MITOCHONDRIAL ALTERATIONS ASSOCIATED WITH AVIAN RETICULOENDOTHELIOSIS VIRUS (STRAIN T) PATHOGENICITY

W. X. Balcavage[1,2]
K. L. Baxter-Gabbard[1]
M. Ko[2]
M. Rea[2]
F. Padgett[1]
A. S. Levine[1]

[1]Indiana University School of Medicine
Terre Haute Center for Medical Education
at Indiana State University
Terre Haute, Indiana 47809
and
[2]Department of Chemistry
Indiana State University
Terre Haute, Indiana 47809

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SUMMARY

This report indicates that reticuloendotheliosis virus (REV) induces morphological, physical and enzymatic alterations in chick target organ mitochondria. Succinoxidase activity of infected mitochondria is decreased at 5 days post-REV-infection, but ADP:O ratios are unchanged. Calcium accumulation is decreased from 100 nMoles/mg of protein in controls to 50 nMoles/mg of protein in infected mitochondria. Mitochondria from infected tissue exhibit a buoyant density of 1.193 gm/cc, significantly greater than the buoyant density of 1.167 gm/cc for control mitochondria. Mitochondria isolated from REV-infected livers were effective in transmitting reticuloendotheliosis when inoculated intraperitoneally into day old chicks. Electron microscopy of tissue sections indicates that infected cell mitochondria are enlarged with a less dense matrix relative to controls. Purified mitochondria from infected livers contained up to 16 particles having the size and shape of unenveloped(naked) C-type RNA tumor virus.

It has previously been shown that viral-induced neoplastic reticuloendotheliosis is associated with reticuloendotheliosis virus (REV) (1). This virus exhibits oncogenic properties and is a well characterized RNA C-type particle (2, 3, 4, 5, 6). Previous attempts to derive a relationship between tumor cell mitochondria and the oncogenic event date from the work of Warburg (7, 8), but have been relatively unsuccessful. Numerous investigators have observed a decrease in the number of mitochondria per neoplastic cell (9, 10, 11, 12), but this characteristic has been attributed to the similar phenomenon seen in rapidly dividing non-neoplastic (e.g., embryonic) tissue.

Other investigators have described an increase in mitochondrial DNA (13) or the rate of incorporation of ^{3}H-thymidine (14) into mitochondria of neoplastic tissue. Chang, et al. (15) have described alterations in mitochondrial membrane proteins from neoplastic cells, while Kara, et al. (16) have observed viral-like inclusions within mitochondria of neoplastic tissue. In addition, Oda (13) has shown that mitochondria or mitochondrial membranes from hepatoma, AH-130, are effective in inducing hyperimmunization and protection against future challenge by AH-130 cells.

In this communication we present preliminary physical, enzymological and morphological evidence which suggests that mitochondria of REV target organs are a locus for REV replication. Highly significant in this respect is that mitochondria prepared from REV-infected target organs contain viral-like inclusions and are capable of transmitting reticuloendotheliosis to newly hatched chicks after intra-peritoneal injection.

MATERIALS AND METHODS

Mitochondria were prepared from the livers of chicks aged one to seven days post-hatching using the method of Schneider (17) and the medium described by Chance and Hagihara (18). Infected tissue was induced by intra-peritoneal injection into day old chicks of 0.25 ml of a 10% gravity settled homogenate of RE-specific liver and spleen tissue. Respiratory studies were performed at 25°C with an Interscience polarograph (19) as previously described (20). Protein was estimated as described by Murphy and Kies (21) with a bovine serum albumin standard. Sucrose density gradients were prepared as described by Baxter-Gabbard (22) and refractive indices of gradient fractions were determined using a Bausch and Lomb 3L refractometer. Tissue samples for electron microscopy were prepared by the procedure of Padgett (23). Electron microscopy of density gradient purified mitochondria was performed by negative staining techniques (4).

RESULTS

Initial observations on mitochondria derived from REV infected tissue involved the polarographic determination of mitochondrial succinoxidase activity.

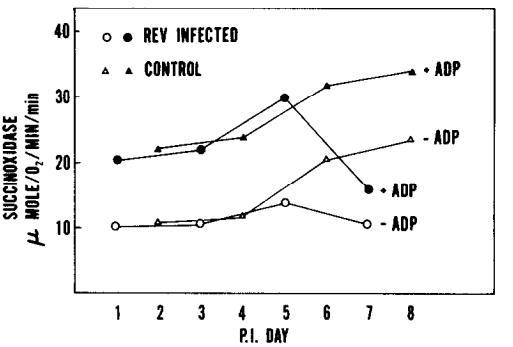

FIGURE 1. Respiratory activity of chicken liver mitochondria. The oxidizable substrate was 6 mM succinate and state 3 activity was initiated with 125 μM ADP (+ADP). The 3 ml assay system contained 10 mg. of mitochondrial protein, 0.25 M Mannitol, 1 mM K Cl, 2 mM Tris, 1 mM KH_2PO_4, 25 M EDTA. PI - Post Infection.

Figure 1 demonstrates that mitochondria from control and REV infected tissues exhibit an initial increase in activity usually associated with rapidly developing tissues. However, after 5 days post-infection (p.i.), mitochondria exhibit a strikingly decreased succinoxidase activity (Fig. 1), while control mitochondria continued to exhibit an increasing level of activity. The major alteration in infected tissue mitochondria is associated with the state 3 (+ADP) respiratory activity, but the energy coupling activity as measured by the ADP:O ratio is largely unaltered. These results suggest that the membrane associated energy coupling process is impaired as a consequence of REV infection. In this context it is of interest to note that Chang, et al. (15) have observed marked alterations in protein content of mitochondrial membranes of neoplastic cells.

Normally, mitochondria possess a variety of other ways of utilizing the energy coupling process. One of these ways is via ion pumping as reflected by their energy dependent accumulation of calcium (Ca^{++}), with the stoichiometric ejection of protons (H^+) (24). Figure 2 illustrates that REV infected liver mitochondria exhibit a greatly decreased potential for H^+ ejection after addition of Ca^{++} to the reaction system. In Fig. 2, it is shown that in response

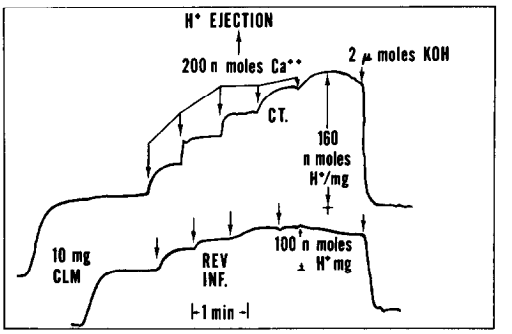

Fig. 2.

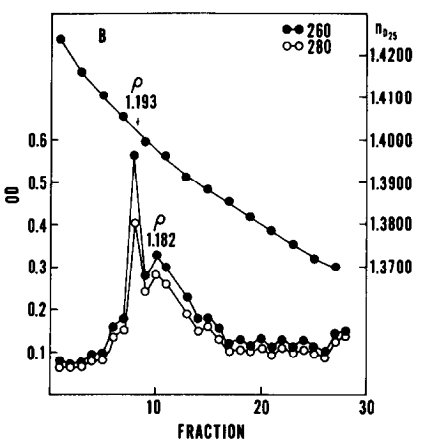

Fig. 3.

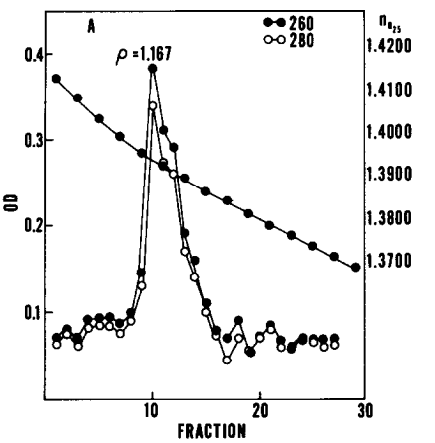

Fig. 4.

FIGURE 2. H^+ ejection by control and REV infected mitochondria. Mitochondria were added to a medium containing 0.25 M Mannitol, 0.001 MKCl, and 1 mM KH_2PO_4, pH 7.2. H^+ ejection was monitored with a recording pH meter. 200 nMoles of calcium were added at each arrow.

FIGURE 3. Sedimentation profile of REV infected mitochondria purified by differential and velocity sedimentation. The gradient was centrifuged to equilibrium and ten drop fractions were collected, diluted to 2.5 ml with buffer and the O.D. measured at 260 and 280 nm.

FIGURE 4. Sedimentation profile of control (non-infected) chick liver mitochondria purified by differential and velocity sedimentation. Other conditions as in fig. 3.

to calcium additions, control mitochondria can eject up to 160 nMoles of H^+X mg^{-1}, while REV infected tissue mitochondria can eject a maximum of 100 nMoles H^+X mg^{-1}, again suggesting an REV associated alteration in energy coupling processes. In separate experiments (not shown), it was observed that the decrease in Ca^{++} induced H^+ ejection parallels the onset of viral reticuloendotheliosis pathology with an 18% and 38% decrease in H^+ejection capacity at 6 and 8 days p.i., respectively. The development of the neoplastic symptoms appears on the 5th or 6th day p.i., and is lethal after 7 to 11 days p.i.

Liver section prepared from tissue excised 5 and 7 days p.i. showed gross changes in mitochondrial morphology, in agreement with previous reports (25). The mitochondria, in situ, appeared swollen with an electron translucent matrix. Control chick tissues exhibited mitochondria with an orthodox conformation (26) and no alterations in the matrix density.

When the buoyant density of control and infected tissue mitochondria was examined by density gradient centrifugation, it was observed that infected tissue mitochondria exhibited a significantly greater buoyant density than control tissue mitochondria (ρ =1. 193 gm/cc vs ρ = 1.167 gm/cc) as shown in Figs. 3 and 4, respectively. The significance of the band at ρ =1.182 gm/cc in Fig. 3 is at the moment undetermined.

When mitochondria from REV infected tissue were purified by the preceding method, negatively stained, and examined by electron microscopy, mitochondria were observed which contained up to 16 internally located particles. These objects approximate in size and shape unenveloped RNA C-type viruses.

These results suggested that viral replication occurred in association with mitochondria. It was proposed that such mitochondria contain all of the elements required for viral replication and would be effective in transmitting the neoplasia into previously unchallenged chicks. To test this proposal, mitochondria were isolated (17) from chick livers 7 days p.i. Mitochondria (3.1 mg protein) were injected (i.p.) into 40, one-day old chicks. After 8 days chicks exhibited symptoms similar to those induced with REV. Dead chicks were autopsied

and found to contain the liver lesions typical of reticuloendotheliosis. Within 14 days, 36 of the 40 chicks had died of reticuloendotheliosis.

DISCUSSION

The results presented here are supported by results of other investigators. Oda (13) has described alterations in nucleic acid content (and presumably density) of hepatoma mitochondria, while Kara, et al. (16) have reported viral inclusions in mitochondria of Rous Sarcoma cells. Oda (13) has also shown that mitochondria or mitochondrial membranes are capable of hyperimmunizing challenged rats and protecting against subsequent lethal doses of AH-130 hepatoma cells.

The preceding reports, when viewed in the context of the results of the present investigation, make it seem reasonable to suggest that mitochondria may be a target organelle for oncogenic modification, at least in the case of REV. The importance of these observations in further elucidation of the viral induced neoplastic event needs little elaboration and experiments are in progress to extend and clarify the observations reported here.

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