EFFECTS OF PHOSPHOTUNGSTIC ACID AND SILICOTUNGSTIC ACID ON RESPIRATION AND INTEGRITY OF RAT LIVER MITOCHONDRIA

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Summary

Phosphotungstic acid and silicotungstic acid, when employed at low concentrations (≤ 0.2 mg/mg protein), were found to markedly inhibit mitochondrial respiration stimulated by either ADP or 2,4-dinitrophenol. At higher concentrations these negative staining agents were found to solubilize significant amounts of mitochondrial protein. The results clearly show that PTA and STA are not biochemically inert and probably have more than a single mitochondrial site of action.

PTA and STA[1] have been widely used as negative staining agents in electron microscopy. Use of PTA in the study of mitochondrial morphology has resulted in visualization of the 90 Å particles protruding from the mitochondrial inner membrane (Fernandez-Moran, 1962; Stoeckenius, 1963) and identification of these particles as the electron microscopical expression of the soluble coupling factor F_1 (Kagawa and Racker, 1966). Racker's group has also used STA to remove the 90 Å particles from submitochondrial membrane preparations (Racker, 1969). Despite the widespread use of PTA in studies of mitochondrial ultrastructure, and the more recent use of STA as a mitochondrial ATPase detaching agent, no detailed studies concerning the biochemical effects of either PTA or STA on mitochondria have been reported. In this communication we report the results of studies of the

1 Abbreviations used are PTA: phosphotungstic acid, $2H_3PO_4 \cdot 24WO_3 \cdot H_2O$; STA: silicotungstic acid, $SiO_2 \cdot 12WO_3 \cdot H_2O$; DNP: 2,4-dinitrophenol.

effects of these agents on the respiration and integrity of intact mitochondria and submitochondrial particles.

Methods

Rat liver mitochondria were isolated as described by Schneider and Hogeboom (1951) and resuspended at a concentration of 50 mg mitochondrial protein/ml in a medium containing 220 mM D-mannitol, 70 mM sucrose, 2.0 mM HEPES buffer, pH 7.4, and 0.5 mg/ml crystalline bovine serum albumin. A submitochondrial particle preparation was obtained by treatment of whole mitochondria with the non-ionic detergent Lubrol WX (obtained from I. C. I. Organics Inc., Providence, R. I.) for 15 minutes at a concentration of 0.16 mg/mg mitochondrial protein followed by centrifugation at 105,000 x g for 1 hour. The sediment fraction was resuspended in the medium described above.

Respiration rates and acceptor control ratios were determined polarographically in a 2.0 ml system using the $MgCl_2$ containing-respiration medium described by Schnaitman and Greenawalt (1968) with the additions specifed in the appropriate figure legends.

Solubilization of mitochondrial protein by STA (obtained from British Drug Co., Ltd., Poole, England) and PTA (obtained from Merck Inc., Rahway, N. J.) was studied by adding appropriate amounts of stock STA or PTA solutions (20% in 0.25 M sucrose, pH 7.0), and, where indicated, 0.16 mg Lubrol WX per mg mitochondrial protein, to a mitochondrial suspension to give a final concentration of 12 mg mitochondrial protein per ml. This mixture was allowed to stand at 0°C for 15 minutes and then centrifuged at 105,000 x g for 1 hour. Protein concentrations in the pellet and supernatant fractions were determined by the biuret procedure.

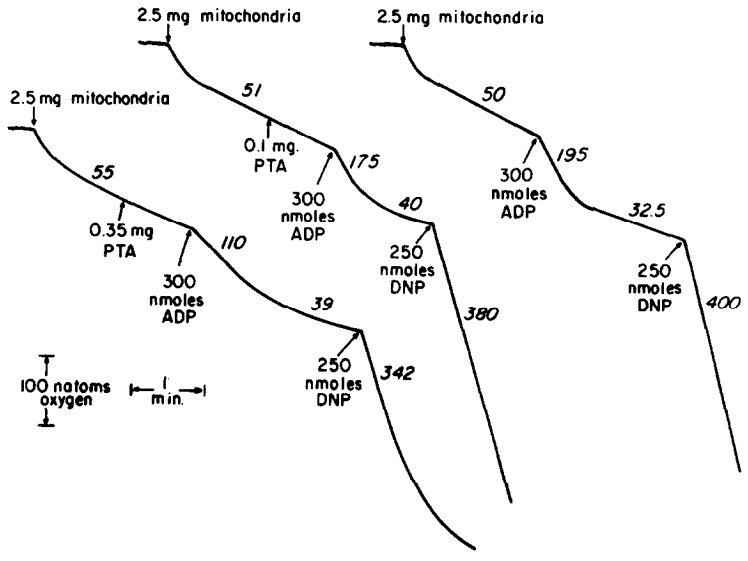

Figure 1

Typical polarographic traces showing the effect of PTA on mitochondrial respiration stimulated by ADP or DNP. Respiration conditions using succinate as substrate are described under Methods. Rates of respiration shown on the traces are expressed as mμatoms O_2 consumed/min. Similar traces were obtained when STA replaced PTA.

Results and Discussion

The polarographic traces presented in Figure 1 show the results consistently obtained on addition of PTA to respiring mitochondria. Similar results were observed with STA. Both the rate of ADP-stimulated respiration and the sharpness of the state 3 to state 4 transition are markedly decreased in the presence of PTA or STA. At concentrations of PTA or STA below 0.2 mg/mg protein the state 4 rate is not noticeably affected suggesting a specific effect of these agents on respiration coupled to the phosphorylation of ADP. Figure 2A illustrates the effect of increasing concentrations of PTA and STA on the rate of state 3 respiration. The results are presented in this way because the slow state 3 to state 4 transition makes determination of acceptor control ratios as defined by Chance and Williams (1955) uncertain.

In addition, the polarographic traces in Figure 1 indicate a significant

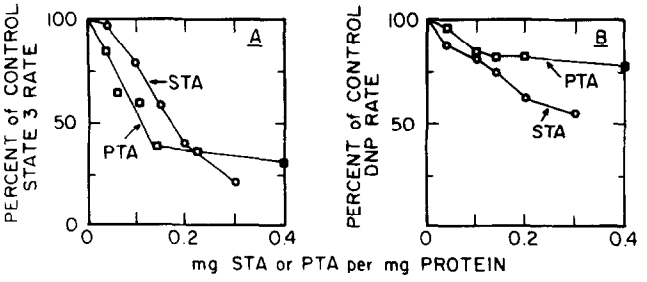

Figure 2

Effect of STA and PTA concentration on ADP- or DNP-stimulated respiration of mitochondria. Respiration condition are as described in Figure 1. Where indicated appropriate amounts of 1% STA or PTA (pH 7.4) was added followed by addition of 300 nmoles ADP and 250 nmoles DNP. Similar results were obtained using 2-hydroxybutyrate as substrate. A, ADP-stimulated (State 3) rate. B, DNP-stimulated (uncoupled) rate.

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decrease in the initial rate of DNP-stimulated respiration when mitochondria are pre-treated with STA or PTA. The dependence of this decrease on concentration of STA and PTA is shown in Figure 2B. Initial rates are presented because the rate of oxygen uptake in the presence of dinitrophenol and STA or PTA declines to zero with a time constant that is dependent on the concentration of STA or PTA (cf. Figure 1). Similar results presented in Figure 3 were obtained with the Lubrol WX submitochondrial particle preparation which also exhibits uncoupled respiration. These results taken together suggest a relationship between the integrity of mitochondrial structure as reflected by coupling of respiration to phosphorylation and the extent of the inhibitory effects of STA or PTA on oxygen uptake. In uncoupled mitochondria, complete cessation of respiration occurs in a time dependent process; in tightly coupled mitochondria, only ADP stimulated oxygen uptake is inhibited.

In experiments not presented here, inhibition of ADP-stimulated respiration by PTA, but not by STA, could be reversed by washing the mitochondria once with isolation medium. Respiration inhibited by either PTA or

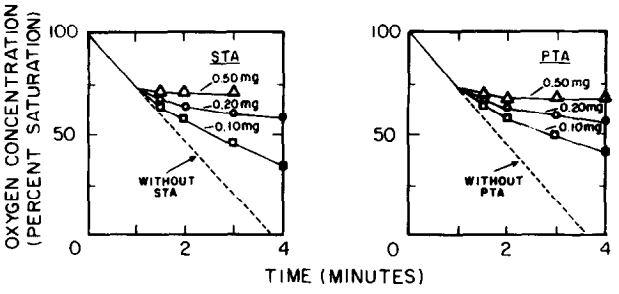

Figure 3

Effect of STA and PTA on the respiration of submitochondrial particles. Respiration conditions are as described in Figure 1 and Methods. Where indicated appropriate amounts of 1% STA or PTA was added. The points plotted were taken from continuous polarographic traces and normalized to the point of addition of STA or PTA.

STA in the submitochondrial preparation could be partially restored by washing. Thus, PTA and STA appear to bind with significantly different affinities to the intact inner membrane of mitochondria, and with more similar affinities to the submitochondrial membrane preparation.

STA and PTA, at concentrations higher than those used in the respiration experiments, also appear to have effects on mitochondrial integrity as reflected by solubilization of protein. The results illustrated in Figure 4A show that STA solubilizes more protein than Lubrol WX alone and, furthermore, that these two agents probably solubilize the same complement of proteins since their effects are not additive. In contrast, PTA does not solubilize large amounts of protein in the absence of Lubrol WX (Figure 4B). However, it increases the amount of protein solubilized in the presence of Lubrol WX from 51% to 84%. The reason for the different behavior of PTA and STA in these experiments is not clear, but one explanation in agreement with the data is that PTA, unlike STA, cannot effectively penetrate (or disrupt) the normal inner membrane structure.

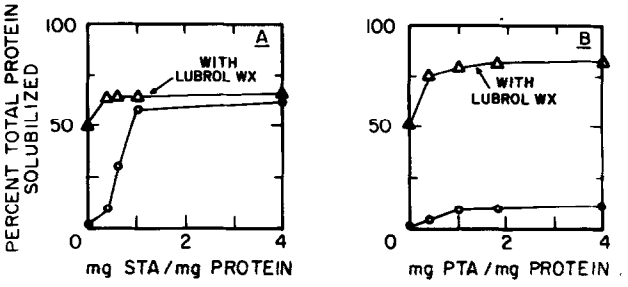

Figure 4

Solubilization of mitochondrial protein by STA and PTA. Intact mitochondria were treated and centrifuged as described under Methods. The proportion of total protein appearing in the 105,000 x g supernatant is plotted.

Although the results presented here are not sufficiently complete to allow speculation on the mechanism of action of these agents, they do clearly indicate that PTA and STA are not inert with respect to the structure and function of the mitochondrial membrane system.

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