EFFECTS OF TANNIC ACID AND 2-THENOYLTRIFLUOROACETONE ON SUCCINATE OXIDATION AND PENETRATION ACROSS THE MITOCHONDRIAL MEMBRANE

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1. Introduction

The penetration of succinate across the mitochondrial membrane is a rate limiting step in the oxidation of this substrate in isolated intact mitochondria [1] whereas in mitochondrial particles the oxidation of succinate is only dependent upon the activity of the succinate oxidase system.

TTA* is a known specific inhibitor of succinate CoQ-reductase activity [2, 3] and also of succinate oxidation both in intact mitochondria [4–6] and in submitochondrial particles [3]. Tannic acid, at concentrations which inhibit succinate oxidation in mitochondria, is devoid of any effect on submitochondrial particles [7]. TTA is a chelating agent for iron [8] and its effect on succinate oxidase has been generally ascribed to interaction with nonheme iron [2–6]. Tannic acid though having the capacity to precipitate ferrous salts is essentially a protein cross-linking reagent [9] and its effect on succinate oxidation (only in intact mitochondria) together with its inhibition of mitochondrial swelling in isotonic ammonium succinate has been explained by suggesting an interaction of this drug with the hypothetic exchange diffusion carrier of succinate [10].

However, in the course of the study on the mechanism of action of tannic acid, it has been observed that increasing the concentration of this drug caused an inhibition of succinate oxidation also in submitochondrial particles [11].

On the basis of this result and of the recent proposal by Tyler and Newton [12] that nonheme iron can be involved in the mechanism of anion exchange of dicarboxylic acids, it was decided to compare the effect of TTA and tannic acid on succinate penetration and oxidation in both intact and disrupted mitochondria.

* *Abbreviation:*
TTA: 2-thenoyltrifluoroacetone.

2. Methods and materials

Rat liver mitochondria were isolated by conventional techniques in a medium of 0.25 M sucrose, 4 mM tris-HCl pH 7.4.

Submitochondrial particles were prepared by ultrasonic disruption of rat liver mitochondria [13] in a medium containing 10 mM succinate and 30 mM phosphate buffer pH 7. Respiration rates were measured with a Clark oxygen electrode [14]. Mitochondrial swelling was followed by measuring absorbancy changes at 546 nm in an Eppendorf photometer equipped with a recorder.

Pure tannic acid was obtained by extraction with solvent from a neutralised solution of commercial tannic acid [15]. The purity was assessed by paper chromatography [16]. TTA was obtained from Eastman Kodak, valinomycin was a gift of Professor V.V. Zakusov (Moscow), all other reagents were analytical grade.

3. Results

3.1. *Intact mitochondria*

The effect of increasing concentrations of tannic acid on (a) succinate oxidation (stimulated by ADP), (b) swelling in ammonium succinate [17], and (c)

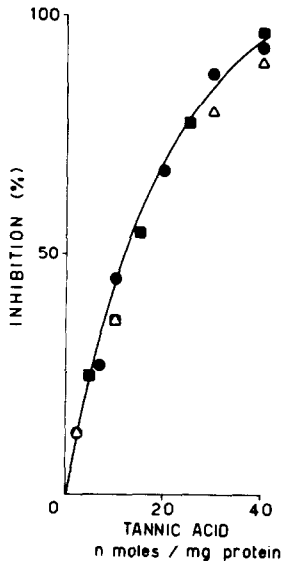

Fig. 1. Inhibition by tannic acid of succinate oxidation (•), swelling in ammonium succinate (△) and valinomycin induced swelling (▪). The reaction medium (2 ml) for respiration contained 100 mM KCl, 20 mM tris-HCl pH 7.4, 0.1 mM EGTA, 5 mM phosphate, 5 mM succinate, 0.25 mM ADP and rotenone (2 μg). Swelling in 100 mM ammonium succinate, 0.1 M EGTA, rotenone (2 μg) and antimycin (2 μg) was initiated by 3 mM ammonium phosphate (final volume 3 ml). Valinomycin (0.2 μg) induced swelling was observed in a medium containing 200 mM sucrose, 10 mM tris-HCl pH 7.4, 0.1 mM EGTA, 2 mM KCN, 5 mM succinate (tris-salt) (final volume 3 ml). Mitochondrial protein was 3–4 mg, temperature 30° in any case.

valinomycin induced swelling in the presence of K^+ [18] with succinate as accompanying anion, is shown in fig. 1.

It can be seen that the sensitivity of the three systems to tannic acid is exactly the same. It should be pointed out that in the experiments of valinomycin induced swelling, succinate was acting only as permeant anion, while the energy requirement of the system was supported by ATP in the presence of KCN.

The most reasonable interpretation of these results is that already proposed [10]: tannic acid inhibits succinate oxidation by preventing its penetration into mitochondria.

Furthermore in fig. 2 it is shown that tannic acid greatly prevented the shrinkage of valinomycin swollen mitochondria [19] due to addition of either an uncoupling agent (FCCP) or an energy transfer inhibitor (oligomycin). This result may be taken as an indication that tannic acid prevents also the exit of succinate from mitochondria, although an interference of the drug with the exit of the cation cannot be excluded.

The effect of increasing concentrations of TTA on succinate oxidation, stimulated either by ADP or by DNP, is shown in fig. 3. Both coupled and uncoupled respiration are almost completely inhibited by 10 nmoles of TTA/mg of mitochondrial protein. Unlikely tannic acid [10], the inhibition of succinate oxidation by TTA was not affected by varying the concentration of succinate from 1 to 10 mM. Amounts of TTA in the range of those giving complete inhibition in intact mitochondria are practically devoid of any effect on succinate penetration as revealed by experiments on swelling in ammonium succinate. However on increasing the concentration of TTA from 10 to 50 nmoles/mg protein inhibition of swelling in ammonium succinate could be shown (table 1). The same result could also be obtained in the case of valinomycin induced swelling.

3.2. *Submitochondrial particles*

Table 2 shows the effect of tannic acid and TTA on the oxidation of succinate in submitochondrial particles. The amount of tannic acid needed to produce 50% inhibition of succinate oxidase activity of submitochondrial particles is six times higher than that producing 50% inhibition of the penetration of cuccinate across the mitochondrial membrane.

Table 1
Effect of TTA on mitochondrial swelling in ammonium succinate.

TTA (nmoles/mg protein)	Extent of swelling (absorbancy units)	Inhibition (%)
0	−0.340	
10	−0.320	5
20	−0.230	32
30	−0.130	60
50	−0.060	82

Experimental conditions as in fig. 1. Extent of swelling was measured 2 min after the addition of 3 mM ammonium phosphate.

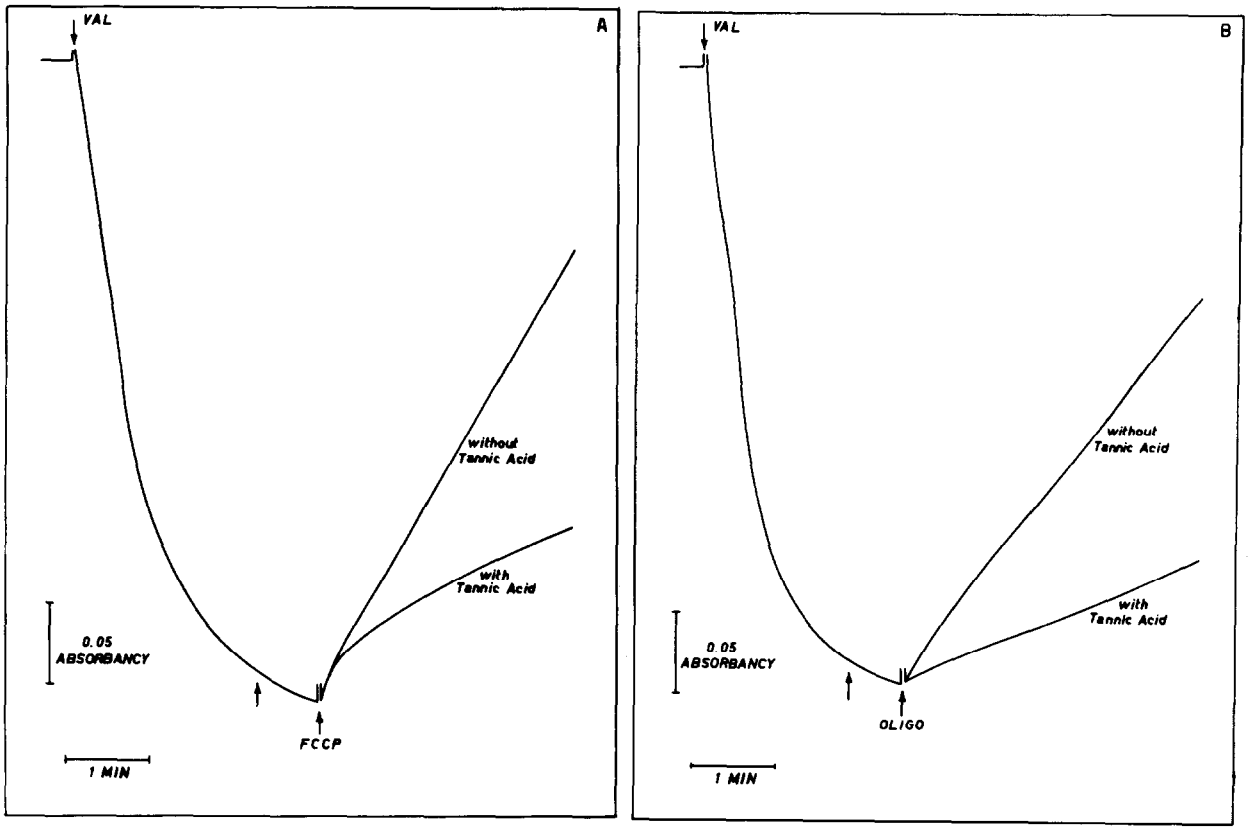

Fig. 2. Effect of tannic acid on the shrinkage of valinomycin swollen mitochondria. FCCP was 2 μM, oligomycin was 2 μg. Tannic acid (15 nmoles/mg protein) was added at the time indicated by the arrow.

By contrast, the concentrations of TTA acting on this system are in the same range as those preventing the swelling in ammonium succinate although above those inhibiting the oxidation of succinate in intact mitochondria. This indicates that the inhibition of succinate penetration, in the case of TTA, cannot explain the inhibition of respiration in intact mitochondria. The observation that succinate oxidation in intact mitochondria (fig. 3) is more sensitive to TTA than in submitochondrial particles (table 2) deserves further attention.

4. Conclusions

The following conclusions can be drawn from the experiments reported above: (a) the amount of tannic acid required to prevent succinate penetration into mitochondria corresponds exactly to that needed to inhibit the oxidation of this substrate in intact mitochondria, strongly indicating a cause–effect relationship between these two phenomena, (b) the concentrations of TTA which inhibit succinate oxidation in intact mitochondria are below those preventing succinate penetration indicating that the two effects are not related.

The results presented in this study are difficult to reconcile with the proposal [12] that nonheme iron is involved in the mechanism of anion exchange of dicarboxylic acids. However, the observation that a specific inhibitor of succinate oxidase activity like TTA interferes with succinate penetration together with the observed inhibition of succinate oxidation in submitochondrial particles by tannic acid may indi-

Table 2
Effect of tannic acid and TTA on succinate oxidation in submitochondrial particles.

Drug (nmoles/mg protein)	Oxygen uptake (natoms O/mg protein/min)	Inhibition (%)
Tannic acid		
0	380	
40	380	
50	320	16
60	200	48
80	160	58
100	110	71
TTA		
10	200	48
20	140	63
40	45	88
80	35	98

Submitochondrial particles were incubated in a medium (2 ml) containing 100 mM sucrose, 30 mM phosphate buffer pH 7.4, 10 mM succinate, cyt. *c* (1 mg) and rotenone (2 μg). Temperature was 37°

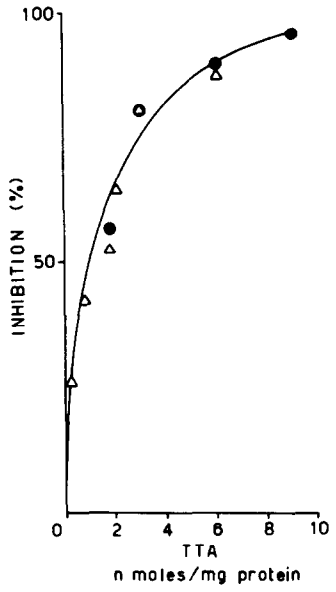

Fig. 3. Inhibition by TTA of succinate oxidation stimulated by 0.25 mM ADP (△) or 0.1 mM DNP (●). Experimental conditions as in fig. 1.

cate that translocation of succinate and its oxidation are not completely independent.

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