis.[12] Therefore, one possible explanation for the results reported in this manuscript may be related to a developmental change in the AMP-deaminase isozymic pattern of the heart, that is, the pattern in the heart

of a 10-year-old *AMPD1* C34T heterozygote differs from that observed in non-affected adults.

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