Interaction of Mg\(^{2+}\) with Human Liver Aldehyde Dehydrogenase

I. SPECIES DIFFERENCE IN THE MITOCHONDRIAL ISOZYME*  

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The dehydrogenase activity of the mitochondrial isozyme (E2) of human liver aldehyde dehydrogenase was stimulated about 2-fold by the presence of low concentrations (about 120–140 \(\mu\)M) of Mg\(^{2+}\) in the assay at pH 7.0 using propionaldehyde as substrate. The stimulation was totally reversible by treatment with EDTA. Maximum stimulation was dependent on the concentration of NAD\(^+\) used in the assay; an increase in \(K_m\) value of NAD\(^+\) was observed to parallel the increase in maximal velocity with increasing Mg\(^{2+}\) concentration, indicating that alterations in the catalytic properties of the E2 isozyme occur in the presence of Mg\(^{2+}\). The presteady state burst of NADH product was observed to decrease in the presence of Mg\(^{2+}\), suggesting that the rate-limiting step of the dehydrogenase reaction is altered by Mg\(^{2+}\). No evidence for Mg\(^{2+}\)-induced alterations in the molecular weight properties of the E2 isozyme was observed using gel filtration column chromatography and fluorescence polarization techniques. In addition, no alterations in the inactivating properties of iodoacetamide or disulfiram were produced by Mg\(^{2+}\). These results suggest that the mechanism by which human mitochondrial aldehyde dehydrogenase (E2) is stimulated by Mg\(^{2+}\) is different from that of the horse enzyme, representing a significant species difference.

Recently, Takahashi and Weiner (1980) have demonstrated that the mitochondrial isozyme of horse aldehyde dehydrogenase exhibits a doubling of specific activity when assayed in the presence of low levels of MgCl\(_2\) (about 500 \(\mu\)M) at pH 7.0 and that the increase in rate is due to dissociation of the tetrameric enzyme functioning with half of the sites reactivity into dimers with concomitant exposure of two additional functioning active sites. On the other hand, it has been shown by Venteicher et al. (1977) that the cytoplasmic enzyme from horse is inhibited at similarly low concentrations of Mg\(^{2+}\). The concentration of free Mg\(^{2+}\) in the liver cell is known to be in the range of 0.6–0.8 mM (Siliprandi et al., 1977), indicating that in vivo regulation of aldehyde dehydrogenase activity may involve Mg\(^{2+}\) ions or perhaps another divalent metal cation such as Ca\(^{2+}\).

In the present study, the interaction of Mg\(^{2+}\) ions with the mitochondrial isozyme (E2) of human liver aldehyde dehydrogenase is examined. While the overall effect of Mg\(^{2+}\) on the activities of the E2 isozyme is similar to that previously observed for the horse enzyme, the results presented demonstrate that the mechanism by which the human mitochondrial isozyme (E2) is stimulated by Mg\(^{2+}\) is distinct from that reported for the horse mitochondrial isozyme. With the human enzyme, Mg\(^{2+}\) alters the catalytic properties of already existing active sites and does not cause an increase in the number of functioning active sites via dissociation of the tetramer into dimers.

**EXPERIMENTAL PROCEDURES AND RESULTS**

**DISCUSSION**

It has been shown that the mitochondrial isozyme (E2) of human liver aldehyde dehydrogenase is stimulated by low levels of MgCl\(_2\) (micromolar range). A 2-fold enhancement in dehydrogenase activity was observed when assayed at pH 7.0 using propionaldehyde as substrate. The stimulation was observed to occur immediately following addition of MgCl\(_2\) to the assay, while complete reversal to the original reaction velocity was obtained almost instantaneously by addition of EDTA. These observations are basically consistent with data previously reported by Takahashi and Weiner (1980) from studies on the mitochondrial isozyme of horse liver aldehyde dehydrogenase. In our study, however, a buffering agent (25 mM PIPES, pH 7.0) which does not form complexes with Mg\(^{2+}\) was used (Good et al., 1966), and this probably accounts for the relatively low concentration of MgCl\(_2\) (about 120–140 \(\mu\)M) required to bring about maximum stimulation of the human enzyme when compared to the horse enzyme (about 400–500 \(\mu\)M; Takahashi and Weiner, 1980). Substitution of CaCl\(_2\) for MgCl\(_2\) caused a significantly greater fold stimulation (2.9-fold for CaCl\(_2\) compared with 2-fold for MgCl\(_2\)) under identical assay conditions. This observation provided the first piece of evidence suggesting that the mechanims for stimulation of the horse and human mitochondrial aldehyde dehydrogenase by divalent metal cations might be different, as both MgCl\(_2\) and CaCl\(_2\) produced a 2-fold enhancement in the activity of the horse enzyme (Takahashi and Weiner, 1980).

Maximum stimulation of E2-catalyzed dehydrogenase activity was dependent on the concentration of NAD\(^+\) used in the assay (Figs. 1 and 2). This phenomenon was not observed

*This work supported by Grant AA-00186 and Research Scientist Development Award AA-00046 from the National Institute of Alcoholism and Alcohol Abuse and the Charles and Johanna Busch Memorial Fund. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Portions of this paper (including "Experimental Procedures," "Results," Table I, and Figs. 1–4) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 850 Rockville Pike, Bethesda, MD 20814. Request Document No. 83M-931, cite the authors, and include a check or money order for $5.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

2 The abbreviation used is: PIPES, 1,4-piperazinediethanesulfonic acid.
by Takahashi and Weiner (1980) with the mitochondrial isozyme of horse aldehyde dehydrogenase. In addition, stimulation curves obtained with E2 isozyme at both concentrations of NAD+ (570 μM and 3 mM, Fig. 1) were different from the curve obtained for the horse enzyme. With E2, a biphasic ascending pattern was observed before maximum stimulation was reached at about 120-140 μM Mg2+, which was then followed by a gradual, steady decrease in specific activity as the concentration of Mg2+ was further increased. In contrast, the horse enzyme showed a hyperbolic stimulation curve with a leveling off pattern after maximal stimulation was reached (Takahashi and Weiner, 1980).

An increase in the Ks value of NAD+ with E2 isozyme was observed to parallel the increase in maximal velocity with increasing levels of Mg2+ (Fig. 2; Table I), indicating that alterations in the catalytic properties of E2 isozyme occur in the presence of Mg2+. That the positions of the stimulation peaks were the same at both levels of NAD+ (Fig. 1), suggests that Mg2+ probably does not combine with NAD+ to form a "true substrate" as a means for stimulation. This observation is consistent with the results of Burkhard (1981), who has calculated the dissociation constants for divalent metal cations with NAD+ to be in the millimolar range. Thus, the requirement for increased concentrations of NAD+ to achieve maximum fold stimulation appears not to be related to formation of an NAD+·Mg2+ substrate complex, but it is instead related to an increased value for the Ks of free NAD+ in the presence of Mg2+.

The parallel series of lines (Fig. 2) generated in the double reciprocal plots of data obtained with varied NAD+ at changing levels of MgCl2 and saturating propionaldehyde suggest that Mg2+ does not combine with free enzyme. It is possible, however, that Mg2+ may combine with the enzyme-NAD binary complex, as suggested by the pattern of intersecting lines (Fig. 3) generated in the double reciprocal plots of data obtained for varied glycolaldehyde, constant NAD+, and changing levels of Mg2+.

The presence of a presteady state burst of NADH product indicates that the rate-limiting step of the E2-catalyzed dehydrogenase reaction occurs after ternary complex interconversion. In the case of horse mitochondrial aldehyde dehydrogenase, the amplitude of this burst was observed to increase in the presence of Mg2+ (Takahashi and Weiner, 1980), and has been used as evidence to support an increase in number of functioning active sites as the mechanism of Mg2+ stimulation for that enzyme. The amplitude of the pre-steady state burst of NADH product formed with the E2 isozyme was, however, observed to decrease, while the steady state reaction velocity increased in response to increasing concentrations of Mg2+ (Fig. 4). In addition, the stimulation constants obtained for Mg2+ from the steady state (about 15 μM) and pre-steady state (about 16 μM) are in good agreement, as are the coefficients for maximum change in maximal velocity (2.25) and burst amplitude (2.30) obtained by extrapolation of the data plotted in Figs. 2 and 4, respectively. Since Mg2+ showed no effect on the unstimulated esterase burst amplitude, and no burst was detected for the coenzyme-stimulated reactions (with or without Mg2+), information regarding esterase active site number, in the presence and absence of Mg2+, using pre-steady state techniques could not be obtained. Since the decrease in dehydrogenase burst amplitude virtually parallels the increase in specific activity (Fig. 4), it appears unlikely that Mg2+ stimulates the E2 isozyme by increasing the number of functioning active sites; these data are more consistent with a mechanism in which Mg2+ alters the properties of already existing sites.

No evidence for Mg2+-induced alterations in the molecular weight properties of the E2 isozymes was observed. Gel filtration chromatography experiments indicated a molecular weight of about 260,000 in the presence and absence of Mg2+, a value which is in fairly good agreement with that determined previously for the tetrameric form of the enzyme (Pietruszko et al., 1977), and which is precisely the same value reported by Takahashi and Weiner (1980) for the tetrameric form of the horse mitochondrial enzyme. Presence of dehydrogenase substrates (and products) together with Mg2+ also appeared to have no effect on the molecular weight of the E2 isozyme, as determined by gel filtration chromatography. In the study by Takahashi et al. (1981), a decrease in the polarization of fluorescence (P = 0.34 to P = 0.26) determined for enzyme-bound NADH was observed to occur in response to Mg2+ and correlated to a decrease in enzyme molecular weight (from tetramer to dimer). In fluorescence polarization experiments on the E2 isozyme (carried out under conditions identical with those of Takahashi et al., 1981), however, no decrease in P value was observed. A value of 0.32 was determined in both the absence and presence of Mg2+, a number closely resembling the value of 0.34 determined for the tetrameric form of the horse enzyme (Takahashi et al., 1981). Since the active site number of the E2 isozyme cannot be determined by conventional titration methods, it is impossible to duplicate experiments of Takahashi et al. (1980) which clearly demonstrate an increase in the number of NADH binding sites. Finally, no alterations in the inactivating properties of either disulfiram or iodoacetamide were observed with E2 isozyme when incubations were carried out in the presence of Mg2+. These results, coupled with the fluorescence polarization, gel filtration, steady state, and presteady state data, indicate that the stimulation of the E2 isozyme by Mg2+ is probably not related to enzyme dissociation or an increase in the number of functioning active sites.

As discussed previously, the presence of a presteady state burst of NADH in the dehydrogenase reaction indicates that the rate-limiting step is after interconversion of the ternary complex (e.g. deacylation or NADH product release). A Mg2+-induced acceleration in the rate of NADH release or deacylation could shift the rate-limiting step to a step which precedes ternary complex interconversion, thereby causing a decrease in the magnitude of the presteady state burst. The 4-fold higher maximal velocity obtained with glycolaldehyde (0.8 μmol/min/mg) than with propionaldehyde (0.2 μmol/min/mg) as substrate suggests further that deacylation rather than NADH dissociation may be rate-limiting in the case of the E2 isozyme, since the greater electron withdrawing capacity of the R group of glycolaldehyde (when compared to propionaldehyde) facilitates this nucleophilic step in the reaction (Weiner, 1979). Deacylation has previously been determined to be the rate-limiting step for horse liver mitochondrial aldehyde dehydrogenase (Weiner et al., 1976). As discussed above, Mg2+ appears to effect the amplitude of the presteady state burst (Fig. 4), with propionaldehyde as substrate, by shifting the rate-limiting step to a step preceding ternary complex interconversion. The relatively small degree of stimulation of the E2 isozyme by Mg2+ with glycolaldehyde as substrate (1.6-fold), coupled with an already accelerated rate of dehydrogenation for the same substrate in the absence of added Mg2+, suggests that the rate of deacylation for glycolaldehyde is already quite fast and another step in the reaction sequence may already be rate-limiting. Preliminary studies revealed a presteady state burst of NADH product with glycolaldehyde that appears to be even smaller in magnitude than the one obtained with propionaldehyde and Mg2+.
(80 μM), indicating that this is indeed a good possibility.

In summary, these results suggest that Mg²⁺ causes E2-catalyzed dehydrogenase activity to increase by altering the catalytic properties of already existing active sites rather than by causing an increase in their functional number. Mg²⁺ changes the kinetic properties of the enzyme relative to $K_m$ values of NAD⁺, but does not appear to cause alterations in the molecular weight properties of the enzyme. This finding represents a significant species difference between mitochondrial aldehyde dehydrogenases from horse and man.

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Human Liver Aldehyde Dehydrogenase I

INTERACTION OF NAD+ WITH HUMAN LIVER ALDEHYDE DEHYDROGENASE

1. SPECIES DIFFERENCE IN THE MITOCHONDRIAL ISOZyme

by Robert C. Vallani and Regina Pietruszko

EXPERIMENTAL PROCEDURES

Material. Nicotinamide adenine dinucleotide (NAD+) was obtained from Sigma Chemical Co., St. Louis, Mo. L-alanine, L-cystine, L-methionine, L-phenylalanine, L-proline, and L-serine were obtained from Sigma Chemical Co., St. Louis, Mo. All reagents used were of assay grade. The mitochondrial (E3) isozyme of aldehyde dehydrogenase was purified from human liver mitochondria as described by Vallani and Pietruszko (1980). The enzyme was assayed by a modification of the procedure of Wallen and colleagues (1976) using 4-methylpyrazole as reagent. The mitochondrial isozyme of aldehyde dehydrogenase was also assayed in the same assay. The enzyme was assayed by a modification of the procedure of Hay and colleagues (1980) using 4-methylpyrazole as reagent. The mitochondrial isozyme of aldehyde dehydrogenase was also assayed in the same assay.

Fig. 1. Effect of NAD+ concentration on the characteristics of NAD+-induced stimulation of mitochondrial and aldehyde dehydrogenase activity.

Stimulation of the E3 isozyme may be achieved either by incorporation of NAD+ into the assay mixture before the reaction is initiated (by addition of 350M NAD+) or by addition of the metal ion to the assay mixture after the reaction is already in progress. Both methods result in instantaneous stimulation of the steady state reaction velocity. Subsequent addition of NAD+ in an enzyme-catalyzed reaction leads to the accumulation of the added NAD+ (Wallen and colleagues, 1976). The enzyme is then included in the reaction mixture and the method of Feldman and Weiner (1977) was used to determine the concentration of NAD+ in the reaction buffer, while the concentration of NAD+ in the assay mixture was determined by the method of Feldman and Weiner (1977).

RELATION BETWEEN NAD+ AND E3 ISOZyme-Catalyzed Dehydrogenase Activity

In Figure 2, the stimulation of mitochondrial dehydrogenase activity by increasing concentrations of added NAD+ is shown at two different concentrations of 350M NAD+ (100 and 1000). While the maximal degree of stimulation occurs at about the same concentration of NAD+ (100 and 1000) at both levels of NAD+ (1.9 fold and 1.9 fold), the degree of stimulation increases proportionally in response to increasing concentrations of NAD+. A second set of the values in the inset gives a series of parallel lines as shown in Figure 2. The parallel pattern indicates that both maximal velocity and Km NAD+ are increased proportionally in response to increasing concentrations of NAD+. The replot of the data in the inset gives a straight line which extrapolated to 2 fold (fold stimulation) with NAD+ concentration and saturation NAD+ concentration constant.

Results

Effect of NAD+ on the Dehydrogenase Activity of the E3 Isozyme

The effect of increasing concentrations of NAD+ on the assay buffer on the dehydrogenase activity of human mitochondrial aldehyde dehydrogenase (E3) is depicted in Figure 1. The specific activity of the enzyme was observed to increase with increasing concentrations of NAD+ to a maximum which appeared to be the optimal concentration of NAD+. As the concentration of NAD+ was increased, the specific activity of the enzyme increased. The specific activity of the enzyme was also observed to increase with increasing concentrations of NAD+ up to the preparation of the assay mixture. The specific activity of the enzyme was observed to increase with increasing concentrations of NAD+ up to the preparation of the assay mixture. The specific activity of the enzyme was observed to increase with increasing concentrations of NAD+ up to the preparation of the assay mixture. The specific activity of the enzyme was observed to increase with increasing concentrations of NAD+ up to the preparation of the assay mixture.
Table 1

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<thead>
<tr>
<th>MgCl₂ (μM)</th>
<th>Km NAD⁺ (μM)</th>
<th>Maximal Velocity (μmol/min/mg)</th>
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<tr>
<td>0</td>
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<td>0.45</td>
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Effect of MgCl₂ on the Kinetic Parameters of Human Liver Aldehyde Dehydrogenase I

Using a stopped flow spectrophotometer, the steady state formation of NADH in the MgCl₂-catalyzed dehydrogenase reaction was investigated in the absence and presence (100 μM) of added NAD⁺. Although we were unable to record the entire steady state phase of the reaction, we were able to obtain a recording of the steady state phase, immediately following the rapid phase of formation of NADH at an extinction coefficient of 6.22. This method has been successfully used with other dehydrogenases in our laboratory, even with the use of an ordinary spectrophotometer and strip chart recorder (Kane et al., 1982; Yashahshi et al., 1982; Kita et al., 1987).

Effect of MgCl₂ on the Presteady-State Burst Amplitude of NADH in the E2 Isozyme-Catalyzed Dehydrogenase Reaction

In Figure 4, the relation between MgCl₂ concentration, presteady state burst amplitude, and maximal steady state velocity of the E2-catalyzed dehydrogenase reaction is plotted. Burst amplitudes (C) and steady state velocities (ν) were determined separately from the stopped flow data using a normal spectrophotometer. The inset, the reciprocal of the change in burst amplitude plotted vs. the reciprocal of the MgCl₂ concentration. The line was drawn to fit the parameters derived from analysis of the stopped flow data by linear regression (r ≥ 0.99).

Figure 4. Relation between MgCl₂ concentration, presteady state burst amplitude, and maximal steady state velocity of the E2-catalyzed dehydrogenase reaction. Burst amplitudes (C) and steady state velocities (ν) were determined separately from the stopped flow data using a normal spectrophotometer. The inset, the reciprocal of the change in burst amplitude plotted vs. the reciprocal of the MgCl₂ concentration. The line was drawn to fit the parameters derived from analysis of the stopped flow data by linear regression (r ≥ 0.99).

Effect of MgCl₂ on the Molecular Weight of the E2 Isozyme

The binding of NAD⁺ to E2 isozyme can be monitored by fluorescence microspectroscopy. The binding of NAD⁺ to E2 isozyme can be monitored by fluorescence microspectroscopy. The binding of NAD⁺ to E2 isozyme can be monitored by fluorescence microspectroscopy. The binding of NAD⁺ to E2 isozyme can be monitored by fluorescence microspectroscopy. The binding of NAD⁺ to E2 isozyme can be monitored by fluorescence microspectroscopy.
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