SHORT COMMUNICATIONS

BBA 43160

ATP formation coupled to photosynthetic $NADP^+$ reduction with artificial electron donors

The photosynthetic reduction of $NADP^+$ by isolated chloroplasts is coupled stoichiometrically to ATP formation[1]. The rate of NADPH formation is therefore increased when the phosphorylating system ($ADP–P_i–Mg^{2+}$) or an uncoupler (NH_4Cl) is added[2]. When $NADP^+$ is reduced at the expense of an artificial electron-donor system such as DCIP–ascorbate[3] or diaminodurol–ascorbate[4] while the oxygen-evolution system is blocked by a specific inhibitor like DCMU, ATP is also formed[4–6]. However, it has been questioned[7,8] whether the ATP is formed during the electron transport from the electron donor to $NADP^+$ or rather by a superimposed cyclic photophosphorylation.

We wish to report experiments which strongly support the view that $NADP^+$ reduction at the expense of diaminodurol–ascorbate is indeed directly coupled to ATP formation. This is based on the findings that: (1) the rate of NADPH formation in the diaminodurol system (and to a lesser extent in the DCIP system as shown by KEISTER[14]) is increased by the addition of the phosphorylating system or by NH_4Cl, and (2) the coupled $NADP^+$ reduction is inhibited by phlorizin, a recently described inhibitor[8] of photophosphorylation. This inhibition by phlorizin is annulled by NH_4Cl.

As Table I shows, $NADP^+$ reduction at the expense of diaminodurol or DCIP,

TABLE I

STIMULATION OF $NADP^+$ REDUCTION IN THREE ELECTRON-DONOR SYSTEMS AT pH 7.4 BY NH_4Cl AND PHOSPHORYLATING SYSTEM AND INHIBITION BY PHLORIZIN

The reaction mixture contained in 3 ml: 80 μmoles Tris buffer, 6 μmoles $NADP^+$, 10 μmoles ascorbate, broken chloroplasts (P_{1s1}) from spinach with 0.2 mg chlorophyll and excess ferredoxin. 10 μmoles ADP and inorganic phosphate and 5 μmoles $MgCl_2$ were added where indicated. Illumination with 35000 lux at 15° in N_2.

Additions to $2 \cdot 10^{-5}$ *M DCMU*	*Electron donor (0.2 μmole)*	*Diaminodurol*		*DCIP*		*TMPD*	
	Time of illumination	*10 min*		*15 min*		*15 min*	
		NADPH	*ATP*	*NADPH*	*ATP*	*NADPH*	*ATP*
		(*μmoles formed*)					
—		2.6		1.1		1.5	
+ 10^{-3} M NH_4Cl		3.6		3.1		1.7	
+ $ADP–P_i–Mg^{2+}$		3.1	3.0	1.5	0.7	1.9	<0.1
+ $ADP–P_i–Mg^{2+}$ + 10^{-3} M phlorizin		2.6	1.1	0.9	<0.1	2.2	<0.1
+ $ADP–P_i–Mg^{2+}$ + 10^{-3} M phlorizin + 10^{-3} M NH_4Cl		3.3	0.6	2.4	0.2	2.2	<0.1

Abbreviations: DCIP, 2,6-dichlorophenolindophenol; DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine.

kept reduced by ascorbate, is stimulated by the addition of the uncoupler NH_4Cl or by the phosphorylating system (ADP–P_i–Mg^{2+}). The coupled $NADP^+$ reduction is inhibited by 10^{-3} M phlorizin down to the rate of the basal (non-phosphorylating) electron transport (as IZAWA, WINGET AND GOOD[9] have described for the ferricyanide system). This inhibition by phlorizin is annulled by the uncoupler NH_4Cl. In the TMPD system, however, which at this concentration of TMPD is not coupled to ATP formation[8,10], $NADP^+$ reduction is only slightly stimulated by NH_4Cl and is not inhibited by phlorizin. Basal as well as coupled electron transport in the diaminodurol system is higher than in the DCIP, and markedly higher than in the TMPD system, showing again the superiority of diaminodurol as electron donor[4]. The high basal rate in the diaminodurol system is probably due to a bypassing of the rate-limiting phosphorylation site, as diaminodurol has indeed two points of entry into the electron-transport chain[4]. In the presence of the phosphorylating system diaminodurol uses the point of entry before the phosphorylation site, since ATP is now formed stoichiometrically with respect to NADPH and the rate of the electron transport has been increased.

The experiments in Table I were run at pH 7.4 since at this pH the stimulation of the electron transport by an uncoupler or the phosphorylating system is more pronounced[2]. As Table II shows, the stimulation of coupled $NADP^+$ reduction with water as the electron donor (and therefore with oxygen evolution) is markedly increased by the addition of NH_4Cl at pH 7.4 but very little at pH 8.0. The stimulation of basal (non-phosphorylating) $NADP^+$ reduction with an artificial donor system (diaminodurol–ascorbate) by NH_4Cl or phosphorylating system is also more clearly seen at pH 7.4 than at pH 8.0 (Table II).

TABLE II

STIMULATION OF $NADP^+$ REDUCTION BY NH_4Cl AND PHOSPHORYLATING SYSTEM AT DIFFERENT pH

Experimental conditions as in Table I; 10 min light.

Additions	*pH 7.4*		*pH 8.0*	
	μmoles NADPH formed	*μatoms oxygen evolved*	*μmoles NADPH formed*	*μatoms oxygen evolved*
Water as electron donor				
+ ADP–P_i–Mg^{2+}	1.7	1.4	4.2	3.8
+ ADP–P_i–Mg^{2+} + 10^{-3} M NH_4Cl	4.4	3.8	4.6	4.5
+ $2 \cdot 10^{-5}$ M DCMU + 0.2 μmole diaminodurol				
—	2.2		3.0	
+ 10^{-3} M NH_4Cl	3.1		3.1	
+ ADP–P_i–Mg^{2+}	3.1		3.3	
+ ADP–P_i–Mg^{2+} + 10^{-3} M NH_4Cl	3.6		3.6	

The rate of $NADP^+$ reduction at pH 7.4 (but not at pH 8.0) in the presence of phosphorylating system is even further increased by NH_4Cl when O_2 is evolved but not when diaminodurol is the electron donor (Table II). This shows that coupled electron transport at pH 7.4 with water as the electron donor is limited by the ATP-formation system which has a pH optimum at pH 8.0; the electron transport is

therefore markedly stimulated by the further addition of an uncoupler (Table II) (as is well known for the ferricyanide system[11–13]). The DCIP system is also more stimulated by an uncoupler than by the phosphorylating system at pH 7.4 indicating that the ATP-forming system is limiting the electron transport (Table I). In the diaminodurol system, however, NH_4Cl and the phosphorylating system give the same stimulation of the basal electron transport (Tables I and II) and further addition of NH_4Cl to the phosphorylating system has only a slight effect (Table II), indicating that the ATP-forming system is not or less limiting. NADPH formation with water or with diaminodurol as the electron donor is coupled to a stoichiometric ATP formation. The absolute rate of ATP formation during $NADP^+$ reduction at pH 7.4 with diaminodurol as the donor is therefore higher than with water as the electron donor. This might be taken as an indication that in the diaminodurol system at pH 7.4 another phosphorylating site is participating which has an optimum at a lower pH.

The research reported in this paper has been sponsored by the Air Force Cambridge Research Laboratories under Contract No. AF 61(052)-716 through the European Office of Aerospace Research (OAR), U.S. Air Force.

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Received December 14th, 1966

Biochim. Biophys. Acta, 131 (1967) 580–582

BBA 43161

On the possible role of structural protein in the binding and translocation of adenine nucleotides in mitochondria

The permeability studies of PFAFF, KLINGENBERG AND HELDT[1] as well as direct evidence obtained by M. KLINGENBERG AND F. PALMIERI (unpublished results) with mitochondria devoid of outer membranes (prepared according to PARSONS[2]) show that the exchange between exogenous and endogenous adenine nucleotides[1,3] occurs at