PRELIMINARY NOTES

BBA 41130

Photosynthetic phosphorylation and associated reactions in the presence of a new group of uncouplers: Salicylanilides

Salicylanilide uncouples oxidative phosphorylation in rat liver[1] and housefly[2] mitochondria. Several years ago in a study of photosynthetic phosphorylation with spinach chloroplasts it was noted that salicylanilide did not act as an electron acceptor[3] and was an inhibitor of photophosphorylation (unpublished data of author, 1964). Recently several derivatives of salicylanilide were shown to be potent uncouplers of oxidative phosphorylation, to inhibit a P_i–ATP exchange reaction, and inhibit an ATPase activity with housefly and rat liver mitochondria[2].

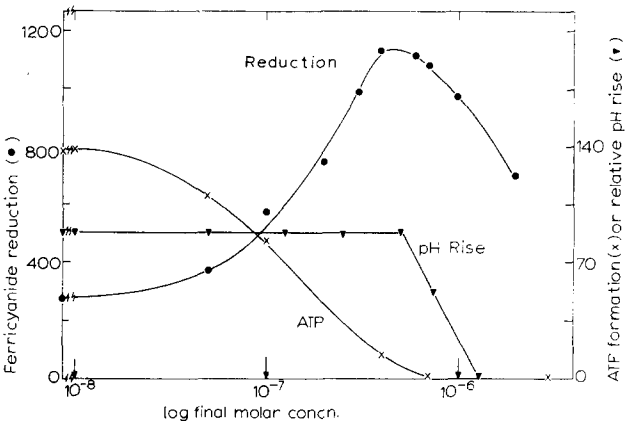

Fig. 1. Effects of Salicylanilide I concentration on photochemical reactions of spinach chloroplasts. Left ordinate: μmoles of ferricyanide reduced per mg chlorophyll per h. Right ordinate: μmoles of ATP formed per mg chlorophyll per h or relative total change in pH. The procedures employed in assaying photoreduction, ATP formation, and preparing chloroplasts have been presented[3]. The pH rise was assayed between pH 6.3 and 6.5 employing the techniques described by NEUMANN AND JAGENDORF[4]. All reaction mixtures were incubated at 21° and illuminated at an intensity of 2500 ft candles obtained from an incandescent lamp.

As seen in Fig. 1 the derivative, 5-chloro,3-(*p*-chlorophenyl),2′,4′,5′-trichlorosalicylanilide (Salicylanilide I), effectively inhibits photosynthetic phosphorylation coupled to the reduction of ferricyanide. Complete inhibition occurs at $4 \cdot 10^{-7}$ M and the maximum rate of ferricyanide reduction occurs at the same concentration indicating that phosphorylation is uncoupled from electron flow. Similar data has been obtained for the photoreduction of $NADP^+$ and the associated photophosphorylation.

Abbreviations: Salicylanilide I, 5-chloro,3-(*p*-chlorophenyl),2′,4′,5′-trichlorosalicylanilide; Salicylanilide II, 5-chloro,3-(*p*-chlorophenyl),2′-chloro,5′-nitrosalicylanilide; PMS, phenazine methosulfate.

Cyclic photophosphorylation catalyzed by phenazine methosulfate (PMS) also is completely inhibited by $4 \cdot 10^{-7}$ M Salicylanilide I. Similar effects on these reactions also were observed using 5-chloro,3-(*p*-chlorophenyl),2'-chloro,5'-nitrosalicylanilide (Salicylanilide II).

Interestingly Salicylanilide I appears to be ineffective in inhibiting the pH rise of chloroplasts[4] at concentrations up to $5 \cdot 10^{-7}$ M even though photophosphorylation is inhibited and the uncoupled rate of electron flow is maximal (Fig. 1). This lack of inhibition is in contrast with other uncouplers of photophosphorylation which inhibit the light-induced pH rise observed with chloroplasts[4]. At higher concentrations of Salicylanilide I the pH rise is inhibited, but concurrently an inhibition of electron flow is occurring which complicates an interpretation of the data (Fig. 1).

Photophosphorylation with chromatophores isolated from the photosynthetic bacterium *Rhodospirillum rubrum* was studied in the presence of Salicylanilide I. Both the endogenous photophosphorylation and the phosphorylation supported by PMS were inhibited by Salicylanilide I. The endogenous photophosphorylation was inhibited 50 % by Salicylanilide I at $4 \cdot 10^{-8}$ M.

TABLE I

EFFECT OF SALICYLANILIDE I ON THE ATP HYDROLYSIS ACTIVITIES OF SPINACH CHLOROPLASTS

The Ca^{2+}-dependent ATPase was induced by trypsin digestion of chloroplasts[5] and the Mg^{2+}-dependent ATPase was induced by illumination of chloroplasts in the presence of dithiothreitol[6]. Salicylanilide was present both during the induction period and the subsequent dark ATP hydrolysis incubation period.

Salicylanilide concn. (*M*)	*ATP hydrolysis* (*μmoles/mg chlorophyll per h*)	
	Ca^{2+}	Mg^{2+}
0	219	200
$1.1 \cdot 10^{-7}$	210	146
$3.3 \cdot 10^{-7}$	213	21
$3.3 \cdot 10^{-6}$	162	12

Spinach chloroplasts which synthesize ATP do not hydrolyze ATP at appreciable rates, but by appropriate treatments two ATPase activities can be induced[5,6]. Salicylanilide I when incubated with chloroplasts, did not appreciably effect the small endogenous ATPase activity of chloroplasts. Likewise it did not effect the Ca^{2+}-dependent ATPase of chloroplasts at concentrations which uncouple photophosphorylation (Table I). But the Mg^{2+}-dependent ATPase is inhibited by Salicylanilide I at concentrations which uncouple photophosphorylation (Table I and Fig. 1). Data of other workers indicate that the uncoupler ammonia (10 mM) inhibits the Ca^{2+}-dependent ATPase 45 % (ref. 5) and that the Mg^{2+}-dependent ATPase can be either stimulated or inhibited by ammonia and the uncoupler carbonyl cyanide phenylhydrazone depending upon the concentration of uncoupler employed[6,7]. Salicylanilide I has little effect on the Ca^{2+}-dependent ATPase and does not stimulate the Mg^{2+}-dependent ATPase (Table I). Thus Salicylanilide I in comparison to other uncouplers has unusual effects on the ATPase activities of chloroplasts.

These derivatives of salicylanilide appear to be among the most effective

materials which act as uncouplers of photophosphorylation in plants and bacteria. Since they appear to have unique effects on other reactions associated with ATP formation, *e.g.* the pH rise and the ATPase activities, they should be useful in elucidation of the mechanism of ATP synthesis in photosynthetic systems.

Dr. P. HAMM of the Monsanto Chemical Co. generously supplied the salicylanilide derivatives.

Department of Biochemistry,
University of Georgia,
Athens, Ga. 30601 (U.S.A.)

CLANTON C. BLACK, JR.

1 M. W. WHITEHEAD, *Biochem. Pharmacol.*, 13 (1964) 319.
2 R. L. WILLIAMSON AND R. L. METCALF, *Science*, 158 (1967) 1694.
3 C. C. BLACK, JR., *Biochim. Biophys. Acta*, 94 (1965) 27.
4 J. NEUMANN AND A. T. JAGENDORF, *Arch. Biochem. Biophys.*, 107 (1964) 109.
5 V. K. VAMBUTAS AND E. RACKER, *J. Biol. Chem.*, 240 (1965) 2660.
6 B. PETRACK, A. CRASTON, F. SHEPPY AND F. FARRON, *J. Biol. Chem.*, 240 (1965) 906.
7 R. E. MCCARTY AND E. RACKER, *J. Biol. Chem.*, 243 (1968) 129.

Received May 20th, 1968