CHEMICAL MODIFICATION OF EHRLICH ASCITES TUMOUR CELLS BY PERIODATE AND SUCCINIC ANHYDRIDE: EFFECTS ON METABOLISM AND MEMBRANE PERMEABILITY

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1. Introduction

Periodate and succinic anhydride are well-known reagents with a rather high degree of specificity [1, 2]: periodate oxidation is used in carbohydrate chemistry for the determination of adjacent hydroxyl groups [3], and succinylation of amino groups is a well-established procedure in protein chemistry [4]. Since these reactions can be carried out under very mild conditions it seemed of interest to study some effects on metabolism and membrane permeability of cells after modification with the reagents. Succinylation has already been used as a means to solubilize the erythrocyte membrane, and it has been found that certain biological properties of the membrane are retained [5]. Intact ascites tumour cells have been shown to exhibit an increased electrophoretic mobility after treatment with succinic anhydride [6]. The interest in periodate has recently grown by the detection of the mitogenic properties of this compound [7, 8].

We investigated the behaviour of various cell types after periodate oxidation and succinylation [9–11]. The present communication deals with some effects on Ehrlich–Lettré ascites tumour cells.

2. Materials and methods

Cells of the glycogen-free strain of the hyperdiploid Ehrlich–Lettré ascites tumour (EAT) were grown in NMRI mice and harvested 7 or 8 days after transplantation. The following preparations of EAT cell suspensions were used: i) unwashed cells containing native serum; ii) cells washed once in physiological saline, resuspended in saline or buffer solution; iii) cells washed three times in the same way. Excess sodium periodate was removed either by addition of small amounts of glycerol, by centrifugation and resuspension, or simply by adding excess glucose as metabolic substrate; all three procedures yielded the same results. With both reagents inhibitory effects of reaction products (formaldehyde, formate, iodate, succinate) could be excluded. The methods for the determination of metabolic rates, potassium and enzyme leakage, and of cell viability have been described [12]. In some experiments we used a new titrimetric method to measure O_2-consumption. The main principle of this 'oxy-stat' method is as follows: when oxygen in the reaction medium has been consumed a Clark electrode starts an autotitrator filled with paraffin oil and connected to a reservoir of oxygen at constant pressure and temperature. This oil displaces the oxygen and pushes it through a capillary into the reaction vessel which is shaken and kept at constant temperature together with the reservoir. CO_2 is absorbed by soda lime. The volume of oil titrated is continuously recorded and gives a direct measurement of the O_2 uptake by the cells. The method offers the advantage of keeping the cells at a constant oxygen concentration [13].

In order to avoid pH changes, all incubations were performed by stepwise addition in buffered solutions or in the pH-stat (pH 7.2 with periodate and 7.0 with succinic anhydride).

Transplantability of the modified cells was tested by intraperitoneal injection into healthy animals.

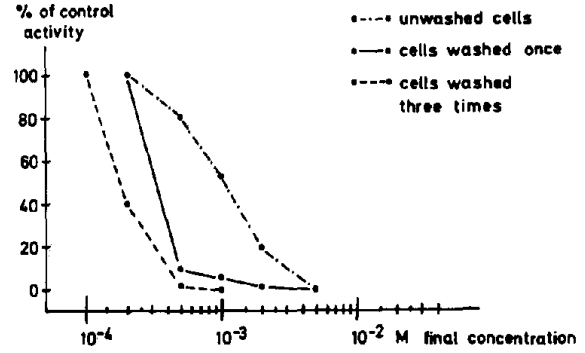

Fig. 1. Anaerobic glycolysis of EAT cells after incubation with $NaIO_4$ for 15 min at 37°C, pH 7.2. n = 14.

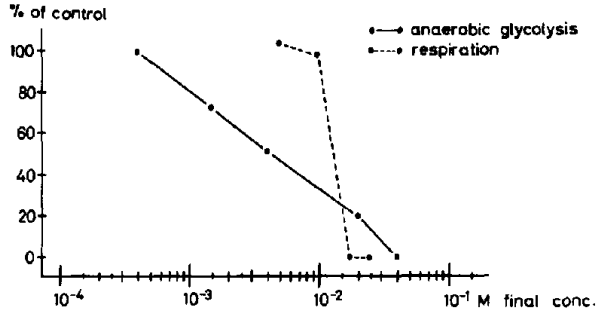

Fig. 3. Anaerobic glycolysis and respiration of EAT cells after incubation with succinic anhydride. Incubation 15 min at 37°C, pH 7.0. n = 14.

3. Results and discussion

As can be seen from figs. 1–3, both periodate and succinic anhydride strongly inhibit anaerobic glycolysis and respiration of EAT cells. With both reagents cell viability and transplantability are lost at those concentrations which block metabolism. Significant leakage of potassium occurs only with periodate in concentrations which abolish metabolic activity (fig. 4). Both reagents affect glycolysis at lower concentrations than respiration. On the other hand, glycolytic activity decreases only gradually, whereas respiratory activity drops suddenly after a threshold concentration has been reached. These findings agree well with the assumption that the primary target for both reagents is the cell membrane which undergoes modifications in some portions essential for transport and/or regulatory functions. The discrepancy between inhibition of glycolysis and of O_2-consumption with succinic anhydride permits an additional explanation: Succinate formed by hydrolysis may cross the cell membrane and serve as metabolic substrate for intact mitochondria; only high concentrations would abolish O_2-uptake by raising the intracellular concentration of succinic anhydride to levels which alter the mitochondrial membrane system.

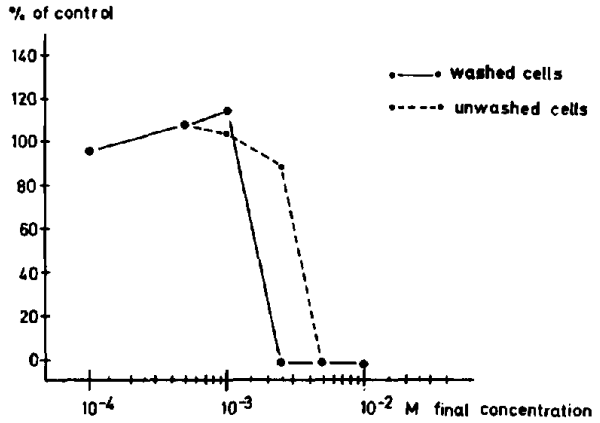

Fig. 2. O_2-consumption of EAT cells after incubation with $NaIO_4$ for 15 min at 37°C, pH 7.2. n = 14.

The view that the cell membrane is the primary site of action of the reagents might be supported by studies with cell-free homogenates. Unfortunately, lysates from EAT cells are very poor candidates for metabolic investigations [12]. Similar studies with hemolysates [9, 10] and lyophilized yeast preparations [11], however, favour the assumption.

Nonspecific oxidation by periodate, especially of essential sulfhydryl groups, could largely be excluded by studies with sulfhydryl reagent sensitive enzymes. Furthermore, our results with cell-free systems indicate for both reagents that intracellular reactions can play only a subordinate role in metabolic inhibition of whole cells.

Washed cells are more susceptible to the reagents than unwashed cells (figs. 1, 2; for succinic anhydride, analogous curves are obtained). This may be related to uncovering of reaction sites or simply to mechanical damage. The finding that succinic anhydride is a less potent inhibitor than periodate may be explained by one or more of the following assumptions: i) the amino groups undergoing modification are 'less essential' for metabolic regulation than certain carbohydrate moieties of the membrane; ii) there are more sites

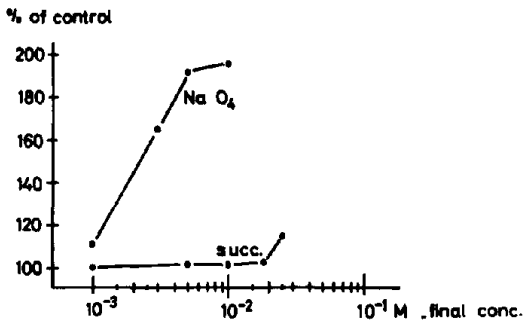

Fig. 4. K^+-efflux from unwashed EAT cells after treatment with succinic anhydride (succ.) and $NaIO_4$. Incubation 15 min at 37°C, pH 7.0. n = 14.

available for periodate oxidation than for succinylation; iii) the two reagents affect different regulatory systems. The latter possibility must be especially considered since only periodate brings about significant potassium leakage from the cells (fig. 4). The same phenomen has been found by us in a much more striking way with red cells [9, 10]. Thus, periodate may act (primarily or additionally) by modification of a carbohydrate portion of the membrane which is involved in cation distribution, thereby leading to a disturbance of the proper ionic equilibria necessary for metabolic function. Further investigations will be necessary to define the molecular sites of action with more accuracy.

Acknowledgement

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