PUMPing plants

SIR — Flowering and fruit ripening are triggered by a burst of respiration and heat production possibly related to a mitochondrial, cyanide-resistant, uncoupled electron transport pathway[1]. In mammals, transient thermogenesis is linked to a mitochondrial uncoupling protein[2] (UcP), believed to be a late evolutionary acquisition[3]. A detailed analysis of respiratory control of plant mitochondria led us to propose the existence of an UcP-like factor in plants[4].

Addition of ATP to potato mitochondria produced a 52% decrease in the rate of state-4 respiration and an increase of about 15 mV in the value of the membrane potential ($\Delta\Psi$) (Fig. 1*a*). The ATP-induced changes in respiration rate and $\Delta\Psi$, compatible with increased mitochondrial coupling, were unaffected by oligomycin (2 μg ml^{-1}) or carboxyatractyloside (20mM) (not shown). The membrane potential of potato mitochondria, isolated and incubated without bovine serum albumin (BSA), increased additively by additions of BSA and ATP (Fig. 1*b*), whereas GTP caused a smaller but significant increase (Fig. 1*b*, dashed line). These results suggested the presence of an H^+ conductance in potato mitochondria similar to that produced by UcP.

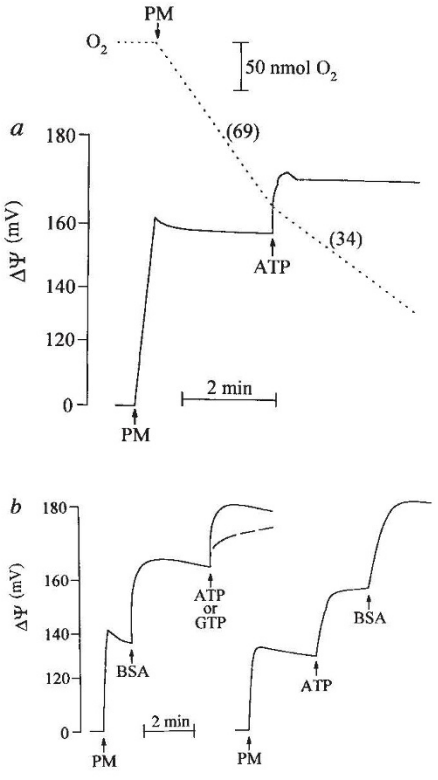

FIG. 1 Effect of ATP, GTP and BSA on transmembrane potential and resting respiratory rate of potato tuber mitochondria (PM). *a*, PM (1 mg protein ml^{-1}) were added to a solution of 300 mM mannitol, 20 mM KCl, 10 mM HEPES buffer pH 7.2, 3 mM tetraphenylphosphonium (TPP^+), 5 mM succinate and 0.1% BSA. ATP (0.1 mM) was added as indicated. The numbers in parentheses are rates of oxygen uptake in nmol per min per mg protein. Oxygen was measured with a Clark electrode in a 1.0-ml magnetically stirred glass chamber. Membrane potential was estimated by measuring extramitochondrial TPP^+ as described by Kamo *et al.*[7]. *b*, PM, isolated in the absence of BSA, were incubated in a medium similar to that in *a*, lacking BSA. BSA (Sigma, fatty acid free) (1%), 0.1 mM ATP or GTP were added as indicated. Results are representative of 5 separate experiments.

We isolated a protein of relative molecular mass 32,000 (32K) from potato mitochondria and purified it following a procedure used to prepare UcP. We call this protein plant uncoupling mitochondrial protein (PUMP). A common property of the mitochondrial P_i carrier, the ATP/ADP translocator and UcP is that these proteins are not retained by hydroxylapatite and at room temperature only UcP is obtained. Polyacrylamide gel electrophoresis revealed the presence of a 32K band both in the brown fat and in plant mitochondrial isolates (Fig. 2, inset).

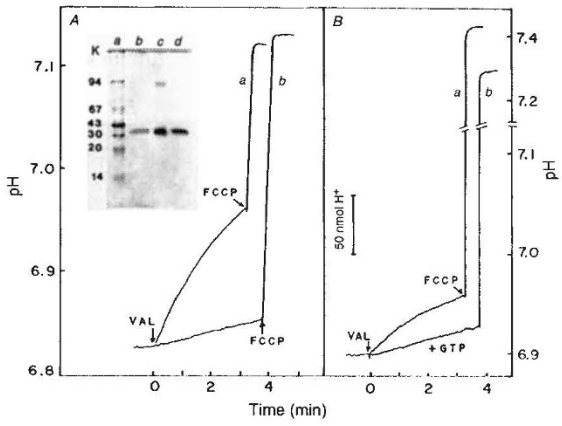

FIG. 2 Variation of external pH during H^+ influx into vesicles prepared with or without PUMP or UcP. UcP from brown fat mitochondria and PUMP from potato mitochondria were purified as described by Klingenberg[5] for UcP. Freshly isolated UcP or PUMP in 90 mM K-phosphate buffer pH 7.5, 2.5% decylpentaoxyethylene ether and 0.2 mM EDTA were used to dissolve ~20 mg ml^{-1} of phosphatidylcholine (PC). Proteoliposomes were obtained after detergent removal by successive treatment with Amberlite XAD-4. External solutes were removed by filtration on a Sephadex G-25 column previously saturated with sonicated PC vesicles and eluted with 0.5 mM PIPES, 0.5 mM HEPES, 0.2 mM EDTA, 0.25 M sucrose pH 7.5. pH was monitored with a combination electrode at 28 °C with a Radiometer PHM82 pH meter equipped with a REC80Servograph. Valinomycin (VAL) (1 μg ml^{-1}) and 1.3 μM carbonylcyanide *p*-trifluoro-methoxyphenylhydrazone (FCCP) were added as indicated. *A*, Vesicles of PC loaded with phosphate buffer pH 7.5, after Sephadex elution, were added to 0.5 mM HEPES, 0.5 mM PIPES, 0.2 mM EDTA, 250 mM sucrose, 5 mM choline chloride, pH 6.8. Line *a*, vesicles of PC (0.46 mM) with PUMP (3.9 mg ml^{-1}); line *b*, vesicles of PC without protein. *B*, Line *a*, vesicles of PC (0.5 mM) with UcP (2.4 mg ml^{-1}) without GTP; line *b*, with 0.05 mM GTP. Inset shows PAGE (10 μg protein) of hydroxylapatite eluate obtained from brown fat adipose tissue[5] containing UcP (lane *c*) and potato mitochondria containing PUMP (lanes *b* and *d*). Lane *a* contains M_r standards.

We incorporated PUMP and UcP into egg phosphatidylcholine liposomes and compared the H^+/OH^- transport properties (Fig. 2). The fast alkalinization (Fig. 2, line *a*) following valinomycin addition indicates increased H^+/OH^- conductance through the incorporated proteins, as it was not observed in protein-free liposomes (Fig. 2*A*, line *b*). Figure 2*B* (line *b*) shows the inhibitory potency of externally added GTP to UcP-containing proteoliposomes. The specific activity of UcP was 7.2 ± 1.4 μmol H^+ per min per mg (n=5) at 28 °C, well within published data[5]. The specific activity of PUMP, determined under similar conditions, was 7.7 ± 1.5 μmol H^+ per min per mg (n=5). As found previously[5], addition of 0.1 mM ATP inhibited 50% of the activity of UcP, whereas 0.05 mM GTP inhibited 80%. Inhibitions of PUMP activity by 0.1 mM ATP or 0.05 mM GTP were 50% and 35%, respectively, showing that the nucleotides inhibited PUMP less than UcP. The inhibition by ATP and GTP in the reconstituted system is compatible with their observed effect in intact mitochondria (Fig. 1). Although at present we have isolated PUMP only from potato tubers, we have observed (not shown) that mitochondria of other plants show the type of respiratory control (Fig. 1) that led us to detect PUMP. As potato mitochondria do not present cyanide-resistant respiration[6], PUMP may be important in this tuber for heat-requiring physiological events.

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