Uncoupling of Respiration-Linked Contraction in Corn Mitochondria

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Abstract. Respiration-linked contraction of corn mitochondria is not noticeably reduced by low, uncoupling concentrations of dinitrophenol. However, if a contraction/respiration ratio is calculated, the contraction proves to be uncoupled. Previous statements that contraction cannot be uncoupled from respiration are in error.

The uncoupling of contraction is consistent with the concept that dinitrophenol attacks a primary non-phosphorylated high energy intermediate (I~X). It is proposed that this intermediate is linked to some contractile mechanism such that the degree of contraction reflects the level of intermediate.

One of the more perplexing aspects of respiration-linked contraction in swollen mitochondria is the ineffectiveness of uncoupling agents on the process. Our observations on corn mitochondria (2, 18, 19, 21) show that only high concentrations of uncoupler (e.g. 100–500 μM DNP) will accelerate spontaneous swelling and reduce contraction. In a phosphorylating medium, such concentrations almost eliminate oxidative phosphorylation and strongly inhibit the respiration. Low concentrations of uncoupler (e.g. 10–30 μM DNP) which are adequate to depress P/O ratios, accelerate ATPase, release acceptorless respiration, and reduce Ca2+ + Pi transport, have little or no effect on respiration-linked contraction. The same insensitivity is found with animal mitochondria (1, 4, 7).

We proposed that contraction is closely linked to respiration and cannot be reduced by uncoupling agents unless they depress respiration (21). When evidence was obtained supporting our contention that contraction is associated with energy conservation, presumably as I~X, the hypothetical non-phosphorylated high energy intermediate, a scheme was presented relating contraction to the level of I~ (13).

Recently we have re-examined the action of DNP on contraction and find our previous statements to be in error. Dinitrophenol does uncouple contraction if expressed on a respiration basis comparable to the P/O ratio. Furthermore, net contraction can be reduced by DNP while the respiration rate is still very high. The findings are in accord with the classical view that DNP attacks a non-phosphorylated intermediate produced at coupling sites.

Methods and Materials

Mitochondria were isolated from 3 day etiolated corn shoots as previously described (13), but with the omission of ADP during washing. Measurements of light absorbancy and of oxygen were made in parallel on separate but identical solutions (13), or by simultaneously recording light absorption and oxygen levels in a specially constructed and thermostated cell at 25°C. Results were the same with both methods. Vessel contents are given with the figures.

Results

Figure 1 shows typical effects of DNP on swelling and contraction. NADH was used as respiratory substrate since it is quickly exhausted permitting observations on the rate of reswelling. Calcium was added to fulfill the divalent cation requirement (9, 11). Inclusion of 50 μM phosphate was to maximize contraction. [A separate report will be made on the requirement for a low level of endogenous or exogenous phosphate in divalent cation catalyzed contraction. The phosphate is actively bound during contraction, but is released upon reswelling. The spurious “contraction” (light absorption) accompanying calcium phosphate accumulation (2) is not involved in these experiments.] The BSA used to protect the mitochondria during swelling lowers the effective concentration of uncoupler about 5-fold (2, 19).

At 20 μM, DNP had little effect on the initial swelling, contraction, or reswelling. However,
respiration was released in that the NADH was consumed in less time as evidenced by the earlier onset of reswelling. With 100 μM DNP there was an acceleration of initial swelling and a reduction in the rate on contraction. The NADH did not last long enough for steady-state contraction to be achieved. There also appeared to be a slower reswelling rate, but it is doubtful if this is a direct effect of DNP. Rates of reswelling seem to be related to the extent of contraction attained, and are dominated by the divalent cation used (11; S. W. Dumford, unpublished thesis research).

Rates of respiration and rates of contraction are directly compared in figure 2. Although the data confirm that contraction is relatively insensitive to DNP, the contraction/respiration ratio clearly shows that uncoupling does occur. In terms of the ratio, contraction rate is just as sensitive to uncouplers as ATP formation and Ca²⁺ + Pi uptake (19, 21). Our past statements about uncouplers not reducing contraction unless they depress respiration are clearly in error.

In other experiments we determined the steady-state contraction levels instead of contraction rates. The results are similar to those of figure 2: significant depression of the level of contraction required 30 to 50 μM DNP, but the contraction/respiration ratio declined with any addition of DNP. By omitting the Ca²⁺ and Pi, thus reducing contraction, we could readily confirm the observation (19) that uncouplers can even increase contraction. With the optimal concentration of 30 μM DNP, the steady state absorbancy change increased from 0.015 to 0.037, while respiration increased 38%.

**Discussion**

Contraction of spontaneously swollen corn mitochondria can be linked to either ATP hydrolysis or substrate oxidation (18, 19, 21). Hence, some energized intermediate or state between electron flow and ATP formation must be involved. In support of this, we have been able to demonstrate energy conservation associated with contraction (13). The formation and stability of the contracted (and energized) state also involves binding of a divalent cation (11).

Figure 3 is a simplified scheme to correlate these observations. Electron flow at coupling sites is dependent on reactive groups I and X, and yields an acid anhydride I—X, the hypothetical non-phosphorylated intermediate. The question of whether I—X is formed by "chemical" or "chemiosmotic" means (6, 14) is by-passed, although Mitchell's
concept of a dehydration is adopted (polar removal of H₂O as H⁺ and OH⁻ not shown) as is our proposal that a divalent cation is chelated. I~X is placed in steady-state equilibrium with the associated contractile mechanism, which can thus contribute to the reservoir of conserved energy. The slow attainment of steady-state contraction (fig 1) will lie with the kinetics of attaining equilibrium between I~X and the unknown contractile mechanism. It is possible, of course, that the cation requirement might be with the mechanism or that I~X might incorporate the mechanism.

With regard to mechanism, contraction equates with water expulsion from the matrix. Electron micrographs (10, 19, and unpublished work) show that with water expulsion the matrix shrinks and becomes very dense. The inner membrane shows no sign of decreasing in surface area, but simply collapses about the condensing matrix. Azzi and Azzone (1) relate the respiration-linked shrinkage of liver mitochondria to active salt expulsion followed by an osmotic equivalent of water. Although we can confirm the loss of salt (R. H. Wilson, unpublished research), salt transport can hardly be the primary event since contraction is related to conservation of energy, whereas active salt transport would consume energy. It is possible, however, that I~X formation may be poised with an enhanced electrochemical potential (14), with the salt efflux during contraction reflecting the establishment of this potential. Conservation of bond energy (I~X) and of electrochemical potential are not exclusive events, and may well have an obligatory relationship.

If this is true, what would be the expected result from addition of an uncoupler? Uncouplers are widely believed to attack I~X, either directly or by permitting entry of water (8, 15, 17). In barley and corn mitochondria, competitive kinetics are shown by Pi and DNP (2, 12), suggesting a direct attack on I~X. Recently, Van Dam and Slater (22) have proposed that uncoupling results from a competitive use of the energy in DNP transport. Regardless of mechanism, the net result will be as illustrated in figure 3; hydrolysis of I~X, recycling I and X for accelerated electron flow.

The problem thus becomes one of kinetics. For any given acceleration of I~X hydrolysis as determined by respiration (fig 2), what level of I~X (and associated contraction) can be expected? No adequate kinetic analysis can be made without identification of the unknowns and their reactions. However, by assuming the reactions of figure 3 to be essentially irreversible, simple rate constants can be used. Electron transport per se should not be rate limiting, and k₁ is assumed much larger than k₂. Dinitrophenol can be visualized as increasing k₂ or increasing (H₂O), thus increasing respiration. Contraction will be lowered in either case since contraction = f(I~X), and (I~X) = k₁/k₂ x (IOH)/(XH)/(H₂O). Preliminary calculations indicate that by assuming the total concentration of X to be several fold greater than I (16), and a steady-state build up of X-DNP with increasing DNP (21), that curves simulating figure 2 can be generated. In view of our ignorance of the biochemistry involved such computations are doubtless premature. However, it is not necessary to postulate that contraction processes have a special affinity for the energy conserved at coupling sites as Blair and Sollars (4) have done in order to explain the relative insensitivity of contraction to uncouplers.

Respiration rates may also be governed by the rate of permeation of NADH or other substrates to oxidation sites (fig 3). Although there is some dispute (20), uncouplers may act to make membranes more permeable (3, 5; see also rapid swelling, fig 1) increasing the rate of membrane permeation by NADH. In this fashion respiration rates could rise more rapidly with low concentrations of DNP than does uncoupling, contributing to the maintenance of a high level of contraction. Some such explanation is needed to account for the net increase in contraction level produced by uncouplers when contraction is limited (19, and text above).

We conclude that the lowered contraction/respiration ratio indicates a true uncoupling of respiration-linked contraction, and that the level of contraction reflects the level of energy conserved in some primary high energy intermediate or state.

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Literature Cited