Detection of the Mitochondrially Encoded Cytochrome c Oxidase Subunit I in the Trypanosomatid Protozoan Leishmania tarentolae

EVIDENCE FOR TRANSLATION OF UNEDITED mRNA IN THE KINETOPLAST*

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With the aim of identification of kinetoplast-encoded proteins we investigated the subunit composition of cytochrome c oxidase (respiratory complex IV) from kinetoplast mitochondria of the trypanosomatid protozoan Leishmania tarentolae. Eleven stoichiometric subunits were visible in Coomassie-stained, two-dimensional Blue Native/Tricine-SDS electrophoretic gels. Their partial amino acid sequences indicated that these polypeptides are nuclear-encoded. The mitochondrial subunit I was detected with the polyclonal antibodies against an internal region of this polypeptide. In two-dimensional (9 vers 14%) polyacrylamide glycine-SDS gels this subunit is found as a series of spots located off the main diagonal, a property that can be explained by abnormal electrophoretic migration and aggregation. In gels loaded with high amounts of the purified, enzymatically active oxidase, the subunit I spots could be visualized by staining. The determined N-terminal amino acid sequence of the putative monomeric subunit I (MFXCLV-CLSVS) matched with the predicted sequence, thus indicating that the corresponding kinetoplast unedited mRNA is translated into a functional protein.

A large amount of molecular genetic data suggests that kinetoplast mitochondrial genes of trypanosomatids (a group of protozoa that includes human pathogens such as trypansomes and leishmanias and several nonpathogenic organisms) encode functional proteins. However, a direct biochemical proof of this in the form of an isolated protein could not be obtained for a long time. Twenty protein-coding genes and two rRNA genes are usually found in the maxicircle component of kinetoplast DNA (1, 2). The encoded proteins include subunits I, II, and III (COI, COII, and COIII) of cytochrome c oxidase (respiratory complex IV), apocytochrome b of ubiquinol-cytochrome c oxidoreductase (complex III), subunit 6 of oligomycin-sensitive ATPase (complex V), several subunits of NADH dehydrogenase (complex I), ribosomal protein S12, and a number of putative proteins with unknown function. Several of these proteins can only be synthesized if the corresponding mRNAs are post-transcriptionally “edited” by insertion and deletion of uridylic residues, a unique process that in extreme cases is capable of creating an entire reading frame out of a cryptic pre-mRNA sequence (3–6).

Although many interesting findings could be expected, the system of kinetoplast translation remained mainly uninvestigated, and the kinetoplast-encoded subunits of respiratory complexes could not be identified (7–9). Because some of the mitochondrial subunits are crucial for the activity and spectral characteristics of complexes III and IV (10, 11), their apparent absence in the corresponding cases was puzzling. The only available evidence for existence of kinetoplast-encoded proteins was the positive reactivity of antisera against synthetic or recombinant COII and NADH dehydrogenase 9 epitopes with proteins in kinetoplast lysates (12–14); however, it was not known whether the immunoreactive polypeptides represent subunits of the corresponding respiratory complexes. In addition, two recent reports (15, 16) indicated that isolated kinetoplasts may be capable of the de novo protein synthesis. Only very recently, we have reported on identification of apocytochrome b, a protein derived from the 5′-edited mRNA, within a functional respiratory complex III, thus providing the first direct evidence for the functional translation in the kinetoplasts (17).

In this work we describe the detection by antibody probing and staining of another kinetoplast-encoded polypeptide, subunit I of cytochrome c oxidase, found within the corresponding purified complex. This identification has been confirmed by determining the N-terminal amino acid sequence, which matched with the sequence predicted by the kinetoplast-encoded COI mRNA. The finding of the COI polypeptide indicates that the kinetoplast mitochondrial system of translation operates with unedited, as well as edited, templates.

EXPERIMENTAL PROCEDURES

Cell Cultivation and Fractionation—Cells of Leishmania tarentolae, strain UC, were cultivated in the BHI medium as described (18). Kinetoplast-mitochondrial fractions of cells disrupted by the hypotonic lysis were obtained using flotation in Renografin density gradients as described earlier (18).

Two-dimensional Gel Electrophoresis—Electrophoretic purification of respiratory complexes from the dodecyl maltoside lysates of purified kinetoplast mitochondria was performed by Blue Native/Tricine-SDS polyacrylamide gel electrophoresis (19). Mitochondrial vesicles (200–400 μg of protein, as determined using a BCA* Protein Assay kit, Pierce) were lysed with 2% dodecyl maltoside (Sigma) in 200 μl of buffer containing 750 mM e-amino-n-caproic acid, 50 mM BisTris-HCl, pH 7.0, at 0 °C for 60 min. The lysate was centrifuged at 14,000 × g in a bench top microcentrifuge for 30 min, and the supernatant was combined with 20 μl of a 5% Coomassie G-250 (Fisher) solution made with 500 mM aminocaproic acid. Separation in the first dimension was performed in 6% Blue Native gels. After incubation of the native gel slices in an...
excess of a solution containing 0.125 M Tris-HCl, pH 6.8, 1% SDS, 1% β-mercaptoethanol for 40 min at 37 °C, proteins were separated in the second dimension using Tricine-SDS polyacrylamide gels (19) or Tris-glycine-SDS gels (20).

Immunological Procedures—Peptides were selected for antibody production on the basis of their antigenic index values as determined by MacVector (version 5.0, Oxford Molecular). Peptide synthesis, conjugation with keyhole limpet hemocyanin, and immunization of rabbits were performed by Research Genetics, Inc. According to the colorimetric enzyme-linked immunosorbent assay titration performed by the manufacturer, a standard level of reactivity against the immobilized peptide (1 µg/well) was observed in 3.0–9.0 × 10⁻⁴ dilutions of the anti-COI serum from different bleed.

The immune sera were further purified by immunoadfinity chromatography using the immobilized antigenic peptide and the QUICKPure system (Sterogene). Coupling of the peptide to activated resin, Actigel ALD, was performed in 0.1 M potassium phosphate buffer, pH 7.0. Other conditions were as recommended by the manufacturer.

For Western blot analyses, proteins were electroblotted onto Hybond ECL nitrocellulose membranes (Amersham Pharmacia Biotec) using a MilliBlot semidy electroblotting system (Millipore) at 1 mA/cm² for 2 h in a cathode buffer containing 300 mM aminocaproic acid, 0.05% SDS titrated with solid Tris-base to pH 8.5–8.6, and an anode buffer containing 150 mM Tris, 50 mM Tricine (pH 8.6), 40% methanol. In some cases, proteins were stained with Ponceau S. After incubating the blot with primary antibodies, the immunoreactive polypeptides were detected by enhanced chemiluminescence using the SuperSignal system (Pierce).

Isolation of Cytochrome c Oxidase—The method represented a development of the procedure utilized previously for isolation of cytochrome c reductase from potato mitochondria (21) and is now allowing for the parallel purification of both cytochrome c reductase and oxidase from Leishmania kinetoplasts. In brief, the dodecyl maltoside lysate of kinetoplasts was subjected to two cycles of the chromatography on DEAE-Phosphorose. During the first chromatographic step, under conditions chosen to purify the reductase as described earlier (21), the oxidase did not bind to the column. The flow-through fraction containing the oxidase was diluted to reduce the concentration of NaCl to 130 mM, re-applied to the DEAE-Phosphorose column, and eluted with a linear gradient of 130–500 mM NaCl. The column fractions were analyzed by gel electrophoresis, and cytochrome c oxidase activity was determined as described previously (7) using horse cytochrome c (Sigma). Active fractions were pooled and concentrated using Amicon Centriprep-10 centrifugal concentrators. Spectral characteristics were assayed as in the previous work (7). Additional details of the procedure will be described elsewhere.

Protein Microsequencing—Proteins were electroblotted onto Trans-Blot PVDF membranes (Bio-Rad) and stained with Coomassie Blue R-250 (Sigma). Partial N-terminal sequencing was performed by Edman degradation at the UCR Protein Sequencing facility using an Applied Biosystems Procise 492 sequencer with an on-line high pressure liquid chromatography system. During the first chromatographic step, under conditions chosen to purify the reductase as described earlier (21), the oxidase did not bind to the column. The flow-through fraction containing the oxidase was diluted to reduce the concentration of NaCl to 130 mM, re-applied to the DEAE-Phosphorose column, and eluted with a linear gradient of 130–500 mM NaCl. The column fractions were analyzed by gel electrophoresis, and cytochrome c oxidase activity was determined as described previously (7) using horse cytochrome c (Sigma). Active fractions were pooled and concentrated using Amicon Centriprep-10 centrifugal concentrators. Spectral characteristics were assayed as in the previous work (7). Additional details of the procedure will be described elsewhere.

RESULTS

Nuclear-encoded Subunits of Cytochrome Oxidase—Isolation of the respiratory complexes from a dodecyl maltoside kinetoplast lysate by Blue Native gel electrophoresis and separation of their subunits in a second dimension gel is illustrated in Figs. 1A and 2A, respectively. Identification of complexes III and IV was performed previously by N-terminal sequencing of some subunits, and complex V was identified by Western blot analysis with an anti-ATPase serum (25). The subunit composition of dissociated complex IV is also shown in Fig. 1B. At least eleven major, apparently stoichiometric bands (numbered from 1 to 11) could be resolved. A very similar pattern was previously observed in a closely related species, *Crithidia fasciculata* (7), except that the smallest subunit was not seen. Because these polypeptides are only found in complex IV (Fig. 2A), they most likely represent the *bona fide* subunits of this complex. A substoichiometric band (labeled with an asterisk) could also be seen in *L. tarentolae* (Figs. 1B and 2A). This component does not seem to be a true subunit of cytochrome oxidase because in two-dimensional gels (Fig. 2A) this polypeptide is found in a broad area overlapping positions of all three complexes. A few minor bands visible in the upper part of Fig. 1B represent contaminating large subunits from complexes III and V.

Because in other organisms the largest subunits of cytochrome c oxidase correspond to the mitochondrially encoded polypeptides COI, COII, and COIII, we determined N-terminal partial sequences of the bands 1 and 2 and internal sequences of these polypeptides, which still may be present in gels, we

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**Fig. 1.** Two-dimensional gel-purification and subunit composition of cytochrome c oxidase from *L. tarentolae*. A, fractionation of kinetoplast mitochondrial lysate in a 6% Blue Native gel. Identities of the respiratory complexes are shown to the right. B, subunit composition of the gel-isolated cytochrome c oxidase (complex IV) resolved in a 12% Tricine-SDS gel. Molecular masses of the markers are indicated to the left.
have used an immunological approach, which is potentially more sensitive than staining. The predicted *L. tarentolae* COI polypeptide contains 549 amino acids, 62% of which are represented by eight nonpolar residues with leucine (15.3%), phenylalanine (14.4%), and isoleucine (9.3%) being the most abundant. The protein contains multiple predicted transmembrane domains and appears to be almost entirely hydrophobic when analyzed by hydropathy plots (26). Two other kinetoplast-encoded polypeptides, COII and COIII, have similar properties, and according to antigenic index analysis, all these subunits contain few potential epitopes (data not shown). Only in COI did we identify an internal undecameric peptide (CDRNFNTS-FFGKGWDNAALDTIF) with a relatively high antigenic index and used it for antibody production. To ascertain the specificity of the assays, we have immobilized peptide on the unpurified serum. The putative sequence produced multiple bands on a Western blot of the kinetoplast lysate; however, after the purification only a subset of minor bands remained (data not shown). Because, as shown below, these bands represent a specific immune reaction, the step of immunopurification was crucial for removing the low specificity or cross-reactive components from the serum.

When the lysate was fractionated in a two-dimensional gel and probed with the purified antibodies, we observed three major immunoreactive bands, *a*, *b* and *c*, in the region of the gel which corresponded to Complex IV (Fig. 2B). The observed bands did not comigrate with the Coomassie-stained bands of nuclear subunits and no other protein bands could be correlated with the immunoreactive bands. This selectivity indicated the presence among the complex IV dissociation products of some minor components bearing the COI epitope. These components were insufficient for staining in a regularly loaded two-dimensional BN gel (200–400 μg of kinetoplast protein/native gel dimension).

A similar set of immunoreactive bands could also be seen on single-dimensional Western blots of kinetoplast lysates, but the intensities of these bands varied between lysates and between gels as illustrated by Fig. 3. In addition, a number of minor bands could be seen on one-dimensional blots of total lysates (Fig. 3) and on two-dimensional blots when higher amounts of the serum or increased gel loads were used (not shown). The pattern of the additional bands, unlike the major bands, was not entirely reproducible. Provided there is no significant cross-reactivity of the antibodies with some other polypeptides, the multiplicity of major immunoreactive bands points toward the possibility of aggregation of the immunoreactive polypeptides.

### Anomalous Electrophoretic Migration of COI—The putative COI polypeptide showed an unusual relative migration; when the Tris-glycine polyacrylamide gel concentration was increased from 9 to 14%, the apparent estimated size of polypeptide *c* also increased from 32 to 38 kDa, and the sizes of components *b* and *a* increased from 49 to 74 kDa and from 59...
corresponding to the running front of the 9% gel (Fig. 4A). The smaller subunits 4–11 are found along the cytochrome oxidase nuclear-encoded bands 1, 2, and 3, whereas the material corresponded to various aggregation products, which may include not only COI but also some other polypeptides. The spot d is possibly a COI breakdown product.

Examination of the gel shown in Fig. 4A reveals the presence of the weakly stained material comigrating with the spots a and b. The putative monomeric COI (component c) could not be seen in stained three-dimensional gels even with the maximally increased gel loading.

To visualize this component by staining, we purified cytochrome c oxidase by DEAE-Sepharose chromatography. Because COI represents a catalytic subunit that contains both hemes of the enzyme, it was important to ascertain a functional and structural integrity of the obtained preparation. The presence of hemes a and a3 and the absence of a significant contamination by cytochrome bc1, followed from the presence of a reduced absorption maximum at 604 nm and the absence of the maxima at 559 and 529 nm (data not shown). The a + a3 heme content calculated from the analysis of dithionite-reduced minus ferricyanide-oxidized spectra (assuming absorbance coefficient of 24 mM–1 cm–1) was 1.9 nmol/mg, representing about 25% of the value reported for a mammalian enzyme (27). The specific activity of the enzyme obtained in several isolations was 0.50–1.37 units/mg. These values were similar or exceeded the corresponding parameters reported previously for the oxidase from C. fasciculata (7), also demonstrating that the isolated enzyme has a high level of purity and structural integrity and, therefore, must contain a full set of catalytic subunits including COI. Additional details of the enzymatic properties, as well as the nuclear subunit composition in comparison with the electrophoretically purified oxidase, will be described elsewhere. Here we only note, that the nuclear subunits 2 and 3 (COV and COVI, respectively) are probably not so tightly associated with the complex compared with other subunits and appear to be underrepresented in the gel shown in Fig. 4C.

With respect to COI, using 50 μg of the purified enzyme/two-dimensional gel we could clearly observe the putative monomeric COI (component c) and the high molecular weight components in their characteristic positions by Coomassie staining (Fig. 4C). The banding pattern of the major COI-related components (a, b, and c) on immunoblots of the isolated enzyme (Fig. 4D) was very similar to the BN-purified oxidase (Fig. 4B), although dissociation of components a and b was not always observed.

Visualization of COI—The property of aberrant migration was used to separate the putative COI polypeptides from all other subunits of complex IV (Fig. 4). The dodecyl maltoside kinetoplast extract was fractionated in a BN gel followed by a 10% Tricine-SDS gel. One lysate was analyzed in lanes 1 and 3 and another in lanes 2 and 4. Immunoreactive components are indicated to the right, and molecular masses are indicated to the left.

to 100 kDa, respectively. This method was, therefore, inappropriate to determine their sizes. In contrast, the relative migration of nuclear subunits was mostly independent of the gel concentration. The size of polypeptide c was then approximately estimated by a Ferguson plot analysis in a series (9, 11, 13, and 15%) of gels concentrations, and the obtained value (65–76 kDa) was close enough to the predicted size of COI (63 kDa). The sizes of components a and b were outside of the calibrated range in Ferguson plots, with component b being larger than 150 kDa and component a even larger. These results suggest that component c is the best candidate for a full size monomeric COI protein, whereas components a and b represent aggregates of COI and, possibly, some other polypeptides. Results of the N-terminal sequencing described below confirmed these conclusions.

Visualization of COI—The property of aberrant migration was used to separate the putative COI polypeptides from all other subunits of complex IV (Fig. 4). The dodecyl maltoside kinetoplast extract was fractionated in a BN gel followed by a 9% Tris-glycine gel in the second dimension. The gel slice, containing resolved subunits of cytochrome oxidase, was then subjected to the third dimension electrophoresis in a 14% Tris-glycine gel. Subunits with an unchanged relative mobility are found on the main diagonal and included the asterisk band and cytochrome oxidase nuclear-encoded bands 1, 2, and 3, whereas the smaller subunits 4–11 are found along the vertical line corresponding to the running front of the 9% gel (Fig. 4A).

When a similar three-dimensional gel was electroblotted and probed with the anti-COI serum, the major immunoreactive components a, b, and c were detected above the diagonal line (Fig. 4B), as was the minor spot c*, migrating just ahead of b, and a high molecular weight smear. Another minor spot d was seen between bands 4 and 5 in the front region of the 9% gel, but this spot was not reproducible.

Unexpectedly, we also detected the minor spot, designated c*, with the same mobility in the 14% gel as the spot c but located in a position that suggests that the component c* may have originated from the component c during the incubation of the 9% gel slice in SDS/mercaptoethanol prior to the separation in the 14% gel. Ferguson plot estimates for c* suggested that it could be a dimer of c. Moreover, the position of another minor spot (c**) suggested that the same component (c) is also released from a higher molecular weight material. Similarly, the position of the spot b may indicate that component a can dissociate releasing component b (and probably c as well). Because component c most likely represents the full size COI, the larger components correspond to various aggregation products, which may include not only COI but also some other polypeptides. The spot d is possibly a COI breakdown product.

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N-terminal Sequencing of the COI—The material corresponding to components c and b could also be seen on Coomassie-stained PVDF blots but the staining intensity was lower, indicating a poor blotting efficiency or a low level retention on the membrane. Because originally no N-terminal sequence of the oxidase by DEAE-Sepharose chromatography. Because COI represents a catalytic subunit that contains both hemes of the enzyme, it was important to ascertain a functional and structural integrity of the obtained preparation. The presence of hemes a and a3 and the absence of a significant contamination by cytochrome bc1, followed from the presence of a reduced absorption maximum at 604 nm and the absence of the maxima at 559 and 529 nm (data not shown). The a + a3 heme content calculated from the analysis of dithionite-reduced minus ferricyanide-oxidized spectra (assuming absorbance coefficient of 24 mM–1 cm–1) was 1.9 nmol/mg, representing about 25% of the value reported for a mammalian enzyme (27). The specific activity of the enzyme obtained in several isolations was 0.50–1.37 units/mg. These values were similar or exceeded the corresponding parameters reported previously for the oxidase from C. fasciculata (7), also demonstrating that the isolated enzyme has a high level of purity and structural integrity and, therefore, must contain a full set of catalytic subunits including COI. Additional details of the enzymatic properties, as well as the nuclear subunit composition in comparison with the electrophoretically purified oxidase, will be described elsewhere. Here we only note, that the nuclear subunits 2 and 3 (COV and COVI, respectively) are probably not so tightly associated with the complex compared with other subunits and appear to be underrepresented in the gel shown in Fig. 4C.

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N-terminal Sequencing of the COI—The material corresponding to components c and b could also be seen on Coomassie-stained PVDF blots but the staining intensity was lower, indicating a poor blotting efficiency or a low level retention on the membrane. Because originally no N-terminal sequence of component c could be obtained, we suggested that the polypeptide could be blocked on the N terminus. To overcome this obstacle, we treated the blotted polypeptides with 0.5 N HCl in methanol at room temperature for 90 min, a deblocking procedure suggested for the removal of a formyl group from the terminal N-formylmethionine of bovine COII (28). The drawback of this procedure is that it seems to introduce internal random cleavages, which result in an increased background during Edman degradation. Nevertheless, we could confidently determine several N-terminal residues. The consensus sequence derived from five independent sequence determinations was: MFCLVCLSVS. To identify the amino acid residues in positions 3, 5, and 8, sequencing was repeated twice more following reduction-alkylation of the PVDF-immobilized material. A clear presence of cysteine residues was determined in positions 5 and 8; therefore, the N-terminal sequence of the identified polypeptide was: MFXLCLVCLSVS. This sequence
shows a very good match with the predicted COI sequence (MFWLCLLCLSLSV), except for tryptophan in position 3, which remained undetermined. It should be mentioned that determination of tryptophan is often problematic, especially when polypeptides are present in low amounts.

Attemps were undertaken to determine internal sequences of COI, but they were unsuccessful. We were able to isolate a sufficient amount of electroforetically homogeneous monomeric COI using two successive (9% followed by 14%) preparative gels. However, there was no detectable digestion of this polypeptide by trypsin under conditions when a control sample (subunit COIV) was completely digested by the enzyme (data not shown). A reason for this resistance is not clear, but it may be related to an increased amount of SDS bound to the polypeptide. We are currently attempting to utilize a chemical cleavage procedure.

It was also possible to determine nine N-terminal residues of component b. As could be expected for this aggregated component, the obtained sequence was heterogeneous. Nevertheless, the N-terminal sequence of COI (except for tryptophan and cysteines) was clearly discernable. Interestingly, the sequence of another mitochondrial subunit, COIII (MFVRVIIFV), could be tentatively recognized in the mixed sequence. These results confirmed that component b is an aggregate and suggested that it contains COIII in addition to COI. The residues derived from the N-terminal sequence of COI (MAFILSFWM) could not be detected, except for the first position methionine and the seventh position phenylalanine, which are shared with COIII. The monomeric forms of COII and COIII with the predicted sizes 24 and 34 kDa, respectively, should migrate ahead of the monomeric forms of COI, but they were unsuccessful. We were able to isolate a sufficient amount of electrophoretically homogeneous monomeric COI using two successive (9% followed by 14%) preparative gels. However, there was no detectable digestion of this polypeptide by trypsin under conditions when a control sample (subunit COIV) was completely digested by the enzyme (data not shown). A reason for this resistance is not clear, but it may be related to an increased amount of SDS bound to the polypeptide. We are currently attempting to utilize a chemical cleavage procedure.

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**DISCUSSION**

In kinetoplast mitochondria of trypanosomatids there is a unique system of RNA processing termed RNA editing. Most, but not all, mRNA species must be processed by editing to become translatable. Investigations of how the systems of editing and translation may interact with each other to produce functional products are of great interest; however, the very existence of a functional translation system could not be unequivocally demonstrated for a long time. The evidence for the kinetoplast translation should include isolation of the putative kinetoplast-encoded proteins from the corresponding molecular complexes and demonstration of their cognate structure. We have recently identified a kinetoplast-encoded protein, apocytochrome b in L. tarentolae, which is translated from an edited mRNA (17). The work presented here describes a protein, cytochrome c oxidase subunit I, translated from an mRNA that is not edited.

With the aim of identifying kinetoplast mitochondrial subunits, we have first analyzed the composition of cytochrome c oxidase directly purified from a kinetoplast lysate by a BN gel electrophoresis. All eleven subunits of the enzyme detected in denaturing gels by staining turned out to be nuclear encoded. A similar result was obtained previously for the enzyme from a closely related organism, C. fasciculata (7, 8). The larger subunits from both species form a very similar banding pattern and subunits 5–10, for which direct comparisons are possible, show nearly identical N termini. The mitochondrial encoded subunits COI, II, and III are not visible in the gels; however, a protein with the predicted molecular mass of COII was detected in C. fasciculata by mass spectrometry (14).

Given the catalytic role of the mitochondrial subunits in cytochrome c oxidase, their apparent absence needs to be explained. It was suggested (7, 8) that these subunits were lost by aggregation and precipitation upon dissociation of the complex in SDS. The mammalian COI and COIII are also prone to aggregation, although they still can be seen in stained gels (29). A similar explanation was also employed to address the apparent absence of the apocytochrome b among the gel-separated subunits of the bc1 complex in C. fasciculata (9).

Results presented in this study and the previous work (17) shed light on this problem. Using specific antibodies we have detected COI in a specific association with the cytochrome c oxidase complex in BN/Tricine-SDS two-dimensional gels. This subunit was observed forming multiple bands suggesting a possibility of aggregation or oligomerization. The detected polypeptides showed aberrant electrophoretic migration in SDS gels, indicating that their charge to mass ratio is different from other subunits, a property that can be explained by a high hydrophobicity leading to an increased binding of SDS. Ferguson plot estimates suggested that one of these polypeptides,
component c, represents a full-size monomeric COI. Then, based on the property of aberrant migration and using denaturing two-dimensional gels, we could separate the monomeric and aggregated forms of COI from the more abundant subunits, which showed a normal migration. Because the minor components were no longer obscured, this procedure also allowed us to visualize COI by staining when higher loads of oxidase were used. It should be noted, that isoelectric focusing could not be used to separate COI molecules, because they apparently require sufficient amounts SDS in order to remain in solution. Replacing SDS with a nonpolar detergent and urea probably leads to precipitation. The loss of COI seems to be complete, because no signal can be detected on the corresponding Western blots. After visualization of COI, the putative monomeric spot was blotted, and we could determine eleven of the twelve N-terminal residues. A match was found with the sequence predicted from the corresponding kinetoplast mRNA.

The COI is encoded by an mRNA, which does not require editing. To resolve the question regarding translation of edited templates, we have recently used a similar two-dimensional gel system for detection in the bc1 complex of the putative apocytochrome b, which is derived from an edited mRNA (17). The second subunit, COII, and the third subunit, COIII, still have to be found. It is likely that the high molecular weight aggregates, and component b in particular, contain not only COI, but COIII as well. With the availability of specific antibodies it will be possible to search for these subunits.

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Detection of the Mitochondrially Encoded Cytochrome cOxidase Subunit I in the Trypanosomatid Protozoan *Leishmania tarentolae*: EVIDENCE FOR TRANSLATION OF UNEDITED mRNA IN THE KINETOPLAST

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