SACCHAROMYCES CEREVISIAE ISO-CYTOCHROMES C : REVISION OF THE AMINO ACID SEQUENCE BETWEEN THE CYSTEINE RESIDUES

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Summary : The N-terminal sequence of iso-1 and iso-2 cytochromes c from bakers' yeast were redetermined with the aid of an automatic sequenator. Errors in the published sequences were detected between the half-cystine residues, which tend to suggest that residue 16 might be invariant in mitochondrial cytochromes.

A recent paper by Sugeno et al. (1) prompts us to report the results we obtained while examining the primary sequence of iso-1 and iso-2 cytochromes c from various strains of *Saccharomyces cerevisiae*.

Since the work of Slonimski et al. (2), it has been known that a single haploid cell of *Saccharomyces cerevisiae* synthesizes two molecular species of cytochrome c, iso-1 and iso-2 cytochromes c, which differ in their amino acid sequence. The primary structure of the cytochrome c from *S. oviformis* was published by Narita and Titani (3) ; by fingerprint analysis, Yaoi (4) showed it to be identical with the sequence of iso-1 cytochrome c from *S.cerevisiae*. The sequence of the chymotryptic hemopeptide of the iso-2 cytochrome c from a commercial bakers' yeast is also published (5). Moreover, Dr. E. Margoliash kindly communicated to us a complete unpublished sequence of that molecule (6).

Materials and Methods

Iso-1 and iso-2 cytochromes c were extracted from a diploid wild type strain D 261 (CY_1/CY_1 CY_2/CY_2) which has been described elsewhere (7). Their purification was carried out according to published procedures (2).

Heme removal was performed by the mercuric salt method of Ambler (8), and carboxymethylation with iodoacetic-acid 2-^{14}C according to Crestfield et al. (9), except that mercaptoethanol was replaced by an equivalent amount

of dithiothreitol. The N-terminal sequence was determined by the automatic Edman degradation method (10) with the Socosi sequenator P.S. 100. Solvents and reagents were purified according to Edman and Begg (10) except for Quadrol which came from Merck or Pierce.

The PTH amino acids were identified by thin layer chromatography on silica gel containing a fluorescent indicator (Merck F254) with solvent E of Edman and Sjöquist (11). After the spots were located under a U.V. lamp, the plates were sprayed with ninhydrin (0.2 % ninhydrin in acetone containing 1 % acetic acid). Identification of hydrophobic amino acids was confirmed in case of doubt by thin layer chromatography on cellulose with solvent system D (11). When a threonine or a serine were suspected, the presence in the U.V. spectrum of an absorption band with a maximum at 320-325 nm was looked for as another reliable indication. PTH-Arg and PTH-His were identified on polyamide plates according to Kulbe (12), or by high voltage electrophoresis on Whatman paper number 1, in 0.025 M phosphate buffer pH 6.2 (13).

Results

After heme removal and S-carboxymethylation of the liberated cysteines, the N-terminal sequences of iso-1 and iso-2 cytochromes c were determined with an automatic sequenator following procedures described by Edman and Begg (10).

For iso-1 cytochrome c, the sequence was determined up to residue 29, for iso-2 up to residue 37, starting from 0.29 and 0.33 µmoles respectively.

For iso-1 cytochrome c, the results were those expected from the sequence published by Narita and Titani, with the exception that residues 20 and 21 (15 and 16 according to the numbering which starts from the N-acetylglycine of mammalian cytochromes) were found to be LEU-GLN instead of the published GLU-LEU.

For iso-2 cytochrome c, we determined the following order :

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ALA-LYS-GLU-SER-THR-GLY-PHE-LYS-PRO-GLY-SER-ALA-LYS-LYS-GLY-ALA-THR-LEU-PHE-
 1                   5                  10                  15

    LYS-THR-    -CYS-GLN-GLN-CYS-    -THR-VAL-GLU-GLU-GLY-GLY-    -ASN-LYS-VAL-
     20                  25                  30                  35
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This sequence only differs from the sequence available to us (5,6) (determined for a commercial yeast) in that residue 24 is glutamine instead of glutamic acid.

For neither sequence could we determine the nature of the water soluble PTH amino acids (the residues immediately before the first and immediately after the second cysteine, respectively arginine and histidine (3,5). The failure to identify proline 34 in iso-2 cytochrome c (5) is

not surprising, considering that the yields were rather low at that stage and that PTH proline usually gives particularly weak spots.

The yield of the various PTH amino acids were determined by measuring the absorbance of a suitably diluted aliquot at 269 nm, and by scintillation counting for PTH-carboxymethyl cysteine. As an example, for iso-2 cytochrome c, PTH-PHE_7 was found to amount to 72 % of PTH-ALA_1, PTH-ALA_{12} to 56 %, PTH-LEU_{18} to 48 %, but PTH-carboxymethyl-CYS_{23} to only 10 %. Even assuming that the yield of residue 18 was overestimated due to a slight overlap from the preceding residue, the drop in yield from residue 18 to 23 is spectacular. The same phenomenon was observed with iso-1 cytochrome c. It is presumably due to poor reaction and/or cleavage at the level of arginine 22 and/or carboxymethylcysteine 23.

Discussion

Two conclusions can be drawn from our results. The most immediate one stems from the determination of errors in the published sequences of iso-1 and iso-2 cytochromes c. This means in particular that iso-1 cytochrome c shows one difference less with most other mitochondrial cytochromes c than was hitherto believed. It should be noted that the recently published sequence of the yeast *Debaryomyces kloekeri* (1) presents the same order : LEU-GLN that we have now established between the cysteines for iso-1 cytochrome c from *Saccharomyces cerevisiae*.

The second conclusion that we would like to draw from our results is somewhat more general.

When one looks at the sequence published up to now for mitochondrial cytochromes c (14), one can compile the following variants for the two residues between the thioether bridges that covalently link the heme to the polypeptide chain :

I	-CYS-ALA-GLN-CYS-
II	-CYS-SER-GLN-CYS-
III	-CYS-LEU-GLN-CYS-
IV	-CYS-GLN-GLN-CYS-
V	-CYS-ALA-GLU-CYS-

Sequence I occurs in a majority of cytochromes, including the plant cytochromes recently studied by Richardson, Boulter and their colleagues in Durham (see for ex. ref. 15). Sequence II occurs somewhat less frequently ; sequence III has been found in two yeasts (this work

and ref. 1), sequence IV in one yeast (this work), and sequence V in two cases, *Neurospora crassa* (16) and *Candida krusei* (17).

Thus, out of around thirty five known sequences, it appears that only the last two mentioned do not comply with the general formula -CYS-X-GLN-CYS- where X is a neutral amino-acid. It is well known that deamidation renders a confusion between glutamic acid and glutamine relatively easy. In this work and in the determination of other sequences with the automatic sequenator, in all cases where glutamine was found, we observed two spots of generally roughly equal intensity, corresponding to the markers glutamine and glutamic acid. One may then wonder whether the sequences for *Neurospora* and *Candida* are not also in error, and whether GLN_{16} (mammalian cytochrome c numbering) should not be counted among the invariant residues in mitochondrial cytochromes c. Investigation concerning this point is in progress in our laboratory.

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