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Oxidative phosphorylation in *Corynebacterium diphtheriae*

A number of strains of *Corynebacterium diphtheriae* differ in the composition of their respiratory chain of enzymes[1-3]. The availability of these strains presents an unusual opportunity to study energy coupling in this organism, with special emphasis on the location of sites of ATP formation in the electron-transfer sequence. For example, the C_7 strain may be regarded as possessing a complete cytochrome system, similar in components to that of mammalian mitochondria[1]; the more slowly growing C_7SC strain appears deficient in menaquinone[4], and the still more slowly growing $PW8_8$(Pd) lacks cytochrome *c* and oxidizes NADH by means of a soluble flavoprotein oxidase[1]. This communication reports studies where the efficiency of ATP synthesis was measured in cell-free extracts from the three strains with a number of respiratory substrates. On the basis of these studies, there appears to be a single phosphorylation site located in the region between cytochrome *b* and *c*, a region previously identified as the locus of menaquinone[4].

Cells were grown, harvested and disrupted exactly as described previously[1]. In the results presented, oxidation was measured manometrically and phosphate esterification measured as the disappearance of inorganic phosphate[5]. In other experiments, phosphorylation was measured enzymatically[6] and no significant differences were noted using the two techniques.

Table I shows substantial phosphate esterification in the C_7 strain with succinate as substrate. Phosphorylation, but not oxidation, required Mg^{2+} and ADP, and was uncoupled by 1 mM 2,4-dinitrophenol. Oligomycin was without significant influence. Phosphorylation coupled to the oxidation of endogenous substrates did not, in these experiments, amount to more than 15 % of that coupled to succinate oxidation and no correction is employed.

Table II shows rates of oxidation and P:O ratios with three strains and three substrates. In both the C_7 (complete respiratory chain) and C_7SC (low menaquinone) strains, phosphorylation efficiency with succinate roughly equals that with NADH

TABLE I

PHOSPHORYLATION COUPLED TO SUCCINATE OXIDATION IN EXTRACTS FROM CELLS OF THE C_7 STRAIN

Respiration was measured manometrically at 25° with a liquid reaction volume of 2.5 ml. The basic reaction medium contained 80 mM Tris–HCl, pH 7.4, 48 mM succinate, 40 mM NaF, 20 mM $MgCl_2$, 8 mM mannose, 6 mM phosphate, 2 mM ADP, 4 units hexokinase, Sigma Type IV, and 5.6 mg bacterial protein. After 30 min the reaction was terminated with 0.2 ml of 40 % trichloroacetic acid. Following centrifugation, the supernatant fluid was neutralized with KOH and assayed for phosphate[5].

Conditions	*ΔO* (*μatoms*)	*ΔP* (*μmoles*)	*P:O* (*μmoles/μatom*)
Succinate	3.36	1.71	0.51
Omit $MgCl_2$	3.04	0	
Omit ADP	3.42	0	
+ 2,4-Dinitrophenol, 1 mM	2.72	0.07	0.03
+ Oligomycin, 16 μg	2.80	1.70	0.61

Abbreviation: TMPD, tetramethyl-*p*-phenylenediamine.

oxidation, although the P:O ratios are lower in the case of C_7SC. The cytochrome *c*-lacking $PW8_8$(Pd) strain not only exhibits a lower ratio with succinate as substrate than either of the other two strains, but is quite unable to couple NADH oxidation to ATP formation. When tetramethyl-*p*-phenylenediamine (TMPD), in the presence of ascorbate, was used as electron source, oxidation comparable in rate to that of NADH was noted, but in no case was phosphorylation detected. Since TMPD passes electrons into the respiratory chain in the vicinity of cytochrome *c* (ref. 7), it appears that there is no energy conservation in the span from cytochrome *c* to oxygen in *C. diphtheriae*.

TABLE II

OXIDATIVE PHOSPHORYLATION IN DIFFERENT STRAINS OF *C. diphtheriae*

Conditions were as described in Table I except that the substrate was either 48 mM succinate, 8 mM NADH or 120 μM TMPD + 36 mM ascorbate. The protein content ranged from 8.2 to 12.2 in the various experiments.

Strain		*Succinate*	*NADH*	*TMPD + ascorbate*
C_7	ΔO (μatoms/mg protein)	0.65	0.92	0.79
	P:O	0.42	0.51	0
C_7SC	ΔO	0.35	0.87	0.72
	P:O	0.23	0.25	0
$PW8_8$(Pd)	ΔO	0.68	0.21	0.28
	P:O	0.16	0	0

The synthesis of ATP must, then, be coupled to electron transfer occurring between substrates and cytochrome *c*. The P:O ratios below unity suggest only one phosphorylation site, and the equal ratios with succinate and NADH in the C_7 strain argue that the one site be between cytochrome *c* and the point where the respiratory chain branches to receive electrons from succinate and NADH. According to previous studies of the diphtherial respiratory chain[1], the two branches converge at cytochrome *b* so that the phosphorylation site would be between *b* and *c*, in the region where menaquinone is reported to act[4]. The apparent close association between oxidative phosphorylation and menaquinone action is in agreement with studies of BRODIE AND ADELSON[8] which point to a central involvement of menaquinone in the coupling process which joins electron flux and ATP synthesis.

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Binding of nucleotides to "structural protein" of wild-type and respiration-deficient yeast mitochondria

One distinguishing feature of "structural protein" isolated from beef heart mitochondria is its capacity to combine with nucleotides such as ATP, ADP and NADH (ref. 1) and a number of other anionic compounds[2]. The binding of nucleotides to "structural protein" was recently shown to be inhibited by atractyloside[3] which is known to block oxidative phosphorylation in intact mitochondria[4,5]. We wish to report that studies on the binding of nucleotides, and its inhibition by atractyloside, provide a valuable means of comparing and identifying mitochondrial "structural proteins" from wild-type bakers' yeast and from its *petite* mutant.

The *Saccharomyces cerevisiae* strain D-273-10B (αP_ρ^+ haploid) and the cytoplasmic *petite* mutant D-273-10B-1 (αPI^- haploid) derived from the wild-type strain by treatment with acriflavin, were grown for 24 h at 26° in the medium described by EPHRUSSI AND SLONIMSKI[6] which was modified to contain 0.3 % yeast extract and 0.8 % glucose. The cells were broken by shaking with glass beads in a MERKENSCHLAGER disintegrator[7] and the mitochondria isolated by differential sedimentation followed by isopycnic centrifugation in a linear sucrose gradient[8]. "Structural protein" was prepared according to the procedure of RICHARDSON, HULTIN AND FLEISCHER[9]. Electrophoresis in polyacrylamide gels revealed that the "structural protein" consisted of several components, one of which was missing in preparations from the non-respiring *petite* mitochondria[10]. Immunological studies also indicated that mitochondrial "structural protein" from the *petite* mutant lacked a component present in a corresponding preparation from the wild-type yeast[10]. Mitochondrial "structural protein" (1.2 mg) suspended in Tris acetate buffer, pH 7.5, was incubated with 10 mμmoles of [^{3}H]ATP. This amount of ATP was much smaller than that required for saturating the binding capacity of "structural protein". After 10 min at 25° the "structural protein" was sedimented by low-speed centrifugation. The amount of [^{3}H]ATP bound to the protein was estimated from the difference between total activity and radioactivity remaining in the supernatant solution (Table I). About 70 % of the added [^{3}H]ATP were bound to the wild-type "structural protein". Similar results were obtained when [^{3}H]ATP was replaced by [^{3}H]TTP. ATP binding was not significantly diminished in the presence of equimolar amounts of ADP or NADH. Even a 10-fold excess of ADP reduced the amount of bound [^{3}H]ATP by not more than one-half. In contrast, an equimolar amount of atractyloside strongly inhibited