COMPETITION BETWEEN MAGNESIUM AND GUANIDINE FOR MITOCHONDRIAL BINDING SITES

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A number of recent, independent investigations have shown that the binding of Mg^{++} (Brierley et al., 1962), Ca^{++} (Vasington and Murphy, 1962) and Mn^{++} (Chappell et al., 1962) to mitochondria share in common a characteristic concomitant binding of phosphate. It has been suggested by Brierley et al. (1962) that the known affinities of these metal ions for phosphate may be involved in the binding and indeed that actual intramitochondrial precipitation of insoluble phosphates is an integral part of the process. We wish to call attention to the similarity of these binding phenomena to that obtainable with guanidine and alkylguanidines (Pressman, 1963). The present communication will extend the similarity by showing that the binding of guanidine shows a common pattern with that of Mg^{++} in response to various inhibitors, and furthermore, that guanidine and Mg^{++} are mutually competetive for mitochondrial binding sites.

METHODS

Mg^{++} and phosphate were determined on $HClO_4$ extracts of mitochondria by the methods of Orange and Rhein (1951) and King (1932) respectively. Bound guanidine was estimated by using C^{14} labeled guanidine (Calbiochem) in the reaction mixture and counting aliquots of the $HClO_4$ extract directly in the Packard liquid scintillation counter. Mitochondria were prepared from rat liver by the method of Schneider (1948) and, prior to $HClO_4$ extraction, were freed from the unbound components of the medium by the method of Pressman (1958).

RESULTS AND DISCUSSION

Although the bulk of our studies have dealt with rat liver mitochondria, qualitatively identical results were obtained with mitochondria prepared from rabbit kidney and guinea pig heart.

Table I

Effect of Inhibitors on the Binding of Guanidine and Phosphate

Guanidine	Absent	.04 M	
μ Mol. bound/gm protein	Phosphate	Phosphate	Guanidine
Control	20	43	23
+3.2 x 10^{-5} M DNP	7.5	8	5
+Amytal 1 mM	21	22	8
+Succinate 6 mM	14.5	35	23
+Amytal +Succinate	15	35	24
+Antmycin A 1.2 γ/ml	16	17	--
+Oligomycin 8 γ/ml	20	55	24

Mitochondria (33 mg) were incubated 5 minutes at 30° in 5 ml of a medium consisting of: phosphate, 4 mM; $MgCl_2$, 6 mM; KCl, 30 mM; ATP, .4 mM; sucrose, 220 mM; final pH, 7.4; additional components as indicated in table. Following incubation, 4 ml were transferred to sucrose gradient tubes and the bound constituents extracted by the method of Pressman (1958).

The effect of a variety of inhibitors is shown in Table I. DNP, amytal and antimycin A all caused a strong inhibition of both guanidine and phosphate binding. Although the binding process was hindered rather than helped by the addition of exogenous substrates such as succinate, it apparently does depend on the low level of endogenous respiration. This is evidenced by the suppression of binding by the electron transport inhibitors amytal and antimycin A. That the inhibition by amytal is indeed due to electron transport inhibition is verified by restoration of the binding process with succinate, the utilization of which is not inhibited by this agent. Oligomycin, which blocks the transfer of energy from substrate to the

ATP synthesizing step (Lardy et al., 1958), probably in the region of activation of inorganic phosphate (Pressman, 1953) fails to inhibit the binding of either guanidine or phosphate. The conclusion arrived at from these inhibitor studies that the simultaneous binding of guanidine and phosphate depends on the availability of substrate derived energy (inhibition by DNP, amytal, antimycin A) prior to its conversion to ATP (lack of inhibition by oligomycin) agrees with the conditions required for Mg^{++} and phosphate binding (Brierley et al., 1962).

More direct evidence for the relationship of the binding of magnesium and guanidine mitochondria is illustrated by Fig. 1. As in the case of heart mitochondria (Brierley et al., 1962), Mg^{++} binding increases with increasing extramitochondrial Mg^{++} concentration. As Mg^{++} accumulates, the ability of mitochondria to bind guanidine is concomitantly reduced. As the phosphate binding sites approach saturation under the influence of guanidine, further binding of phosphate with increasing Mg^{++} is strongly curtailed. This is evidence for the identity of the Mg^{++} induced and guanidine induced phosphate

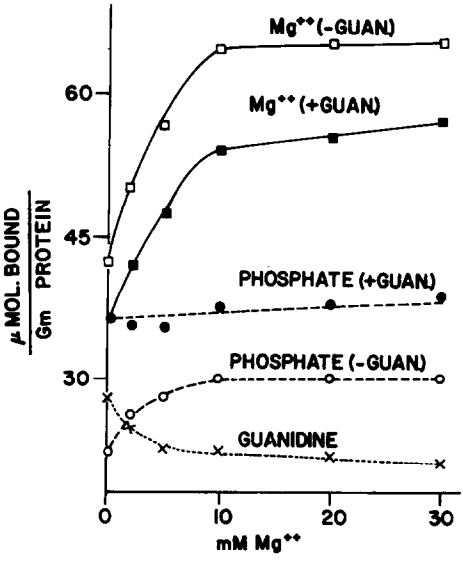

Fig. 1. Effect of Extramitochondrial $[Mg^{++}]$ on the Binding of Mg^{++}, Phosphate and Guanidine. Guanidine 20 mM when present. The basic medium (except for Mg^{++}) and procedure were the same as in Table I.

binding. In this experiment it can also be seen that the amount of Mg^{++} bound at a given concentration of extramitochondrial Mg^{++} is reduced by the addition of guanidine.

These observations suggest that guanidine and Mg^{++} can compete for a common mitochondrial site and by implication that the binding processes are quite similar. Since guanidine forms no known insoluble phosphates, this, in our mind, mitigates against the presumption that the concomitant phosphate accumulation observed is related to the chemical properties of the binding cation. We regard it rather as a characteristic of the binding site or some other specific attribute of the mitochondrion which underlies the binding process.

The possibility that guanidine enters into a ternary complex including phosphate within the mitochondria has been checked by looking for new compounds forming under the influence of guanidine which could acquire a label upon incubation with P^{32}. Mitochondria treated with guanidine and P^{32} were dissolved in the non-ionic Triton-urea reagent (Pressman, 1957) and passed through a Dowex-1-formate column. No increase in P^{32} was observed in the original protein-containing column eluant (essentially duplicating the conditions under which Suelter et al. (1961) first used to extract a P^{32} labeled protein from mitochondria) nor did any major unidentified band show up on elution of the column with neutral ammonium formate.

If one grants the likely close similarity of the Mg^{++} and guanidine binding mechanisms then the similarity of the Mg^{++}/phosphate (Brierley et al., 1962) and guanidine/phosphate (Pressman, 1953) binding ratios, both reported about 1.5, poses an additional problem. It is difficult to conceive of a cation-anion chemical affinity which would fail to distinguish between the singly charged guanidinium ion and the doubly charged Mg^{++}.

The observed competition between Mg^{++} and guanidine suggest a mechanism for explaining the intriguing inhibition of the energy transfer reactions of oxidative phosphorylation by guanidine and its derivatives (Hollunger, 1955). Guanidine could exert its inhibition by displacing Mg^{++} (or Mn^{++}) from a

mitochondrial binding site the occupancy of which by Mg^{++} is essential for activation of the energy transfer process of oxidative phosphorylation. Guanidine could exert its inhibition by competing with Mg^{++}, either preventing the formation, or forming an inactive form, of a complex with a component of the energy transfer process of oxidative phosphorylation. In accordance with the previously postulated locus of guanidine inhibition of oxidative phosphorylation (Pressman, 1963), this would also imply that the locus of this function of Mg^{++} would be prior to the formation of any phosphorylated intermediate. This does not preclude the possibility that Mg^{++} plays an additional role as the activator of a subsequent reaction leading to the formation of ATP which does involve phosphorylated intermediates. If an analogous binding competition obtains between guanidine and Ca^{++}, this could be involved in the inhibition by guanidine of the Ca^{++} induced ATP-ase which some evidence (Pressman, 1963) indicates may not be identical with the mechanism by which guanidine inhibits oxidative phosphorylation.

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