Nature and origin of an inhibitor of oxidative phosphorylation present in freshly isolated sarcosomes from blowfly thoraces

In mitochondria from larvae of the wax-moth, *Galleria mellonella*, the stimulation of the ATP-orthophosphate exchange reaction by albumin has been shown to be due to the binding of a fat-soluble inhibitor by the albumin[1]. In experiments which will be reported elsewhere we have shown that a similar explanation can account for the stimulation by albumin of oxidative phosphorylation in sarcosomes from the thorax of the blowfly, *Calliphora erythrocephala*. Furthermore, we have been able to extract an inhibitor from freshly isolated sarcosomes with iso-octane. Thus it appeared that the inhibitor might be present in the thorax of the living insect. In order to test this possibility extracts were prepared both from thoraces which had been boiled for 1 min in iso-octane before being extracted, and from an equal number of thoraces which were ground for about 1 min before boiling iso-octane was added. The effect of these extracts on the P/O ratio obtained with fresh sarcosomes was then examined. For this purpose the sarcosomes were isolated from thoraces which were crushed in a sucrose medium to which albumin had been added to remove the inhibitor normally present in freshly isolated sarcosomes. The albumin was removed by washing and resuspending the sarcosomes in albumin-free medium. The results (Table I) show that an inhibitor is present in the crushed thoraces, and that its action is reversed by albumin. Extracts of thoraces which were boiled before being crushed did not contain the inhibitor, and therefore we conclude that it is produced by enzymic action when the tissue is crushed, and that it is not present in the living insect.

It has been suggested that when liver mitochondria are aged long-chain fatty acids are released, and that these are responsible for the low P/O ratio obtained with aged mitochondria[4,5]. In view of this suggestion, and of the known ability of albumin

TABLE I

INHIBITION OF OXIDATIVE PHOSPHORYLATION BY EXTRACTS OF BLOWFLY THORACES AND ITS REVERSAL BY ALBUMIN

Additions	*ΔO* *μatoms*	*ΔP* *μmoles*	*P/O*
None	3.9	6.4	1.6
Iso-octane extract of boiled thoraces	3.8	6.3	1.7
Iso-octane extract of crushed thoraces	2.6	1.8	0.7
Bovine plasma albumin fraction V (1 %)	6.0	11.1	1.9
Iso-octane extract of boiled thoraces + albumin (1 %)	5.5	10.6	1.9
Iso-octane extract of crushed thoraces + albumin (1 %)	4.7	10.0	2.1

Reaction medium: sucrose, 198 μmoles; glucose, 66 μmoles; $MgCl_2$, 6.6 μmoles; Tris-HCl buffer (pH 7.3), 11 μmoles; ethylenediaminetetraacetate, 2 μmoles; ADP, 3.3 μmoles; α-ketoglutarate, 12 μmoles; hexokinase, 550 Berger units; phosphate (pH 7.3), 13 μmoles; sarcosomes (2.2 mg protein); final volume, 1 ml. 0.1 ml 10 % KOH in centre well, temp. 25°, incubation time 20 min. O_2 uptake measured with differential manometers; phosphate uptake estimated from change in orthophosphate measured by method of MARTIN AND DOTY[2]. Sarcosomes isolated, by procedure similar to that described by LEWIS AND SLATER[3], in medium (3 ml for 50 thoraces) containing 0.32 *M* sucrose, 0.01 *M* Tris buffer (pH 7.3), 0.01 *M* ethylenediaminetetraacetate, 2 % albumin, both washed (2.5 ml) and suspended (0.75 ml) in albumin-free medium. Iso-octane extract equivalent to 2 thoraces evaporated in flasks.

Abbreviations: ADP, ATP, adenosine di- and triphosphate; Tris, tris(hydroxymethyl)-aminomethane.

to bind long-chain fatty acids[6], it seemed likely that the inhibitor produced in the blowfly thoraces may be of a similar nature. The results shown in Table II confirm the acidic nature of this inhibitor, in that it is completely removed from the iso-octane extract when this is shaken with K_2CO_3 solution, and may be recovered from the latter by acidification and extraction with iso-octane or ether. This procedure effects a considerable purification of the inhibitor from non-acidic substances, particularly neutral fat. In experiments designed to establish its identity an ethereal solution of the inhibitor, partially purified as described above, was treated with diazomethane, and the methylated products were examined by gas chromatography. This revealed the presence of methyl esters of C_{14}, C_{16}, and C_{18} acids in the proportions 4:72:24.

TABLE II

REMOVAL BY ALKALI OF AN INHIBITOR OF OXIDATIVE PHOSPHORYLATION FROM ISO-OCTANE EXTRACT OF BLOWFLY THORACES

Expt.	*Additions*	*ΔO µatoms*	*ΔP µmoles*	*P/O*
1	None	3.0	3.4	1.1
	Iso-octane extract of crushed thoraces	2.0	0.4	0.2
	Iso-octane extract after shaking with K_2CO_3	2.4	3.4	1.4
2	None	2.0	4.8	2.4
	Iso-octane extract of crushed thoraces	1.9	1.6	0.8
	Iso-octane extract of acidified K_2CO_3	1.3	1.1	0.8

Reaction medium and conditions as in Table I. Iso-octane extract shaken twice with an equal volume of 1 *N* K_2CO_3, and washed with water. K_2CO_3 acidified with HCl and extracted twice with equal volume of iso-octane.

In a similar extract prepared from an equal number of boiled thoraces a trace of C_{16} acid only was detected, equivalent to $< 4\,\%$ of the total long-chain acids in the inhibitory extract.

It appears then that the low P/O ratio obtained with blowfly sarcosomes isolated in the absence of albumin is due in part to the presence of free long-chain fatty acids released enzymically when the tissue is crushed.

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