THE STIMULATION OF THE MITOCHONDRIAL OXIDATION OF A QUINOLPHOSPHATE BY POLYLYSINE

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1. Introduction

Quinolmonophosphates have been considered for some time on the basis of model reactions to be attractive candidates for the role of high-energy intermediates in oxidative phosphorylation [1–4] and have been incorporated into several theoretical schemes [5–7]. However, spectroscopic studies have failed to find any evidence for the presence of high-energy derivatives of ubiquinone [8,9], although what appears to be a phosphorylated derivative of vit K has been isolated from *M. phlei* [10]. One of the objects of the present investigation has been to study the mitochondrial oxidation of simple quinolphosphates, and in particular 2,3-dimethylnaphthoquinolmonophosphate (DMNQP) **, as models for ubiquinolphosphate and vit K phosphate, the synthesis and stability of which offer some difficulty [11]. A brief report [12] that ubiquinolphosphate, but not vit K phosphate, is oxidised by mitochondria, with specific transfer of the phosphoryl residue to ADP, has not yet been confirmed. However, fractions from *M. phlei* have been shown to catalyse the oxidation of a quinolphosphate [13] and a chromanylphosphate [14].

DMNQP is oxidised by a variety of mitochondrial preparations, but only at slow rates compared to those of the normal substrates. This communication describes the striking stimulation by polylysine of the oxidation of DMNQP by beef-heart mitochondria. Polylysine has been described [15–17] as stimulating specifically the oxidation of tetrahalobenzoquinols, e.g., tetrachlorobenzoquinol (TCBQ), by mitochondria and by isolated cytochrome oxidase. Evidence is presented that polylysine promotes a direct interaction between DMNQP and the cytochrome oxidase portion of the respiratory chain.

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** Abbreviations: DMNQP, 2,3-dimethylnaphthoquinolmonophosphate; TCBQ, tetrachlorobenzoquinol, TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine.

2. Materials and methods

DMNQP was synthesised by the method of Andrews [18]. Heavy-layer beef-heart mitochondria were prepared by the method of Sanadi and Fluharty [19] and stored at −20°. Cytochrome oxidase was prepared as described by Sun and Jacobs [20]. Protein was estimated by the method of Gornall et al. [21] after solubilisation with deoxycholate (0.2% w/v).

Poly-L-lysine hydrobromide (M.W. approx. 195,000) was obtained from Sigma.

3. Results

The effect of the addition of polylysine on the rate of oxidation of DMNQP, TCBQ-ascorbate and other substrates by beef-heart mitochondria is shown in table 1. The maximum stimulation is achieved with about 0.15 mg polylysine/mg mitochondrial protein. Increasing the concentration of DMNQP above 2 mM

Table 1
The influence of polylysine on mitochondrial oxidations

Substrate	Rate of oxidation	Rate after polylysine
	(mμgatoms O_2/min/mg protein)	
DMNQP	2	53.5
TCBQ-ascorbate	34	58
Pyruvate-malate	53	0
Succinate	70	3.5
TMPD-ascorbate	56	6 *

Incubations: 250 mM sucrose, 50 mM tris-HCl pH 7.6, 3.6 mg mitochondrial protein, 0.9 mM DMNQP or 0.7 mM TCBQ–9 mM ascorbate or 3 mM Na pyruvate–3 mM tris-malate or 3 mM Na succinate or 30 μM TMPD–9 mM ascorbate, and 0.5 mg poly-L-lysine. Where pyruvate-malate, succinate and TMPD-ascorbate served as substrates, 6 mM KPO_4 and 1 μmole ADP were also present (total volume 3.2–3.45 ml). Oxygen uptake was determined polarographically at 30°.

* Cyanide-insensitive. A similar rate of oxygen consumption remains after addition of KCN to the stimulated oxidation of TCBQ-ascorbate.

leads to a decline in the rate of oxidation. The extent of the inhibition of the stimulated oxidation by various compounds is given in table 2.

The mitochondrial oxidation of DMNQP is also stimulated by the addition of cytochrome c. Under the conditions of table 1, 10 mμmoles cytochrome c produce approximately the same stimulation as the polylysine. However, free cytochrome c is reduced rapidly and non-enzymatically by DMNQP.

The ability of polylysine to stimulate the oxidation of DMNQP by a cytochrome oxidase preparation is

Table 2
Inhibition of the polylysine-stimulated oxidation of DMNQP

Compound	Concentration	% inhibition
KCN	0.7 mM	100
Antimycin a	2.5 μg/mg protein	3
ADP	0.3 mM	40
Pi	6 mM	40
Acetate	6 mM	0

Incubations: as table 1, with 0.9 mM DMNQP as substrate.

demonstrated by the data in table 3. The effect of added cytochrome c, both alone and in combination with polylysine, and the influence of polylysine on the oxidation of TCBQ-ascorbate and TMPD-ascorbate are also shown.

The presence of polylysine has no apparent effect on the reduction by TMPD-ascorbate of cytochromes $c + c_1$ (wavelength pair 550–540 mμ on the Aminco-Chance Dual-Wavelength spectrophotometer) or cytochrome a (605–630 mμ) bound in the respiratory chain. However, polylysine does greatly decrease the rate of reduction of cytochromes $c + c_1$ by pyruvate-malate. The non-enzymatic reduction of free cytochrome c by DMNQP is not affected.

4. Discussion

Polylysine is an inhibitor of cytochrome oxidase [22] and consequently blocks the usual mitochondrial oxidations (cf table 1). The results of the dual-wavelength spectroscopy demonstrate that this con-

Table 3
The oxidation of DMNQP by isolated cytochrome oxidase

Substrate	Rate (mμgatoms O_2/min)	Addition	Rate	Addition	Rate
DMNQP	3	Polylysine	142	KCN	13
DMNQP	0	Cyt c	690	Polylysine	125
DMNQP	0	Polylysine	112	Cyt c	112
TCBQ-ascorbate	75	Polylysine	192	KCN	21
TMPD-ascorbate	19	Cyt c	253	Polylysine	24

Incubations: 250 mM sucrose, 50 mM tris-HCl pH 7.6, cytochrome oxidase (1 mg protein), 1.75 mM DMNQP or 0.7 mM TCBQ–9 mM ascorbate or 30 μM TMPD–9 mM ascorbate, 1 mg poly-L-lysine and, where appropriate, 50 mμmoles cytochrome c (except in the experiment with TMPD-ascorbate, where 10 mμmoles were added) or 0.7 mM KCN (total volume 3.2–3.4 ml). Oxygen consumption was measured polarographically at 30°.

centration also inhibits the reduction of cytochromes c + c_1 by pyruvate-malate. However, TMPD-ascorbate enters the respiratory chain at the level of cytochrome c and although polylysine blocks the oxygen uptake, it appears to have no effect on the reduction of cytochromes c + c_1 and cytochrome a. Thus oxidations via reduced cytochrome c are disrupted at some point within the cytochrome oxidase complex. Consequently, since the same concentration of polylysine stimulates the oxidation of DMNQP, the changes produced appear to allow a direct interaction between DMNQP and the oxidase complex.

This hypothesis is given strong support by the ability of polylysine to promote the oxidation of DMNQP by isolated cytochrome oxidase. Cytochrome c, in the absence of polylysine, stimulates an even more rapid oxidation, but in this case, as with whole mitochondria, the rapid oxidation depends on the non-enzymatic reduction of free cytochrome c by DMNQP, the reduced cytochrome c then being reoxidised by the cytochrome oxidase. However, in the presence of polylysine the pathway via cytochrome c is not functional since addition of cytochrome c to the polylysine-stimulated oxidation fails to increase the rate, even though the non-enzymatic reduction of cytochrome c is unaffected.

The rates in table 1 demonstrate that polylysine promotes the oxidation of DMNQP as effectively as that of TCBQ. However, in contrast to TCBQ, in the absence of polylysine DMNQP is oxidised only very slowly by mitochondria and generally not at all by cytochrome oxidase, thus making the stimulation even more marked.

The inhibition of the stimulated oxidation by ADP and by Pi, but not by acetate, is of interest in view of the role which has been postulated [1–7] for quinolphosphates in phosphoryl transfer to ADP. However, the ability of Pi to partially reverse the effects of polycations has been described by Person and Fine [23] and the decrease in the rate of oxidation as the concentration of DMNQP is increased beyond 2 mM probably reflects a similar antagonism of the effects of polylysine by the quinolphospohate itself. In any case, since polylysine also acts as an uncoupler of oxidative phosphorylation [24], the significance of the stimulated oxidation of DMNQP remains uncertain.

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