Temperature Dependence of the Mitochondrial Inner Membrane Anion Channel

THE RELATIONSHIP BETWEEN TEMPERATURE AND INHIBITION BY MAGNESIUM*  

Andrew D. Beavis‡§ and Mary Powers¶  
From the ‡Department of Pharmacology, Medical College of Ohio, Toledo, Ohio 43614-5804 and ¶University of Toledo, Toledo, Ohio 43606

The mitochondrial inner membrane anion channel (IMAC) carries a wide variety of anions and is postulated to be involved in mitochondrial volume homeostasis in conjunction with the K⁺/H⁺ antiporter, thus allowing the respiratory chain proton pumps to drive salt efflux. How it is regulated is uncertain; however, it is inhibited by matrix Mg²⁺ and matrix protons. Previously determined values for the IC₅₀ suggested that the channel would be closed under physiological conditions. In a previous study (Liu, G., Hinch, B., Davatollah-Hag, H., Lu, Y., Powers, M., and Beavis, A. D. (1996) J. Biol. Chem. 271, 19717–19723), it was demonstrated that the channel is highly temperature-dependent, and that a large component of this sensitivity resulted from an effect on the pIC₅₀ for protons. We have now investigated the effect of temperature on the inhibition by Mg²⁺ and have found that it too is temperature-dependent. When the temperature is raised from 20 °C to 45 °C, the IC₅₀ increases from 22 to 350 μM at pH 7.4 and from 80 to 1.5 mM at pH 8.4, respectively. The Arrenhius plot for the IC₅₀ is linear with a slope = −80 kJ/mol. The IC₅₀ is also strongly pH-dependent, and at 37 °C increases from 90 μM at pH 7.4 to 1230 μM at pH 8.4. In view of the extremely rapid fluxes that IMAC is capable of conducting at 37 °C, we conclude that inhibition by matrix Mg²⁺ and protons is necessary to limit its activity under physiological conditions. We conclude that the primary role of Mg²⁺ is to ensure IMAC is poised to allow regulation by small changes in pH in the physiological range. This control is mediated by a direct effect of H⁺ on the activity, in addition to an indirect effect mediated by a change in the Mg²⁺ IC₅₀. The question that remains is not whether IMAC can be active at physiological concentrations of Mg²⁺ and H⁺, but what other factors might increase its sensitivity to changes in mitochondrial volume.

The mitochondrial inner membrane anion channel (IMAC) is a non-selective anion channel that carries a wide variety of anions ranging from small, singly charged ions, such as Cl⁻ and HCO₃⁻, to multicharged anions, such as citrate, ferrocy-

* This work was supported in part by the American Heart Association, Ohio Affiliate, Columbus, Ohio. 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.  
‡ To whom correspondence should be addressed. Tel.: 419-383-4125; Fax: 419-383-2871; E-mail: abeavis@mco.edu.  
§ The abbreviations used are: IMAC, inner membrane anion channel; MOPS, 3-N-morpholino)propanesulfonic acid; TAPS, N-tris(hydroxy-methyl)methyl-3-amino propanesulfonic acid.

Received for publication, September 22, 2003  
Published, JBC Papers in Press, November 13, 2003, DOI 10.1074/jbc.M310475200  

© 2004 by The American Society for Biochemistry and Molecular Biology, Inc. Printed in U.S.A.
The data presented show that the IC\textsubscript{50} for Mg\textsuperscript{2+} is strongly dependent upon temperature increasing from about 30 \textmu M at 25 °C to about 100 \textmu M at 37 °C; moreover, at 37 °C it increases further to 1.2 mM when the pH is raised to 8.4. From these results, we estimate that, in the presence of 0.5 mM Mg\textsuperscript{2+} at 37 °C and pH 7.4, IMAC may be about 7% active. In view of the very high intrinsic activity of this channel, this represents a very significant flux of 400 nmol/min/mg.

EXPERIMENTAL PROCEDURES

Assay of Anion Transport—Anion transport was assayed by following the swelling that accompanies net salt transport, using the light-scattering technique as described in detail elsewhere (9–11). In brief, reciprocal absorbance, as a function of mitochondrial volume, is monitored and normalized for mitochondrial protein concentration in the assay to yield a parameter referred to as \( \beta \); its rate of change can be converted to a rate of ion transport in nmol/min per mg mitochondrial protein. In a previous study of the effect of temperature on IMAC (7), we used malonate as the substrate anion; however, to investigate inhibition by Mg\textsuperscript{2+}, we chose to use Cl\textsuperscript{−}, because the association between Mg\textsuperscript{2+} and Cl\textsuperscript{−} is much weaker than between Mg\textsuperscript{2+} and malonate, and much less Mg\textsuperscript{2+} must be added to obtain a given concentration of free Mg\textsuperscript{2+}.

Pretreatment of Mitochondria with A23187—To deplete the mitochondria of endogenous Mg\textsuperscript{2+}, the normal stock suspension was pretreated as described in Ref. 7, except that the amount of A23187 was treated as described in Ref. 7, except that the amount of A23187 was increased to 10 nmol/mg mitochondrial protein to allow rapid equilibration of Mg\textsuperscript{2+} across the inner membrane after transfer to the assay medium. In brief, the mitochondrial stock suspension (50 mg/ml) was diluted 1:5 into a medium containing potassium salts of MOPS (25 mM) and EDTA (5 mM), adjusted to pH 7.4 at 25 °C, and maintained at 0 °C. A23187 (10 nmol/mg), nigericin (0.5 nmol/mg), and rotenone (0.5 \mu g/mg) were added, and at least 10 min was allowed to elapse before transfer of aliquots to the assay medium.

Assay Media for Anion Transport—The potassium chloride medium for assay of IMAC contained the potassium salts of Cl\textsuperscript{−} (55 mM), EGTA (0.1 mM), and MOPS (5 mM for assays at pH 7.4) or TAPS (5 mM for assays at pH 8.4). After the addition of the pretreated mitochondria, the final assay medium also contained EDTA (50 mM) and sucrose (0.5 mM). All media were 110 mossmolar. For experiments in which the temperature was to be varied, the pH of the medium was adjusted at 25 °C to a value, calculated on the basis of −0.0095 for ΔpK\textsubscript{a}C for MOPS and −0.021 for the ΔpK\textsubscript{a}C for TAPS, that would yield the desired pH at the assay temperature. Thus, separate assay media were prepared for each temperature to be studied. The value of ΔpK\textsubscript{a}C was determined experimentally in the assay media described above. The temperature of the medium in the assay tube was measured during the experiment to ensure that a steady value had been achieved. The free concentration of Mg\textsuperscript{2+} was calculated for each temperature and pH value using the program WinMaxC (courtesy of C. Patton, Stanford University). The same program was used to calculate the total Mg\textsuperscript{2+} to be added to obtain a specific free concentration.

Drugs, Reagents, and Mitochondria—Most drugs were obtained from Sigma. Valinomycin, nigericin, and rotenone were dissolved in ethanol, and valinomycin, nigericin, and rotenone were dissolved in ethanol, and A23187 (20 mM stock) was dissolved in dimethyl sulfoxide. Rat liver mitochondria were prepared from 30 to 35-day-old Sprague-Dawley rats and A23187 (20 mM stock) was dissolved in dimethyl sulfoxide. Rat liver mitochondria were prepared from 30 to 35-day-old Sprague-Dawley rats and A23187 (20 mM stock) was dissolved in dimethyl sulfoxide. Rat liver mitochondria were prepared from 30 to 35-day-old Sprague-Dawley rats and A23187 (20 mM stock) was dissolved in dimethyl sulfoxide.

RESULTS

Effect of Temperature on Inhibition of IMAC by Mg\textsuperscript{2+}—In a previous paper (6), we showed that when the mitochondrial IMAC is assayed at 25 °C, it is inhibited by matrix Mg\textsuperscript{2+}, and that the IC\textsubscript{50} rises from 38 \textmu M at pH 7.4 to 250 \textmu M at pH 8.4. The values obtained were not affected by the method used to remove endogenous Mg\textsuperscript{2+} nor by the time of addition of Mg\textsuperscript{2+} to the assay medium. In the present study, because the rate of swelling is very rapid at high temperature, the mitochondria were pretreated with A23187 and EDTA to ensure complete depletion of endogenous Mg\textsuperscript{2+}, and Mg\textsuperscript{2+} and valinomycin were added to the assay at zero time, i.e. before the mitochondria. The data contained in Fig. 1 show typical traces obtained at three temperatures: 25 °C for comparison with previous data, 37 °C to determine the flux at physiological temperatures, and 15 °C to illustrate behavior at low temperatures. In each case, there is a short acceleration phase before maximum swelling rates are observed. Mg\textsuperscript{2+} inhibited the fluxes at all three temperatures and the IC\textsubscript{50} values obtained from the dose response curves shown in Fig. 2 are 114 \mu M, 37 \mu M, and 17 \mu M at 37 °C, 25 °C, and 15 °C, respectively. Note, however, that the Hill coefficients tend to increase with the temperature. Consistent with our previous studies (7), the control rates are strongly temperature-dependent, with values equal to 4.0, 1.2, and 0.23 \mu mol Cl\textsuperscript{−}/min/mg at 37 °C, 25 °C, and 15 °C, respectively.

Relationship Between Mg\textsuperscript{2+} IC\textsubscript{50} and Temperature—To examine more closely the relationship between the Mg\textsuperscript{2+} IC\textsubscript{50} and temperature and how this might be affected by pH, we carried out dose-response studies at a series of temperatures ranging from 5 to 45 °C and at two pH values, pH 7.4 and 8.1. For each temperature, the pH of the assay medium was adjusted
separately to ensure that the assay pH did not vary with temperature.

The data contained in Fig. 3 show Arrhenius plots of the control rates of Cl⁻ transport in the absence of Mg²⁺ at the two pH values. Non-linear relationships are observed, consistent with the data obtained previously using malonate as the substrate for IMAC (7). Note that there is a 275-fold increase in rate when the temperature is raised from 10 to 45 °C at pH 7.4, and at 10 °C, there is a 15-fold increase in rate when the pH is raised from 7.4 to 8.1.

The curves are drawn using Equation 1 (equation 9 of Ref. 7), which was derived on the basis of a model in which the open probability of the channel is temperature-dependent.

$$\ln J = A + T \frac{\Delta H_{\text{flux}}}{R} + \frac{\Delta S_{\text{flux}}}{R} - \ln \left( 1 + \exp \left( \frac{\Delta H_{\text{open}}}{R} \left( \frac{1}{T} - \frac{1}{T_{50}} \right) \right) \right)$$

(Eq. 1)

where $\Delta H_{\text{flux}}$ and $\Delta S_{\text{flux}}$ are the activation enthalpy and entropy of the transport process, and $T_{50}$ is the temperature at which 50% of the channels are open, which is given by $T_{50} = \frac{\Delta H_{\text{open}}}{\Delta S_{\text{open}}}$, where $\Delta H_{\text{open}}$ and $\Delta S_{\text{open}}$ are the enthalpy and entropy of channel opening, respectively.

Like the transport rate, the Mg²⁺ $IC_{50}$ is strongly dependent upon the temperature increasing exponentially as the temperature is raised (Fig. 4A). In contrast to the Arrhenius plots for the transport rates, the Arrhenius plots of the $IC_{50}$ values are essentially linear. Using values interpolated from the curves fitted to the data, the ratio of $IC_{50}$ values range from about 4.6 at 45 °C to 5.3 at 20 °C. The narrow temperature range and scatter in the data do not allow one to determine to what extent the effect of pH results from a change in the slope or intercept. However, whatever the cause of the shift, there is about a 5-fold increase in Mg²⁺ $IC_{50}$ as the pH is raised from 7.4 to 8.1 over the temperature range examined.

Influence of Temperature on the Relationship between Mg²⁺ $IC_{50}$ and pH—To examine further the effect of temperature on the relationship between the $IC_{50}$ for Mg²⁺ and pH, the IC⁵₀ was determined at a series of pH values ranging from 7.35 to 8.35 at 25 °C and 37 °C. The results of two independent experiments contained in Fig. 5 reveal that the $IC_{50}$ increases 10-fold and 16-fold at 25 °C and 37 °C, respectively. The curves drawn were fitted to the data using a model, previously shown to describe the relationship between the Mg²⁺ $IC_{50}$ and pH at 25 °C (6), in which

$$IC_{50} = K_{50}\left( 1 + \left( K_{p} / [H]^{p} \right) \right)$$

(Eq. 2)

Because the intercept on the ordinate is so close to the origin, the data do not allow one to determine whether the effect of temperature is mediated by means of an effect on $K_{50}$ or the $pK$ for the protonation site. The slopes, however, which are determined by the product $K_{50}K_{p}$ and $n$ differ by a factor of 7. Using the constants provided in the legend, values of $IC_{50}$ can be calculated. Thus, at 37 °C, the $IC_{50}$ rises from 90 μM at pH 7.4 (compare 114 μM in Fig. 1) to 250 μM at pH 7.8. This latter value is considerably closer to published estimates of the physiological free matrix Mg²⁺ concentration (8).

Estimate of IMAC Activity at Physiological Mg²⁺ Concentration—It is now well accepted that the physiological concentration of free Mg²⁺ in the mitochondrial matrix is close to 0.5 mM (8). It is also likely that dramatic changes in the free concentration do not occur under most physiological conditions. Thus, the temperature dependence of IMAC in the presence of this fixed concentration of Mg²⁺ is of interest. Because at pH 7.4 rates are extremely low at low temperatures, especially with Mg²⁺ present, we carried out this experiment at both pH 7.4 and pH 8.4. The data presented in Fig 6A show that at pH 8.4, both in the presence and absence of Mg²⁺, the Arrhenius plot is...
The Mg\textsuperscript{2+} \textit{IC}_{50} was determined in KCl assay media adjusted to pH values ranging from 7.3 to 8.3 at 25 °C and 37 °C. A, exponential increase in IC\textit{C}_{50} as the temperature is raised. B, Arrhenius plots of the data. White and black symbols represent data from two independent experiments using different mitochondrial preparations. See “Experimental Procedures” and text for further details.

29 °C in the presence of 0.5 mM Mg\textsuperscript{2+}. The latter curve was fitted using the Δ\textit{H}_{\text{bax}} obtained from the control curve. The narrow temperature range and scatter in the data do not permit one to determine whether this change results from a change in ΔH\textsubscript{open} or Δ\textit{S}_{\text{open}}. Because of the fact that it is difficult to measure accurately the very low rates observed when both pH and temperature are low, only rates measured between 45 and 30 °C are shown for pH 7.4. To allow comparison of the T\textit{so} with the other values, the curve was fitted by assuming that ΔH\textsubscript{open} and Δ\textit{S}_{\text{open}} were the same as at pH 8.4, thus yielding a value of T\textit{so} = 49 °C.

To better illustrate the effect of Mg\textsuperscript{2+} and temperature on the activity of IMAC, we have plotted the data as percent inhibition of the rate at 45 °C in the absence of Mg\textsuperscript{2+} (Fig. 6B). The control curve (pH 8.4, no Mg\textsuperscript{2+}; Fig. 6B, black circles) shows the “inhibitory” effect of lowering temperature. Between 45 and 25 °C, IMAC is inhibited by about 75%, with a Q\textit{so} = 2. In Fig. 6B, the white circles and white squares show the inhibition of the flux by temperature plus 0.5 mM Mg\textsuperscript{2+}. At pH 8.4, the flux increases dramatically between 35 and 45 °C, whereas at pH 7.4, the channel is almost completely inhibited. This effect is most conveniently expressed as a change in T\textit{so}, brought about by the preferential binding of H\textsuperscript{+} and Mg\textsuperscript{2+} to the closed state of the channel. The curves fitted indicate that the T\textit{so} increases from about 10 °C in the control curve to about
effects of pH and temperature on the Mg\(^{2+}\) IC\(_{50}\) and their direct effects on the activity of IMAC, the data presented provide strong evidence that IMAC may have considerable activity under physiological conditions.

The data presented demonstrate that Mg\(^{2+}\) is an efficacious inhibitor over a temperature range that is associated with a 350-fold change in anion flux. We have observed, however, that the Hill coefficient for the inhibition tends to increase with temperature. It is quite likely that this reflects the involvement of more than one inhibitory Mg\(^{2+}\)-binding site at high temperatures. A similar but more pronounced phenomenon was observed for inhibition by protons (7), which was explained by the presence of a second inhibitory site with a lower temperature dependence. The Hill coefficient of less than unity seen at 15 °C may reflect the presence of a small uninhibitable component, which only becomes significant at the very low fluxes observed at the low temperatures. For consistency, however, all IC\(_{50}\) values were determined on the basis of the assumption that the flux could be completely inhibited.

The values for Mg\(^{2+}\) IC\(_{50}\) increase quite dramatically as the temperature is raised. At 45 °C, values of 1.5 and 0.35 nmol were obtained at pH 8.4 and 7.4, respectively. It is also evident that the IC\(_{50}\) remains pH-dependent over the whole temperature range. We have previously reported (6) that the IC\(_{50}\) may be expressed as a function of the binding constants for a magnesium-binding site (K\(_{MB}\)) and a proton binding site (K\(_{HP}\)) (see Equation 2). Consequently, the slope of the Arrhenius plot for the IC\(_{50}\) which is linear and equal to −77 to −81 kJ/mol, is dependent upon the enthalpies of binding for both Mg\(^{2+}\) and H\(^{+}\) (ΔH\(_{MB}\) and ΔH\(_{HP}\)). From these data, the interpolated values for the Mg\(^{2+}\) IC\(_{50}\) at 37 °C are 114 and 550 μM at pH 7.4 and 8.1, respectively.

The dependence of the Mg\(^{2+}\) IC\(_{50}\) on pH (Fig. 5) is consistent with the model previously proposed (6), although the Hill coefficient seems to increase slightly with temperature, so that the increase in IC\(_{50}\) with pH is greater at 37 °C than it is at 25 °C. Because both the curves extrapolate to values of IC\(_{50}\) very close to the origin, it is not possible to determine whether K\(_{MB}\) or K\(_{HP}\) is affected by temperature. Both curves shown were fitted by setting K\(_{MB}\) = 8 μM and allowing pK\(_{HP}\) and the Hill coefficients (n) to vary. Using this approach, the effect of raising the temperature from 25 to 37 °C is explained by a decrease in the value of pK\(_{HP}\) from 6.9 to 6.5, and an increase in n from 0.89 to 1.17. Curves can be fit equally well, however, if it is assumed that pK\(_{HP}\) = 6.7 at both temperatures, and K\(_{MB}\) and n are allowed to vary. In this case, the increase in IC\(_{50}\) can be explained by an increase in K\(_{MB}\) from 6.3 to 13 μM, together with the above-mentioned increase in n. Both models allow the Mg\(^{2+}\) IC\(_{50}\) to be calculated. For example, at 37 °C, interpolated values of the IC\(_{50}\) are 90, 250, and 1230 μM free Mg\(^{2+}\) at the pH values of 7.4, 7.8, and 8.4, respectively.

These data indicate that at 37 °C, as the pH rises from 7.4 to 8.4, the IC\(_{50}\) for Mg\(^{2+}\) passes from values that are about 15% of the free Mg\(^{2+}\) to values that are at least 2-fold higher than the free Mg\(^{2+}\). Thus, as the pH changes over this range, the activity of IMAC not only increases because of the diminished inhibition by protons, but also because of a decrease in the IC\(_{50}\) for Mg\(^{2+}\). Needless to say, the flux through a pathway is not simply dependent on the percent inhibition, but also the intrinsic activity of the process. Because IMAC has a very high J\(_{\text{max}}\), even when Mg\(^{2+}\) and H\(^{+}\) concentrations are significantly above their IC\(_{50}\) values, IMAC still has considerable activity. From the data presented here and the pH dependence described previously (7), we estimate that at 37 °C, the J\(_{\text{max}}\) ([H\(^{+}\)]\_free\) = 0, [Mg\(^{2+}\)]\_free\) = 0) is 6–7 μmol Cl\(^{−}\)/min-mg. To place this value in perspective, it should be noted that the maximum rates of respiration on succinate are of the order of 200 nmol O/min-mg. Moreover, because the net H\(^{+}\)/O stoichiometry of proton pumping is 6–7 (11), this respiration rate corresponds to a proton flux of about 1400 nmol H\(^{+}\)/min-mg, which is less than 25% of the capacity of IMAC. Thus, to avoid uncoupling of oxidative phosphorylation, in vivo IMAC must be tightly controlled and could have sufficient activity even at just a few percent of its maximum activity to play an important role in volume homeostasis. For the respiratory chain to drive net salt efflux, activity of both IMAC and the K\(^{+}\)/H\(^{+}\) antiporter is required (1). In a preliminary study, we have found that at 37 °C and pH 8.4 the K\(^{+}\)/H\(^{+}\) antiporter has a J\(_{\text{max}}\) ([Mg\(^{2+}\)]\_free\) = 0) of 1.3 μmol/min-mg and a Mg\(^{2+}\) IC\(_{50}\) of 220 μM. Thus, under physiological conditions, the K\(^{+}\)/H\(^{+}\) antiporter could also have significant activity. Note that the relatively high activity of IMAC would ensure that respiration-driven (H\(^{+}\) pump) anion efflux would elevate matrix pH and further activate both the K\(^{+}\)/H\(^{+}\) antiporter and IMAC, which are both regulated by matrix protons (1, 14). Note also that the very high J\(_{\text{max}}\) for IMAC relative to the rate of mitochondrial respiration is also consistent with the suggestion by Aon et al. (4) that IMAC is responsible for depolarization of mitochondria in myocytes observed in their studies.

To illustrate better the potential roles of H\(^{+}\) and Mg\(^{2+}\) in the regulation of IMAC, we have plotted the Cl\(^{−}\) flux calculated on the basis of the Mg\(^{2+}\) dependence presented here and the pH dependence reported previously (1). Fig. 7A shows the pre-

---

*Fig. 7. Effects of Mg\(^{2+}\) and pH in regulating IMAC at 37 °C.* Theoretical curves are plotted using the equation $J = J_0/[1 + ([\text{Mg}^{2+}]_{\text{free}}/\text{IC}_{50})^n]$ in which the values of $\text{Mg}^{2+}$ IC\(_{50}\) and n were determined from Figs. 2, 4, and 5, and the values of $J_0$ for each pH were determined using equation 10 of Ref. 7 with the value of pK\(_{J_0}\) = 7.1 to fit the data presented in this paper. A. Flux versus free $\text{Mg}^{2+}$ at pH values: a, 7.0; b, 7.2; c, 7.4; d, 7.5; e, 7.6; f, 7.7; g, 7.8; B, flux versus pH at free $[\text{Mg}^{2+}]$ (mM) of: a, 0; b, 0.2; c, 0.3; d, 0.5; e, 0.7; f, 0.9; g, 1.2. Note: the range of y axis values is limited to focus on the maximum range that could be coupled to the respiratory chain proton pumps. The maximum value shown represents the theoretical rate that would be seen if all proton pump activity were coupled to the anion flux through IMAC.

---

A. D. Beavis and M. Powers, unpublished data.
dicted flux as a function-free Mg$$^{2+}$$ at different pH values, taking into account the direct effects of pH on the flux as well as its effect on the Mg$$^{2+}$$ IC$_{50}$. Given that estimates of physiological free Mg$$^{2+}$$ are 0.5–0.6 mM, only as the pH becomes alkaline do small changes in Mg$$^{2+}$$ significantly affect the rate. Fig. 7B shows the predicted flux as a function of pH at various free Mg$$^{2+}$$ concentrations. Note that the effect of physiological concentrations of Mg$$^{2+}$$ is to move the pH dependence to a point that the flux will be very sensitive to pH changes in the 7.2–7.8 range. It should also be noted that the range of fluxes shown have been limited to the maximum that could be sustained by the proton pumps of the respiratory chain. The flux at pH 7.4 and 0.5 mM Mg$$^{2+}$$ is 400 nmol/min·mg, which is only 6.5% of the $J_{\text{max}}$ ([H$^+$] = 0, [Mg$^{2+}$] = 0), but still high enough so that in vivo, some other factor probably limits the flux further.

Even though changes in Mg$$^{2+}$$ may not be sufficient to regulate IMAC, the major function of Mg$$^{2+}$$ may be to ensure that the channel is poised to be responsive to other factors that fine tune its activity and that it does not have excessive activity under physiological conditions. The large number of inhibitors that have so far been identified (1, 12, 13) suggests that there is a very delicate balance between the open and closed states of IMAC. Many unidentified factors could be involved in regulating the open probability of IMAC. In addition to pH, these factors could include changes in matrix volume. As also suggested by others (8), volume per se could exert effects via cytoskeletal proteins that might be involved in control of mitochondrial shape and membrane folding. In view of the complex convoluted nature of the mitochondrial inner membrane and its cristae, this could provide a much more sensitive sensor of mitochondrial volume changes. Aon et al. (4) have recently proposed that superoxide may also activate IMAC. Thus, the question that remains is not whether IMAC can be active at physiological concentrations of Mg$$^{2+}$$ and H$^+$, but what other factors might increase its sensitivity to changes in mitochondrial volume.

Acknowledgment—We thank Dr. Elizabeth Tietz for the generous donation of rat livers.

REFERENCES