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Studies on the assembly of cytochrome oxidase in isolated rat hepatocytes

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1. The assembly of rat liver cytochrome oxidase was studied in isolated hepatocytes and isolated liver mitochondria labelled with L-[^{35}S]methionine. 2. Labelled subunits II and III appeared in the immunoabsorbed holoenzyme within minutes after the initiation of a pulse label. In contrast, labelled subunit I appeared in immunoabsorbed holoenzyme only after a subsequent 2h chase or after an additional 2h of labelling. Subunit I was heavily labelled, however, in intact mitochondria after 10min. 3. A similar pattern of labelling was observed in holo-cytochrome oxidase which was chemically isolated by a small scale procedure adapted for this purpose. The appearance of subunit I in the holoenzyme was delayed for 1.5–2h after a 60min pulse with labelled methionine. 4. Incubation of hepatocytes for 4h in the presence of cycloheximide had no effect on the labelling pattern described above. 5. Methods were developed in which newly translated, presumably unassembled, subunits of cytochrome oxidase could be separated from the holoenzyme by fractionation in Triton X-114. Short-term pulse experiments indicate that subunits II and III are associated with the holoenzyme fraction immediately after their completion, whereas subunit I is not. 6. The data are consistent with a model in which cytochrome oxidase assembly is viewed as an ordered and sequential event.

Cytochrome oxidase contains seven subunits, three of which are translated on mitochondrial ribosomes; the remaining subunits are translated on cytoplasmic ribosomes. Little is known, however, about the mechanism of assembly of these subunits into holocytochrome oxidase. The advantageous use of yeast mutants has allowed some insight into this process in lower eukaryotic cells. These studies demonstrated a role for haem (Saltzgarber-Müller & Schatz, 1978), oxygen (Woodrow & Schatz, 1979) and nuclear genes (Cabral & Schatz, 1978) in the assembly of mitochondrially-translated subunits with certain cytoplasmic subunits. Furthermore, lower eukaryotic cells have been used to demonstrate the presence of pools (Schwab *et al.*, 1972) and precursor molecules (Werner & Bertrand, 1979; Machleidt & Werner, 1979) of mitochondrially-translated subunits of cytochrome oxidase.

Investigations of this nature are more difficult in mammalian cells due to the lack of available mutants. However, in the present paper we describe our initial studies on the assembly of cytochrome oxidase in isolated rat hepatocytes and mitochondria. The results suggest that transfer of various mitochondrially-translated subunits into the holoenzyme might be an ordered and sequential event.

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Materials and methods

Materials

Chloramphenicol, cycloheximide and phenylmethanesulphonyl fluoride were purchased from Sigma. Protein A–Sepharose was a product of Pharmacia. L-[^{35}S]Methionine at the highest available specific activity (over 800Ci/mmol) was purchased from The Radiochemical Centre, Amersham, and the scintillation solution (Luma Gel) was from Lumac, Sweden. All other chemicals were of reagent grade.

Labelling of rat hepatocytes in vitro

Rat hepatocytes were isolated from 180g male, Sprague–Dawley rats as described by Gellerfors & Nelson (1979). Hepatocytes (10mg of protein/ml) were incubated in the medium described by Gellerfors *et al.* (1979) in either the presence or absence of 0.5mM-cycloheximide. Labelling was initiated by the addition of L-[^{35}S]methionine (35–45μCi/ml) and the cells were incubated for up to 4h at 37°C in spinner flasks (Gellerfors *et al.*, 1979). An oxygen

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concentration of 20 μM was maintained in the incubation medium by adjusting the flow rates of O_2 and N_2/CO_2 (19:1). Labelled cells were washed in a solution containing 0.25 M-sucrose, 10 mM-Tris/HCl, pH 7.5, 10 mM-unlabelled methionine and 1 mM-phenylmethanesulphonyl fluoride. Mitochondria were isolated as described by Gellerfors & Nelson (1979) after a brief sonication.

Labelling of rat liver mitochondrial proteins in vitro

Mitochondria were isolated from rat livers as described by Johnson & Lardy (1967). Mitochondrial protein synthesis was measured in a medium containing 50 mM-Bicine [*N,N*-bis(2-hydroxyethyl)-glycine], 5 mM-KH_2PO_4, 1 mM-EDTA, 90 mM-KCl, 1.2 mM-ATP, 4.4 mM-phosphoenolpyruvate, 15 mM-$MgCl_2$, 1 mM-cycloheximide, 0.1% (w/v) bovine serum albumin, 10 i.u. of pyruvate kinase, a 1.5 mM mixture of amino acids (Roodyn *et al.*, 1961) lacking methionine, L-[^{35}S]methionine (30 μCi/ml) and rat liver mitochondria (3 mg of protein/ml). The pH of the medium was 7.6. Incubations were conducted at 30°C for 30 min.

For the measurements of radioactivity incorporated into protein, 30 μg of protein was placed on Whatman 3MM filter papers presoaked with trichloroacetic acid and the filters were subsequently washed with cold trichloroacetic acid (7%, w/v), hot trichloroacetic acid (7%, w/v) containing unlabelled methionine (0.1% w/v), ethanol/diethyl ether (3:1, v/v) and finally with diethyl ether alone. The filters were dried and counted in a scintillation counter.

Purification of rat liver cytochrome oxidase

A small scale procedure which allowed the isolation of cytochrome oxidase from as little as 15 mg of rat liver mitochondrial protein was adapted from the methods of Ades & Cascarno (1977). Labelled mitochondria were removed from the incubation medium by centrifugation (5000 *g*) and were washed with phosphate buffer as described (Ades & Cascarno, 1977). The washed mitochondria were suspended to 15 mg of protein/ml in a buffer containing 50 mM-potassium phosphate, pH 7.4, and 3.3% (v/v) Triton X-114. The suspension was centrifuged at 48000 *g* for 1 h to form a supernatant (Triton X-114 soluble fraction) and a pellet (Triton X-114 insoluble fraction). The pellet was dissolved in 100 mM-sodium phosphate buffer, pH 7.4, containing 5% (v/v) Triton X-100. This fraction was chromatographed over DEAE–Bio-Gel as described by Ades & Cascarno (1977), with the exception that all phosphate buffers were made with Na^+ salts instead of K^+. For small scale preparations, however, the dimensions of the chromatographic column were reduced to 0.5 cm × 4.0 cm, and all elutions were done at a flow rate of 0.1 ml/min. The elution was followed simultaneously at 270 nm and 417 nm using a LKB 2089 Uvicord III. Glycerol (final concn. 40%, v/v) was added to the fractions containing cytochrome oxidase, and the enzyme was stored at −80°C.

Immunological methods

Antibodies against rat liver cytochrome oxidase were produced in rabbits as described by Mendel-Hartvig & Nelson (1977). Solubilization of labelled mitochondria and immunoabsorption of labelled cytochrome oxidase with Protein A–Sepharose have been described by Kolarov *et al.* (1981).

Miscellaneous methods

Electrophoresis was carried out on 12.5% (w/v) polyacrylamide gels containing sodium dodecyl sulphate with the buffer system described by Laemmli (1970). Gels used for fluorography were processed in 1 M-sodium salicylate (Chamberlain, 1979) and exposed for 10–20 days to X-Omat R Kodak film stored at −80°C. In some experiments the distribution of radioactivity on the gels was determined by slicing the gels (1 mm slices) and measuring radioactivity in a scintillation counter.

ATPase activity was measured according to Glaser *et al.* (1980). Protein was determined by the method of Lowry *et al.* (1951), except in the presence of high concentrations of Triton when modifications of the method were made according to Peterson (1977). Cytochrome aa_3 haem was measured at 605 − 630 nm after reduction with dithionite, using an absorption coefficient of 12 mM·cm^{-1} (Van Gelder, 1966). Cytochrome *b* haem was determined at 562 − 575 nm using an absorption coefficient of 19 mM·cm^{-1} (Hatefi *et al.*, 1962).

Results

Assembly of cytochrome oxidase was studied in isolated hepatocytes pulsed for 1 h with L-[^{35}S]-methionine or after a subsequent 2 h chase either in the presence (Fig. 1) or absence (Fig. 2) of cycloheximide. Mitochondrial inner membranes were isolated and the translation products were analysed by electrophoresis. All of the major mitochondrial translation products are present in the inner membrane after a 1 h pulse (Fig. 1*a*) (Kolarov *et al.*, 1981). As described earlier (Gellerfors *et al.*, 1979), the translation products are relatively stable in isolated hepatocytes and, with the exception of subunit I, there are no significant changes observed after a 2–2.5 h chase with unlabelled methionine. The reason for the continued apparent increase in subunit I during a chase (Fig. 1*c*) (Gellerfors *et al.*, 1979) is not known, but is, in fact, due to an accumulation of radioactivity in this peak and not to a decrease in subunits II and III (Gellerfors *et al.*, 1979). This has been observed only in mitochon-

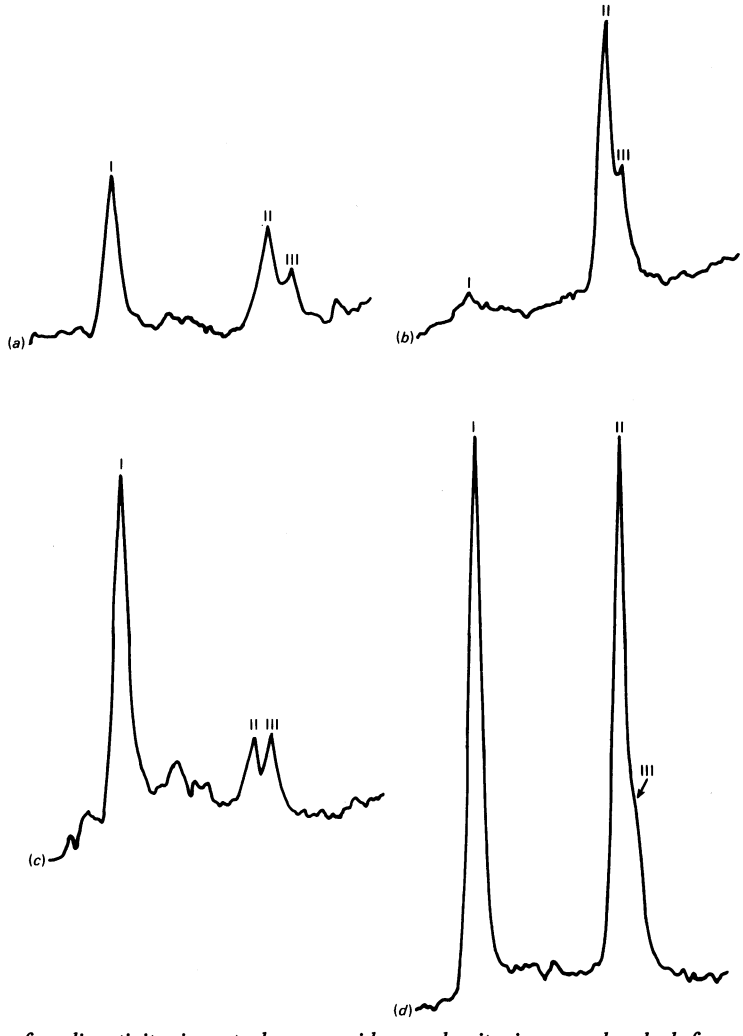

Fig. 1. *Distribution of radioactivity in cytochrome oxidase subunits immunoabsorbed from cycloheximide-treated hepatocytes*

Hepatocytes were pulsed for 1h with L-[^{35}S]methionine (40μCi/ml) and then chased for 2h in the presence of 5mM-unlabelled methionine. Cycloheximide (0.5mM) was present throughout the experiment. Mitochondrial inner membranes were isolated and cytochrome oxidase was immunoabsorbed as described in the Materials and methods section. Samples were electrophoresed and fluorographs were prepared. The Figure shows densitometric tracings of the exposed film. (*a*) Mitochondrial inner membranes, after a 1h pulse. (*b*) Cytochrome oxidase immunoabsorbed from (*a*). (*c*) Mitochondrial inner membranes after a 2h chase. (*d*) Cytochrome oxidase immunoabsorbed from (*c*). The location of cytochrome oxidase subunits I–III is indicated.

drial inner membrane preparations and might indicate a delay in the transfer of this peptide into the membrane. This problem has not been pursued further.

The situation is different, however, if analysis is extended to cytochrome oxidase isolated by immunoabsorption (Kolarov *et al.*, 1981). In spite of the fact that subunit I in inner membranes is heavily labelled relative to subunits II and III after a 1h pulse (Fig. 1*a*), it is not labelled in immunoabsorbed samples from these membranes (Fig. 1*b*). Subunit I appears in an immunoabsorbable form after a 2h chase (Fig. 1*d*). Similar findings were also obtained with hepatocytes labelled in the absence of cycloheximide (Fig. 2). These results were also confirmed in more quantitative experiments in which the immunoabsorbable samples were electrophoresed and the subunits were sliced from the gels and counted for radioactivity (results not shown).

The above results, as well as earlier observations

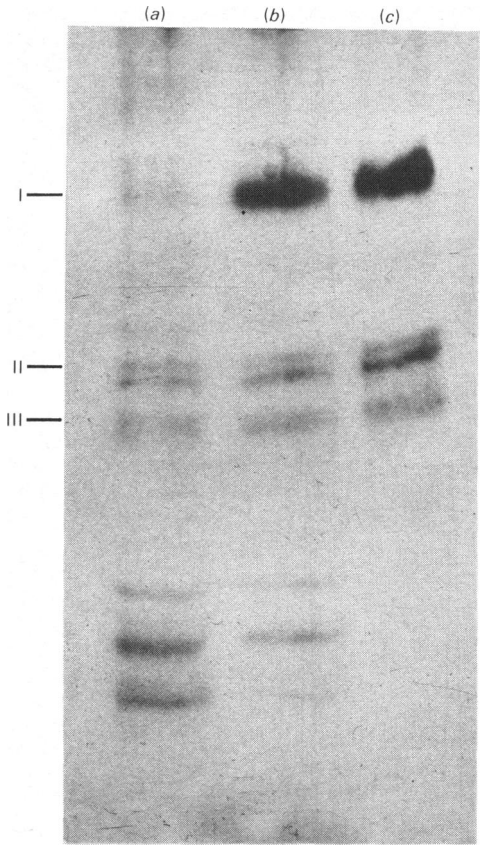

Fig. 2. *Distribution of radioactivity in cytochrome oxidase immunoabsorbed from hepatocytes labelled in the absence of translation inhibitors*

Hepatocytes were labelled with L-[^{35}S]methionine and chased as described in Fig. 1, but in the absence of cycloheximide. Mitochondria were isolated, immunoabsorbed and fluorographs were prepared as described in the Materials and methods section. (*a*) 1 h pulse. (*b*) 1 h pulse followed by 2 h chase with unlabelled methionine. For comparison, mitochondria from cells labelled continuously for 3 h in the presence of cycloheximide were also immunoabsorbed (*c*). The location of the cytochrome oxidase subunits I–III is indicated.

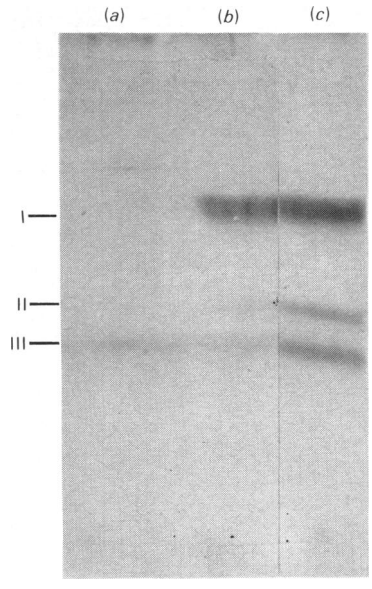

Fig. 3. *Distribution of radioactivity in the subunits of chemically-isolated cytochrome oxidase*

Isolated hepatocytes were labelled and chased in the absence of cycloheximide as described in Fig. 2. Mitochondria were prepared (15 mg of protein) and cytochrome oxidase was isolated by the small scale procedure described in the Materials and methods section. Fluorographs are shown. (*a*) Cytochrome oxidase isolated from hepatocytes labelled with L-[^{35}S]methionine for 1 h. (*b*) Cytochrome oxidase isolated from hepatocytes labelled for 1 h and chased for 2 h. For comparison, cytochrome oxidase was also isolated from cells continuously labelled for 3 h in the presence of cycloheximide (*c*). Subunits I–III are indicated. The cytoplasmically translated subunits are too weakly labelled to be seen in this fluorograph.

from this laboratory (Kolarov *et al.*, 1981), suggest that the process of assembly, or the association, of different cytochrome oxidase subunits can be investigated in isolated hepatocytes. It is not clear at what level the assembly is occurring, however. The antisera used in the above experiment were found to remove rather specifically subunit II during immunoabsorption of labelled mitochondria. Thus, the co-absorption of subunits II and III from cells labelled for 1 h, and subunits I, II and III after a 2 h chase, suggests a time-dependent association of subunits I and III with subunit II.

This conclusion was confirmed by the chemical isolation of cytochrome oxidase from mitochondria prepared from labelled heptocytes (Fig. 3). The same general result was obtained as with the immunoabsorbed enzyme. Labelled subunits II and III were associated with the isolated holoenzyme after a 1 h pulse, whereas all three subunits were labelled in the isolated enzyme after a 2 h chase with methionine either in the presence or absence of cycloheximide (Fig. 3). Chemical isolation probably represents a more advanced stage of assembly than the immunoabsorption experiments, since in the former the subunits are, by definition, associated with the holoenzyme, whereas the immunoabsorbed subunits need not be (see below).

As shown in Figs. 1 and 2, the time-dependent

association of subunits I–III is not impaired in isolated hepatocytes incubated in the presence of cycloheximide. These findings show that the association of subunits I–III is not coupled to the concurrent synthesis of cytoplasmic peptides. This, in turn, indicates either that the association of cytochrome oxidase subunits I–III occurs in the absence of cytoplasmic peptides or that mitochondria always contain an excess of very stable, unassembled cytoplasmic subunits.

The above discussion, and the data obtained from chemical isolation of cytochrome oxidase (Fig. 3), raise the possibility of different stages of assembly and, thus, different 'pools' of cytochrome oxidase subunits. These 'pools' are most easily defined operationally as those newly translated peptides exhibiting holoenzyme-like properties (associated with chemically isolated holoenzyme), and those which do not. The data in Table 1 show, indeed, that the holoenzyme can be separated from newly labelled translation products by extraction of labelled mitochondria with Triton X-114. Whereas 75–90% of the radioactivity is solubilized by Triton X-114, most of the holoenzyme (represented by haem aa_3) remains in the insoluble fraction (Table 1). Extraction of mitochondria labelled *in vitro* or of mitochondria isolated from hepatocytes labelled for 3h in the absence of inhibitor yielded similar results (Table 1). These findings again underline the possible lack of requirement for newly synthesized cytoplasmic peptides.

Results in Table 1 also indicate that the transfer of labelled peptides into the holoenzyme fraction is a slow process. Only 1% of the total radioactivity co-purifies with chemically isolated cytochrome oxidase (Table 1), even though the recovery of haem aa_3 was approx. 50%.

Electrophoretic analysis of the newly synthesized peptides in the Triton X-114 soluble and insoluble fractions is shown in Fig. 4. Isolated mitochondria were pulsed with L-[^{35}S]methionine for 10min and then chased for 20min with unlabelled methionine. The Triton X-114 soluble and insoluble fractions were prepared from each sample.

The results from this experiment show that the nascent chains, which dominate the labelling pattern after a 10min pulse, appear as completed translation products after a 20min chase (Fig. 4*a*). The salient feature of this experiment, however, is that within 10min after formation of these completed translations products there is a clear segregation of the products into either Triton X-114 soluble (Fig. 4*b*) or insoluble (Fig. 4*c*) fractions. Newly translated cytochrome *b* and the ATPase subunits are concentrated in the Triton X-114 soluble fraction. This is in keeping with the distribution of the holoenzymes to which these peptides belong. Cytochrome oxidase subunits exhibit, on the other hand, a different partitioning (Fig. 4). After a 20min chase nearly all of cytochrome oxidase subunit II is in the Triton X-114 insoluble fraction with the holoenzyme (Fig. 4*c*, Tables 1 and 2). In contrast,

Table 1. *Separation of cytochrome oxidase from newly translated mitochondrial peptides*

Mitochondria were labelled with L-[^{35}S]methionine for 30min *in vitro*. Hepatocytes were labelled *in vitro* for 3h in the absence of inhibitors. Mitochondria, the Triton X-114 soluble and insoluble fractions, and cytochrome oxidase were isolated as described in the Materials and methods section. Numbers in parentheses represent % of total radioactivity or haem aa_3 recovered.

Preparation labelled	Fraction	10^{-5} × Total radioactivity (c.p.m.)	Total haem aa_3 (nmol)
Mitochondria	Mitochondria	52.0 (100)	8.9 (100)
	Triton X-114 soluble	48.0 (92)	2.1 (24)
	Triton X-114 insoluble	3.0 (6)	5.4 (61)
	Isolated cytochrome oxidase	0.6 (1)	4.2 (47)
Hepatocytes	Mitochondria	198.0 (100)	2.6 (100)
	Triton X-114 soluble	148.0 (75)	0.4 (15)
	Triton X-114 insoluble	16.0 (8)	1.9 (72)
	Isolated cytochrome oxidase	1.9 (0.9)	1.5 (58)

Table 2. *Distribution of mitochondrial inner membrane components in the Triton X-114 soluble and Triton X-114 insoluble fractions*

Isolated mitochondria were labelled *in vitro* with L-[^{35}S]methionine for 30min at 30°C, and then extracted with Triton X-114 as described in the Materials and methods section. Numbers in parentheses represent the % of the total recovered.

Fraction	Protein (mg)	10^{-5} × Radioactivity (c.p.m.)	Haem aa_3 (nmol)	Cytochrome *b* haem (nmol)	Total ATPase activity (μmol/min)
Triton X-114 soluble	13.1 (82)	22.5 (94)	0.9 (18)	2.1 (88)	6.4 (97)
Triton X-114 insoluble	2.9 (18)	1.4 (6)	4.1 (82)	0.3 (12)	0.2 (3)

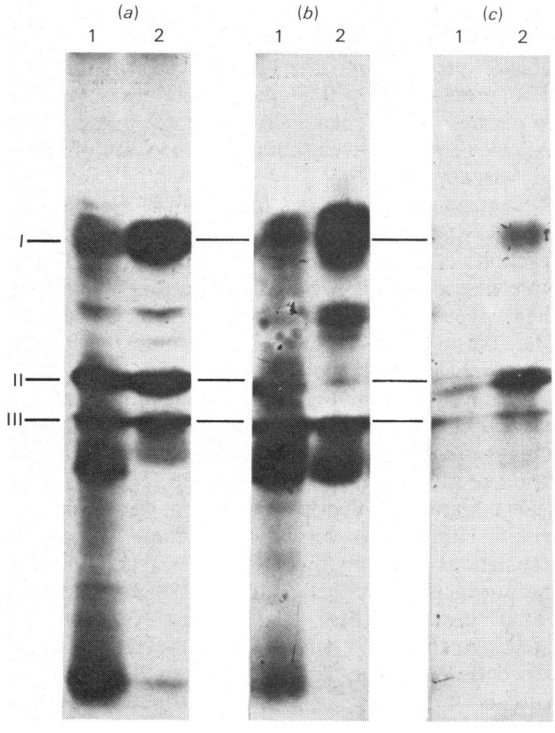

Fig. 4. *Distribution of the mitochondrial translation products into Triton X-114 soluble and insoluble fractions*

Rat liver mitochondria were labelled *in vitro* with L-[^{35}S]methionine (30 μCi/ml) as described in the Materials and methods section. Labelling was terminated by addition of a stop solution which contained 0.25 M-sucrose, 10 mM-Tris/HCl, pH 7.5, 0.5 mM-chloramphenicol and 10 mM-unlabelled methionine. Mitochondria were removed by centrifugation and then processed as described (Ades & Cascarano, 1977, and the Materials and methods section) to give Triton X-114 soluble and insoluble fractions. The samples were electrophoresed and fluorographs were prepared. Track 1, mitochondria pulsed for 10 min; track 2, mitochondria pulsed for 10 min, followed by a 20 min chase with unlabelled methionine. (*a*) Mitochondrial inner membrane fraction; (*b*) Triton X-114 soluble and (*c*) Triton X-114 insoluble fractions prepared from the sample in (*a*). Roman numerals I–III are cytochrome oxidase subunits I–III, respectively.

subunit I is concentrated in the soluble fraction (Fig. 4*b*). The small amount of subunit I which is found in the pellet (Fig. 4*c*) is not assembled with holoenzyme, as shown by results in Figs. 1 and 3. The distribution of subunit I seen in Fig. 4 is in keeping with data in Fig. 1, which shows a lag before the appearance of this peptide in the holoenzyme.

Discussion

The present study describes our initial attempts to investigate the assembly of cytochrome oxidase in isolated rat hepatocytes. Although the problem is far too complex to present a complete analysis of the process, our findings allow the following conclusions. (*a*) Mitochondrially translated subunits associated with the holoenyzme on very different time scales. For example, only newly labelled subunit II and, to some extent, subunit III are associated with the immunoabsorbed or chemically isolated holoenzyme after 30–60 min of labelling. In contrast, subunit I, though highly labelled in intact mitochondria after 10 min, first appears in the holoenzyme after 1.5–2 h. (*b*) Newly synthesized, unassembled subunit I can be separated from the holoenzyme by extraction into Triton X-114. Pulse–chase experiments show that there is very little transfer of subunit I from the Triton X-114 soluble fraction, whereas subunits II and III are associated with the holoenzyme fraction within 10 min after their completion.

Two explanations can be offered for the different rates of transfer of subunits I–III to the holoenzyme fraction. One is that the rates reflect different pool sizes of the unassembled subunits (Schwab *et al.*, 1972). In this case, the pool of unassembled subunit I should be large relative to that of subunit II, implying either a relatively slower breakdown or a more rapid synthesis of subunit I. However, kinetic experiments in which mitochondrially translated products were chased from ribosomes reveal no preferential synthesis of subunit I (Fig. 4) (Kuźela *et al.*, 1981). If anything, subunit II appears to accumulate most rapidly. Furthermore, all mitochondrial translation products in isolated hepatocytes are stable for at least 4 h (Gellerfors *et al.*, 1979) and probably longer. Thus, there is no evidence for different pool sizes as reflected in differential degradation of the subunits.

A second explanation for the slow assembly of labelled subunit I could be that the assembly process is ordered, and that subunit I is the last of the mitochondrially translated subunits to assemble. Pulse–chase experiments (Fig. 4) show that subunit II is the first to associate with the holoenzyme fraction. In fact, all newly labelled subunit II is located with the holoenzyme fraction within 10 min after removal from the ribosomes (Fig. 4). Rapid transfer of subunit II to the holoenzyme could, of course, be fortuitous, reflecting unique chemical properties of this peptide. This seems somewhat unlikely, however, in view of the fact that all other newly synthesized peptides, including the nascent chains, fractionate into the Triton X-114 soluble fraction. However, to determine if subunit II is actually assembled with the holoenzyme will require studies with specific antibodies.

The tentative working hypothesis which is drawn from the present findings is that cytochrome oxidase assembly in rat hepatocytes is probably an ordered

event in which subunit II associates first and subunit I associates last with holoenzyme fraction. It is not known if this 'assembly' we have observed requires the participation of unassembled cytoplasmically-translated subunits. It should be pointed out, however, that transfer of newly labelled subunit I into holoenzyme takes place even after several hours of incubation of hepatocytes in the presence of cycloheximide (Fig. 3), as well as in isolated mitochondria (Hundt *et al.*, 1980). This could occur only if mitochondria contained highly stable pools of unassembled cytoplasmic subunits. A possibility which has not been explored is that the pre-existing holoenzyme can act as a template on which assembly of mitochondrially translated peptides can occur. For example, if the holoenzyme provided a membrane recognition site for any one of the three (I–III) subunits, partial assembly could occur without the involvement of cytoplasmically translated subunits.

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References

Ades, I. Z. & Cascarano, J. (1977) *J. Bioenerg. Biomembr.* **9**, 237–253

Cabral, F. & Schatz, G. (1978) *J. Biol. Chem.* **253**, 4396–4401

Chamberlain, J. P. (1979) *Anal. Biochem.* **98**, 132–135

Gellerfors, P. & Nelson, B. D. (1979) *Anal. Biochem.* **93**, 200–203

Gellerfors, P., Wielburski, A. & Nelson, B. D. (1979) *FEBS Lett.* **108**, 167–170

Glaser, E., Norling, B. & Ernster, L. (1980) *Eur. J. Biochem.* **110**, 225–235

Hatefi, Y., Haavik, A. G. & Griffiths, D. E. (1962) *J. Biol. Chem.* **237**, 1681–1685

Hundt, E., Trapp, M. & Kadenbach, B. (1980) *FEBS Lett.* **115**, 95–99

Johnson, D. & Lardy, H. (1967) *Methods Enzymol.* **10**, 94–96

Kolarov, J., Wielburski, A., Mendel-Hartvig, I. & Nelson, B. D. (1981) *Biochim. Biophys. Acta* **652**, 334–346

Kużela, S., Wielburski, A. & Nelson, B. D. (1981) *FEBS Lett.* **135**, 89–92

Laemmli, U. A. (1970) *Nature (London)* **227**, 680–685

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Machleidt, W. & Werner, S. (1979) *FEBS Lett.* **107**, 327–330

Mendel-Hartvig, I. & Nelson, B. D. (1977) *Eur. J. Biochem.* **80**, 267–274

Peterson, G. L. (1977) *Anal. Biochem.* **83**, 346–356

Roodyn, D. B., Rejs, P. J. & Work, T. S. (1961) *Biochem. J.* **80**, 9–21

Saltzgaber-Müller, J. & Schatz, G. (1978) *J. Biol. Chem.* **253**, 305–310

Schwab, A. J., Sebald, W. & Weiss, H. (1972) *Eur. J. Biochem.* **30**, 511–516

Van Gelder, B. F. (1966) *Biochim. Biophys. Acta* **118**, 36

Werner, S. & Bertrand, H. (1979) *Eur. J. Biochem.* **99**, 463–470

Woodrow, G. & Schatz, G. (1979) *J. Biol. Chem.* **254**, 6088–6093