SHORT COMMUNICATIONS

BBA 43151

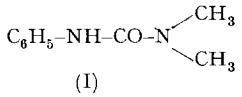
Inhibition and uncoupling of photophosphorylation

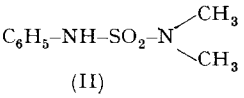
Inhibitors of photophosphorylation[1] in chloroplasts which inhibit both electron transport and simultaneous phosphorylation have been described, as well as uncouplers[2] of photophosphorylation.

Among the inhibitors, some phenylureas act at concentrations of 10^{-7} M by inhibiting steps in the photolytic cleavage of water which lead to the evolution of oxygen. Other substances having marked structural differences such as triazines[3,4], substituted uracils[5], chloroacylanilines[3], *etc.*, are inhibitors of the HILL reaction. They affect the same overall process and, having certain structural features in common, they may act in a similar way. The most active inhibitors of the HILL reaction have a free imino hydrogen atom which is considered to be involved in binding at the active sites of the chloroplasts through hydrogen bonds[4,6]. The phenylamide inhibitors possess, in addition to this structural feature, a carbonyl oxygen atom and an unsaturated ring. Nevertheless, certain inhibitors such as 2,6-bis(ethylamine)-4-chlorotriazine contain no free imino groups and others form hydrogen bonds to only a limited extent, even though they substantially inhibit photosynthesis. Other properties, such as electronic and steric factors and partitioning characteristics, must be of primary importance for blocking the receptor site.

Ammonium ions at concentrations of 10^{-3}–10^{-4} M and monofunctional amines were until recently[8] the only known uncouplers[2] of photophosphorylation, but several compounds, *viz.*, atebrin, chlorpromazine, gramicidin S, octylguanidine, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone and thiophosphate, have since been found to act as uncouplers.

This paper describes the influence of chemical structure on the inhibiting and uncoupling properties of members of the series of substituted phenylureas (I), *N*,*N*-dimethyl-*N'*-phenylsulphamides (II) and phenyl-*N*,*N*-dimethylcarbamates (III),


(I)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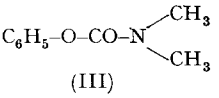

(III)

in which the "hydrogen-bonding" groups differ greatly.

The influence of chloro-substitution in the 3 series of compounds on the inhibition of the HILL reaction has already been studied[9]. A few representatives of each series have been chosen in order to test their influence on the reduction of pyridine nucleotides and on photophosphorylation. Results are given in Table I.

Incorporation of ^{32}P has been followed without any further treatment of the supernatant fluid. ATP, ADP and P_i are well enough separated by paper chromatog-

TABLE I

HILL REACTION, PYRIDINE NUCLEOTIDE REDUCTION AND PHOTOPHOSPHORYLATION FOR VARIOUS SUBSTITUTED PHENYLUREAS, CARBAMATES, SULPHAMIDES AND PHENOLS

Experiments were carried out under N_2 in 15-ml conical flasks shaken in an illuminated bath cooled at 15°. Illumination by photoflood lamps was approx. 4000 ft-candles of white light. Oxygen release, NADPH formation and phosphorylation were measured concurrently. All vessels contained, in μmoles: $MgCl_2$, 5; ADP, 10; $NADP^+$ (pH 8), 4; $K_2H^{32}PO_4$, 10 (10^4 counts/min); dialyzed $NADP^+$-reductase[10], 0.6 ml; chloroplast preparation[11] containing 0.8–1 mg chlorophyll; acetone or 10^{-3} M compound solution, 0.2 ml; Tris (pH 8), 80 μmoles. Total vol., 3 ml. Illumination time, 8 min. The absorbance at 340 mμ of samples centrifuged at 0° and 18000 × *g* was measured. ATP was determined in the reaction mixture after adding 0.3 ml of 20% trichloroacetic acid and centrifuging in the cold. The supernatant was evaporated to dryness under vacuum for 15 min; the residue was taken in 0.5 ml of water and 0.01-ml aliquots were chromatographed on Whatman No. 1 paper using a mixture of 10 vol. of acetic acid–1 M NH_4OH–0.1 M EDTA (51:50:1.5) and 8 vol. of isopropyl alcohol. ATP is well separated from phosphate; the distribution of radioactivity on the paper was measured with an Atomic automatic scanner. Each result is the average of at least 3 individual determinations.

Chloro-substitution in the phenyl ring	*Per cent of control*		
	O_2 evolution	*NADPH formation*	*ATP*
2,3 (I)	15.8	29.7	0
3,5 (I)	13.6	29.0	0
4 (II)	82.0	94.6	27
2,3 (III)	88.7	97.8	0
3,5 (III)	58.5	73.6	0
2,6 (III)	89.0	89.3	16
2,3-dichlorophenol	78.2	95.2	0
3,5-dichlorophenol	23.8	52.4	0
2,6-dichlorophenol	100.0	100.0	74

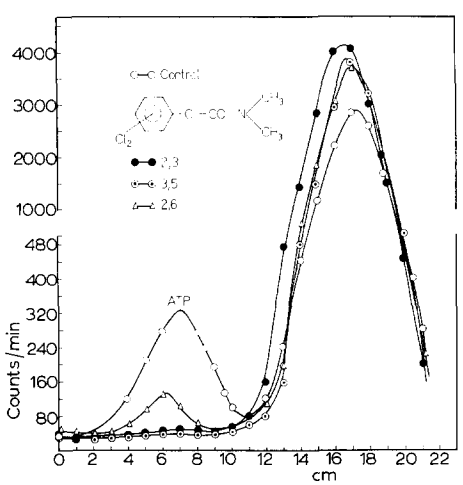


Fig. 1. Inhibition of ATP formation in the presence of chlorophenyl-*N*,*N*-dimethylcarbamates.

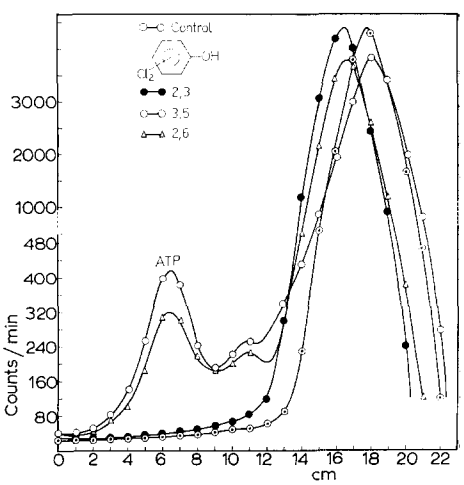
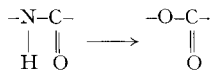


Fig. 2. Inhibition of ATP formation in the presence of different chlorophenols.

raphy for registering the radioactivity in an automatic recorder. In Figs. 1 and 2 examples are given of the method used for estimating the radioactive ATP.

As can be seen in Table I, 2,3- and 3,5-dichlorophenylureas (I) are true inhibitors and the structural change,



makes carbamates (III) exhibit a weak inhibitory effect on electron transport and a chloro-substitution-dependent effect on phosphorylation. The 2,3-dichlorophenylcarbamate (III) is a strong uncoupler but the chloro-substitution in both ortho positions decreases the effect on phosphorylation. From the foregoing it is clear that the lack of N–H...O bonding leads to a considerably lower inhibitory effect on electron transport and that hydrogen bonding is not primarily responsible for the inhibition of phosphorylation. This last effect could be due to a steric factor rather than to hydrogen bonding. This is, moreover, in agreement with the behaviour of chlorophenols. Although the hydrogen bonds increase with increasing polarity of the A–H bond in A–H...B (the mean energy of the hydrogen bonds O–H...O and N–H...O are, respectively, 6 and 2.3 kcal/mole), the electronegativity of A can be increased by orbital hybridization and the hydrogen-bonding capacity of ureas (I) can be greater than that of phenols. The influence of chloro-substitution in the chlorophenols is similar to that in the carbamates; 2,3-dichlorophenol exhibits a strong uncoupling activity in contrast to the decreased effect on phosphorylation due to the substitution in both ortho positions.

3,5-Dichloro-substitution maintains a high inhibitory activity on phosphorylation for both phenols and carbamates, but in general there is a greater tendency for the derivatives to act as inhibitors because of an increase in the electron transport inhibition. A free ortho position seems to be necessary for a high inhibition of photophosphorylation and *m*-chloro-substitution leads to increased hydrogen bonding which is more evident in phenols than in carbamates.

Of all the chlorosulphamides (II) previously assayed[9], the 4-chloro derivative is the one which exhibits only a slight inhibitory effect on the HILL reaction. Its effect on electron transport is to be correlated with the difference between C=O and S=O and their respective influences on N–H bonding.

Institute of Chemistry, A. ALCAIDE
Madrid (Spain) A. M. MUNICIO

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Received September 12th, 1966