# OLIGOMYCIN AND ADP SENSITIVITY OF *IN VITRO* ALKYLATION OF SH-GROUPS IN RAT LIVER MITOCHONDRIA

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## 1. Introduction

As shown in a preceding paper [1] radioactivity of *in vitro* $^{14}C$- or $^{35}S$-maleimide labelled mitochondrial SH-groups appeared in basic structural proteins and not significantly in the oligomycin insensitive ATPase protein ($F_1$).

In order to characterize further the labelled proteins, sensitivity of the labelling process to oligomycin and ADP was investigated. This was performed in analogy to the procedure of Azzi and Azzone [2], who found that there was no swelling of KCN-inhibited, ATP-supported liver mitochondria in the presence of oligomycin or ADP and that even high concentrations of NEM did not releave this block of swelling.

This paper shows that, in the presence of oligomycin, the labelling with $^{14}C$-NEM is decreased to about 52% of the control values. ADP leads to an increase of the labelling up to about 30% when compared with the control in the absence of added ADP. The implications of these findings are discussed.

## 2. Materials and methods

$^{14}C$-NEM was purchased from Schwarz Bioresearch Inc., New York, specific activity: 10.3 mCi/mmole. NEM from Schuchardt, München, was purified by sublimation. Oligomycin was obtained from Serva, Heidelberg. ATP was the disodium salt from Sigma, St. Louis, ADP the trisodium salt from Boehringer, Mannheim.

Wistar rats of a local strain, weighing 150–250 g were used. The mitochondria were prepared essentially in the conventional manner described previously [3]. The final stock solution was made up to about 2–3 ml.

Sufficient mitochondrial suspension was added to 20 ml of the incubation medium to obtain an absorbancy of 0.45–0.55 at 546 nm. The composition of the incubation medium is given in table 1. The protein concentrations, estimated by the method of Lowry et al. [4], were about 0.18 mg/ml incubation medium. After incubation for 30 sec at 24° the mitochondrial suspension was centrifuged for 1 min at 27,000 *g* at 5° in a Serval RC2b centrifuge. The mitochondrial pellets were superficially washed immediately afterwards and were resuspended with 0.25 M sucrose. The time from the start of the incubation to washing of the pellets was 6 min. Sonification of the mitochondria was carried out in 40 ml volumes of 0.25 M sucrose at 3–4 A for 15 min at 5° with the Branson S-75 sonifier. Subsequently, differential centrifugation of mitochondria and membranes was performed at 13,500 *g* and 100,000 *g* as described previously [3]. The membrane pellets were dissolved in 0.4 ml of 1% sodium dodecylsulfate. 0.2 ml aliquots were counted in a Tricarb scintillation counter in dioxane scintillation fluid.

## 3. Results and discussion

Reactivity of the mitochondrial SH-groups in the presence of oligomycin is decreased (table 1) if $^{14}C$-NEM is added to KCN-inhibited, ATP-supported mitochondria, a procedure analogous to that of Azzi and Azzone [2]; this indicates oligomycin sensitivity and thus, as previously shown [1], our labelling procedures could lead to the characterisation of OSCP (oligomycin-sensitivity-conferring-protein) [5].



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Table 1
SH-group labelling with $^{14}$C-NEM in the presence of either oligomycin or ADP in KCN-inhibited, ATP-supported mitochondria, according to Azzi and Azzone [2].

| Exp. | Concentration of $^{14}$C-NEM ($\mu$M) | Conditions | cpm/5 min* | % Inhibition |
|---|---|---|---|---|
| 1 | 10 | oligo omitted | 54 119 | |
| | 10 | oligo present | 28 186 | 48 |
| 2 | 5 | oligo omitted | 25 573 | |
| | 5 | oligo present | 17 942 | 30 |
| | | | | % activation |
| 1 | 10 | ADP omitted | 54 119 | |
| | 10 | ADP present | 64 567 | 19 |
| 2 | 5 | ADP omitted | 25 573 | |
| | 5 | ADP present | 34 022 | 33 |

The incubation medium contained: Tris 0.02 M, KCl 0.125 M, pH 7.5 with HCl, KCN 1 mM, ATP 0.5 mM.
Where present oligomycin was 0.68 $\mu$g/ml and ADP was 167 $\mu$M (exp. 1) and 330 $\mu$M (exp. 2). Serum albumin and respiratory substrate were omitted, see [2].

* The mitochondrial membrane pellets were dissolved in 0.4 ml 1% sodium dodecylsulfate; radioactivity was determined in 0.2 ml of the dissolved membranes.

The rate of swelling caused by 10 $\mu$M $^{14}$C-NEM was decreased by the addition of oligomycin (table 2). At 5 $\mu$M NEM, the rate of swelling was greater in the presence than in the absence of oligomycin. This finding is in accordance with preceding investigations, revealing an alternation of swelling-activating and swelling-inhibiting phases at these concentrations of maleimide [3]. The differential effects of the alkylation of SH-groups on swelling under these conditions are shown in table 2.

Table 2
Differential effects of $^{14}$C-NEM labelling on swelling.

| Conditions | $^{14}$C-NEM labelling in the mitochondrial membranes | Swelling | |
|---|---|---|---|
| | | 10 $\mu$M | 5 $\mu$M |
| Oligo omitted | + | + | −** |
| Oligo present | − | −* | + |
| ADP omitted | + | + | −** |
| ADP present | ++ | −* | −** |

The other conditions were the same as those shown in table 1.
* "−" means that there was a surplus of >60% in the time necessary to attain the same decrease in absorbancy at a given period of swelling, when compared with "+".
** "−" means that there was a surplus of >85% in time necessary to attain the same decrease in absorbancy at a given period of swelling, when compared with "+".

As Knight et al. [6] reported an increased reactivity of the "inner" versus the "outer" mitochondrial membrane SH-groups with mercurial reagents was accompanied by contraction and KCl extrusion. We have reported [7] that an increase of contraction of rat liver mitochondria can be achieved with low concentrations of maleimide in the presence of ATP and $Mg^{2+}$. It is reasonable to assume that both types of SH-group blockade, leading to mitochondrial contraction, took place on the same 'sort' of SH-groups, namely, the "inner" ones. Similar concentrations of maleimide to those used in the contraction studies [7], in the absence of ATP and $Mg^{2+}$, activated swelling, while the "outer" mitochondrial SH-groups were alkylated [3]. Klingenberg et al. [8] have shown that in the presence of $Mg^{2+}$, ATP is translocated mainly as ADP. As shown in tables 1 and 2, in the presence of ADP, there is an increase in reactivity of the membrane SH-groups together with an inhibition of swelling. This change in reactivity of the mitochondrial membrane sulfhydryl groups in the

presence of ATP and $Mg^{2+}$ [7] or ADP (table 1 and 2) is interpreted as a further indication of the conformational change in the mitochondrial membrane proteins.

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