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The two oxygen-regulated subunits of cytochrome *c* oxidase in *Dictyostelium discoideum* derive from a common ancestor

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The two smallest polypeptide components of *D. discoideum* cytochrome *c* oxidase, whose alternative expression depends on oxygen concentration [Schiavo, G. and Bisson, R. (1989) J. Biol. Chem. 264, 7129–7134], have been partially sequenced. They show 45% homology and are isoforms of the same subunit, which must be encoded on two different genes.

Cytochrome *c* oxidase; Isoenzyme; Subunit sequence; Evolution; (*Dictyostelium discoideum*)

1. INTRODUCTION

Cytochrome *c* oxidase is the terminal enzyme of the respiratory chains of eukaryotes and most aerobic bacteria. It catalyzes electron transfer from cytochrome *c* to oxygen coupled to proton translocation across the membrane. In eukaryotes, mitochondrial genes code for the 3 largest polypeptides [1,2] each with significant homology to one of the 3 subunits that make up the bacterial aa_3 type oxidases [3–5]. These mitochondrially-coded subunits contain the prosthetic groups and probably provide the catalytic functions of the enzyme (reviewed in [6]).

The subunit composition increases in complexity from bacteria to higher organisms, the difference being the number of nuclear coded subunits present [6,7]. *Dictyostelium discoideum*, the slime mold, has 4 nuclear coded subunits [8] while yeast has six [9] and mammals ten [10]. Adding to the complexity, several nuclear coded subunits are present in different isoforms [11–13].

The role(s) of the nuclear coded subunits remain to be defined. Studies to establish the functions of these subunits in cytochrome *c* oxidase of mammals are proving difficult [14]. For this reason there is renewed interest in the enzyme from lower eukaryotes, where genetic and molecular biology approaches are more easily applied. It has been reported recently that there are alternative forms of cytochrome *c* oxidase in some lower eukaryotes. Thus yeast has two forms of the enzyme, whose expression is influenced by factors such as oxygen and heme deprivation [15,16]. The two forms contain different isologues of subunit V [16,17].

Two different forms of cytochrome *c* oxidase have been isolated from *D. discoideum* [18], a strictly aerobic lower eukaryote that exhibits some of the features typical of cell types of higher organisms. Single amoebae feed on bacteria and grow and multiply as vegetative or non-social cells. Upon exhaustion of the food supply, the amoebae undergo a developmental transition to form multicellular pseudoplasmodia and begin differentiation [19].

When cells enter the stationary phase of growth, during the vegetative stage of the slime mold life cycle, the smallest cytochrome *c* oxidase subunit, termed VIIe (M_r 5000), is replaced by a larger polypeptide, termed VIIs (M_r 6000). In contrast to yeast, the switching between the two forms of the enzyme is complete and depends on a single environmental parameter: the oxygen tension [20].

Both forms are present during the development, possibly as consequence of a limited diffusion of oxygen into the aggregates [20].

Subunit VIIe and VIIs could be different polypeptides, possibly homologues of two subunits common to the yeast and mammalian enzymes, or they could be isologues of the same subunit. To decide between these possibilities, we have obtained partial sequences of the two polypeptides.

2. MATERIALS AND METHODS

Cytochrome *c* oxidase was prepared from *D. discoideum* cells in exponential and stationary phase of growth as reported previously

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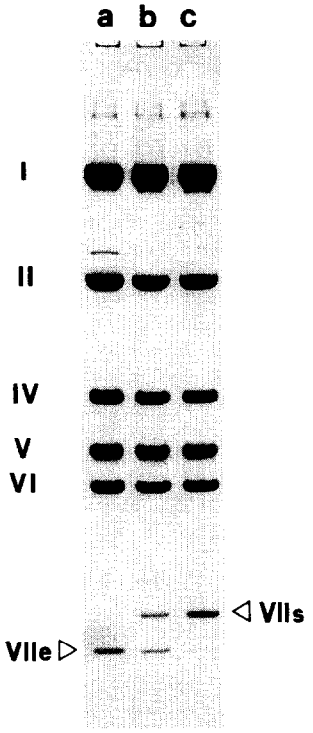

Fig.1. Coomassie blue SDS-gel electrophoretic pattern of *D. discoideum* cytochrome *c* oxidase as isolated from cells in (a) exponential, (b) early stationary and (c) late stationary phase of growth [18]. The same results can be obtained from cells grown under normal and reduced (up to 20 μM) oxygen concentrations, respectively [20]. The two subunits VIIe and VIIs, whose concentration is determined by the oxygen tension, are marked by arrowheads. Nomenclature and other experimental conditions are as previously described [18].

[8,18]. $NaDodSO_4$ polyacrylamide gel electrophoresis was performed according to Zhang et al. [21] using gels 1.5 mm thick and 25 cm in length. Polypeptides were electroeluted from gels onto poly(vinylidene)difluoride membrane (Immobilon, Millipore) using the procedure of Matsudaira [22]. Subunits were localized by staining the solid support with Coomassie brilliant blue, the areas containing VIIe and VIIs were excised, and the polypeptides sequenced. Sequence analysis was performed by a gas-phase protein sequencer (Applied Biosystem Model 470A) equipped with an on-line PTH analyzer (Applied Biosystems Model 120A).

3. RESULTS AND DISCUSSION

For reference, the subunit composition of the two forms of cytochrome *c* oxidase in *Dictyostelium discoideum* are shown in fig.1. It can be seen that the migration of subunits I–VI are identical in the two forms: only the migration of subunit VII is different. Previous studies have shown that subunit VIIs and VIIe have different amino acid compositions [18] and are immunologically distinct ([18] and Bisson, R., unpublished results). The primary structures of the two subunits (fig.2A) show a significant similarity, establishing that the two polypeptides are isoforms. The identical residues are dispersed throughout the sequence, ruling out the possibility that the two polypeptides are alternatively spliced products of the same gene. Rather, two different genes must be present, which presumably arose from a gene duplication event. The low degree of overall similarity (45%) suggests that the duplication event occurred very early in eukaryotic evolution and/or the divergence of the polypeptides was not severely limited by functional constraints. The sequence

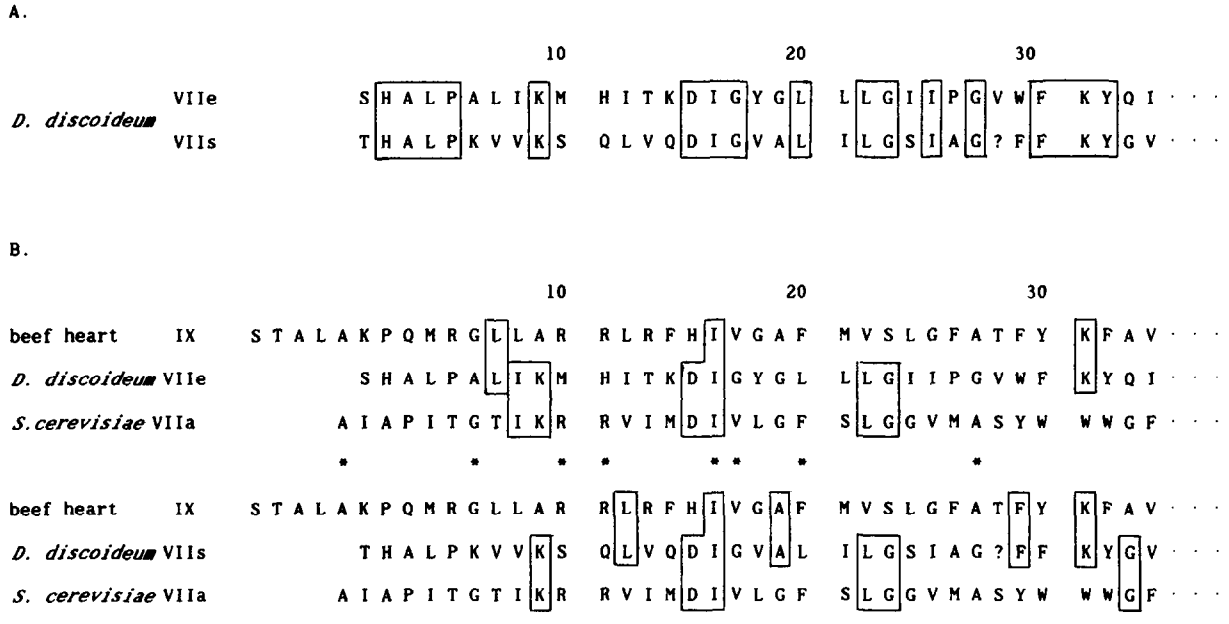

Fig.2. (A) Primary structure and sequence alignment for the amino-terminal portion of subunit VIIe and VIIs. The data, obtained by protein sequencing as reported in section 2, refers to approximately two-thirds of the complete subunit sequence. (B) Similarities among the *D. discoideum* isoforms and the homologous polypeptides from a mammal (beef) and a yeast (*S. cerevisiae*). Identities are indicated by asterisks (between beef and yeast) and by rectangles (between slime mold and the latter species). Nomenclatures are according to Buse et al. [23], to Power et al. [9] and to Bisson et al. [18] for beef, yeast and slime mold, respectively.

data are sufficient to identify subunit VIIe and VIIs as homologues of a subunit found in common in all other organisms examined so far, and numbered VIIa in yeast [9] and VIc [10], STA [11] or IX [23] in mammals (fig.2B). There is no evidence of isoforms of this subunit in organisms other than in *Dictyostelium*.

The complexity of the pattern of isoforms of cytochrome *c* oxidase is remarkable. Different sets of subunits appear to be present as isoforms in different organisms. Even in mammals there are variations. For example, subunit VIII (nomenclature of Kadenbach and Merle [10]) is tissue specific in rat, pig [14] and beef [24] but not in human [25,26]. This variability implies that the function(s) of certain isologues subunits is highly specific to the organisms or can be achieved in a variety of ways.

D. discoideum with its clear-cut change in subunit expression, coupled to a relatively complex life style, can be an interesting model system to investigate these problems. The partial sequence data presented here are a necessary first step in the isolation and analysis of the genes for the *Dictyostelium* enzyme.

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