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The formation of mitochondrial ribonucleoprotein complexes involving guide RNA molecules in *Trypanosoma brucei*

(kinetoplastid RNA editing/RNA–protein interaction)

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ABSTRACT **Transcripts from mitochondrial (maxicircle) genes of kinetoplastid organisms undergo RNA editing characterized by a series of reactions that insert and delete uridine nucleotides within the sequence of the pre-mRNAs. Guide RNAs, which complement fully edited mRNAs, provide the information for the edited sequence by an unknown mechanism. We report here that guide RNA molecules associate with other mitochondrial components to form four specific, stable ribonucleoprotein complexes. The complexes form very rapidly at a low monovalent cation concentration, and their formation is blocked by heparin or pretreatment of the mitochondrial lysate with SDS. ATP hydrolysis is not required but slightly stimulates complex association up to concentrations of 5 mM. The results are suggestive of a sequential assembly of the ribonucleoprotein complexes, and their possible involvement during the kinetoplastid RNA editing is discussed.**

The expression of several mitochondrial genes in the parasitic protozoa *Trypanosoma, Leishmania, Crithidia*, and perhaps all kinetoplastids requires a type of RNA processing termed kinetoplastid RNA (kRNA) editing (1). During editing, pre-messenger RNA molecules (pre-mRNAs) are converted into translatable messages by the insertion and deletion of a precise number of uridine nucleotides. The mechanism of kRNA editing and the mitochondrial machinery that catalyzes this process are not known to date. The only known component other than the pre-mRNA substrate that may participate in the editing reactions is a class of small, stable RNA molecules called guide RNAs (gRNAs) (2–4). These molecules are complementary to edited domains of the fully processed mRNAs and contain posttranscriptionally added poly(U) tails at their 3′ ends (5, 6). A current paradigm for the general chemical mechanism of editing requires formation of a gRNA–pre-mRNA hybrid and two subsequent transesterification reactions where the 3′-OH of the gRNAs terminal uridine serves as the initial nucleophile (7–10).

A nearly ubiquitous feature of RNA molecules involved in a variety of biological processes is their association with proteins to form functionally active, macromolecular ribonucleoprotein (RNP) complexes. Ribosomes, spliceosomes, and the apolipoprotein B (apoB) "editosome" are examples of such complexes. They consist of intricately structured RNA molecules associated with multiple different proteins. They interact with substrate mRNAs or pre-mRNAs and other cofactors when they function. The assembly of these machineries is a precisely controlled process involving RNA–RNA, RNA–protein, and protein–protein interactions (11–13). We wished to determine whether gRNA–protein complexes that may participate in the catalysis of kRNA editing can be identified in *T. brucei*.

Nondenaturing gel electrophoresis under conditions of low ionic strength has been used extensively to experimentally describe nucleic acid–protein association, including RNA–protein binding (14–16). Using this experimental approach, we demonstrate here that four different mitochondrial RNP complexes form upon addition of exogenous gRNA molecules to *T. brucei* mitochondrial extracts. The formation of the complexes depends on the input of mitochondrial lysate in a manner that correlates with theoretical binding curves. We describe the kinetics of complex formation, temperature and ATP requirements, and monovalent and divalent ion dependencies. Complex association can be inhibited by the addition of heparin or SDS. These data provide evidence for a gRNA–RNP complex that is specific for *T. brucei* mitochondria (17–19) and are suggestive of a sequential assembly of the complex.

MATERIALS AND METHODS

Cell Growth and Mitochondria Isolation. Procyclic *T. brucei* clone IsTaR1 stock EATRO 164, VAT 1.7a-1 (20) was grown as described (21). Cells were harvested at a cell density of 10^7 cells per ml and mechanically disrupted in a hypotonic buffer (1 mM Tris·HCl, pH 8/1 mM Na_2EDTA). Mitochondrial vesicles were isolated from the washed and DNase I-treated cell lysate by isopycnic centrifugation on preformed linear 20–35% (vol/vol) Percoll gradients as in ref. 22.

Mitochondrial RNA (mtRNA) Isolation and gRNA Capping. Mitochondrial vesicles were lysed by using an acidic phenol/$CHCl_3$ extraction in the presence of 4 M guanidinium isothiocyanate and 2% (wt/vol) sodium *N*-lauroylsarcosinate (23). After extraction, the RNA material (mtRNA) was precipitated, washed, and resuspended in 10 mM Tris·HCl, pH 7.5/1 mM Na_2EDTA. A standard capping reaction contained 15 μg of mtRNA, 5 units of guanylyltransferase, 40 units of RNasin, and 150 μCi of [α-^{32}P]GTP (3000 Ci/mmol; 1 Ci = 37 GBq) in 50 μl of capping buffer (50 mM Tris·HCl, pH 7.5/1.5 mM $MgCl_2$/6 mM KCl/2.5 mM dithiothreitol). After a 15-min incubation at 37°C the reaction was terminated by ethanol precipitation in the presence of 2 M NH_4OAc and 10 μg of linear polyacrylamide as a carrier. Labeled gRNAs were further purified by denaturing gel electrophoresis on 10% (wt/vol) polyacrylamide gels.

Construction and Synthesis of Synthetic gRNA. The gene for *T. brucei* gRNA gA6-14 (24) was constructed by self-assembly of five overlapping synthetic oligodeoxynucleotides, according to Reyes and Abelson (25), and cloned into plasmid pBS− (Stratagene). The construct was designed in such a way that T7 run-off transcription yielded RNA mol-

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Abbreviations: RNP, ribonucleoprotein; kRNA, kinetoplastid RNA; gRNA, guide RNA; mtRNA, mitochondrial RNA.

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ecules with 5–7 uridine nucleotides at the 3′ end and 10 additional vector-derived bases at the 5′ end. Transcripts were uniformly labeled with [α-^{32}P]UTP (800 Ci/mmol) according to standard procedures and purified by denaturing gel electrophoresis on 6% (wt/vol) polyacrylamide gels.

Preparation of Mitochondrial and Nuclear Extracts. Mitochondrial vesicles were concentrated by centrifugation and resuspended in 0.1 ml of 6 mM Hepes, pH 7.5/30 mM KCl/0.5 mM dithiothreitol. The lysis of the vesicles was done at 4°C with 0.2% (vol/vol) Nonidet P-40 in the presence of leupeptin at 1 μg/ml, 1% (wt/vol) phenylmethylsulfonyl fluoride, and bovine trypsin inhibitor at 10 μg/ml. The procedure was designed to generate reproducible preparations of mitochondrial extracts, although variations in the RNP-binding activity of different extracts prepared from separate isolates of mitochondrial vesicles were apparent. The protein concentration of the extracts was determined as in ref. 26 by using bovine serum albumin as a standard. ATP concentrations were measured by using a luciferin–luciferase light-emission system (27) and usually were <1 μM. A further reduction was achieved by depleting the extracts according to ref. 28. *T. brucei* nuclear extracts were prepared essentially as described (29) and stored at −70°C.

Gel-Retardation Assay. Radiolabeled gRNAs (1–3 × 10^4 cpm; ≈5–15 ng) were incubated with increasing amounts of mitochondrial lysate (0.1–25 mg of total protein) in 15 μl of binding buffer [6 mM Hepes, pH 7.5/30 mM KCl/2.1 mM $MgCl_2$/0.5 mM dithiothreitol/1.5 mM ATP/5 mM creatine phosphate/0.1 mM Na_2EDTA/yeast tRNA at 10 μg/ml/6% (vol/vol) glycerol]. Complex formation was allowed for 30 min at 27°C, and samples were immediately loaded onto a 4% (wt/vol) polyacrylamide gel (acrylamide/methylene bisacrylamide ratio 50:1 or up to 80:1) in 50 mM Tris·glycine, pH 8.8. Electrophoresis was done at 4°C at 5–10 V/cm. The gels were fixed in 10% (vol/vol) acetic acid/10% (vol/vol) methanol, dried, and autoradiographed. Nonsaturated autoradiograms were scanned for quantitation (Bio-Rad model 620 densitometer). In competition experiments, increasing amounts of competitor RNA were included in the binding reaction before addition of the mitochondrial extract.

RESULTS

Mitochondrial Lysates from *T. brucei* Form Four Stable gRNA-Binding Complexes. The total mitochondrial gRNA population was chosen as a target for the association of mitochondrial proteins and other possible mitochondrial components. The molecules have an average length of ≈60 nt and migrate below the tRNA fraction on a denaturing polyacrylamide gel (Fig. 1, lane a). They possess di- or triphosphate 5′ ends and thus can be capped using guanylyltransferase and [α-^{32}P]GTP (2, 3) (Fig. 1, lanes b and c). Purified ^{32}P-end-labeled gRNAs were incubated with mitochondrial lysates of procyclic-form mitochondria with yeast tRNA as a nonspecific competitor. The mixture was electrophoresed through a low-percentage, nondenaturing, polyacrylamide gel, and stable gRNA–protein complexes that migrated with lower electrophoretic mobility than unbound input gRNA were visualized by autoradiography (Fig. 1, lanes e and f). Four distinct complexes could be identified and were termed G1–G4 (fastest to slowest). Complexes G1 and G3 migrated within the gel matrix as tight, homogeneous bands in contrast to G2, which appeared as a heterogeneous smear of two or more bands. G4 did not even enter a 4% (wt/vol) polyacrylamide gel (acrylamide/methylene bisacrylamide ratio 80:1). Complex formation was specific both for the mitochondrial lysate and for input gRNA. A *T. brucei* procyclic, nuclear extract was unable to form the four complexes with added gRNA (Fig. 1, lanes g and h) and similarly, the incubation of a 56-nt, nonspecific RNA transcript with mitochondrial extract did not result in the characteristic binding pattern (Fig. 1, lane i). There was proportionately more G1, less heterogeneous G2, and less G3 and G4 with a uniformly labeled synthetic gRNA compared with heterogeneous total mitochondrial gRNA population (Fig. 1, lanes j and k). The formation of the G1 and G4 complexes was reproducible for all mitochondrial extracts tested. Variability in complex-forming activity of different lysates was only seen for G2 and G3, and in a few nonreproducible cases we saw an additional band between G3 and G4.

The complexes formed at low lysate concentrations (mitochondrial protein at 0.2–0.5 mg/ml). Both G1 and G4 formed at similar concentrations and at concentrations below which detectable G2 and G3 formed (Fig. 2*A*). This concentration-dependent order of appearance of the RNPs suggests that the G1 may be an intermediate in a pathway leading to assembly of the G2 and G3. This result could also be true of G4, but its location in the well indicates that at least some G4 signal may be due to trapping or aggregates. The most active mitochondrial extracts could assemble all ^{32}P-labeled gRNA

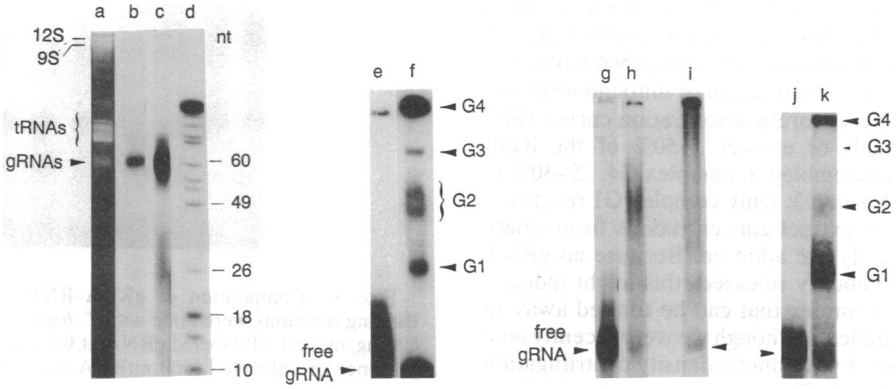

FIG. 1. Visualization of gRNA RNP complexes (arrowheads). (Lane a) Electrophoretic separation of *T. brucei* mtRNA (10 μg) on a 10% (wt/vol) denaturing polyacrylamide gel stained with ethidium bromide. The free gRNA population, as well as tRNA molecules and ribosomal RNAs, are marked accordingly. (Lane b) Separation of ^{32}P-labeled gRNAs after 5′-end-labeling with guanylyltransferase and [α-^{32}P]GTP. (Lane c) Identical separation as in lane b after prolonged exposure. (Lane d) RNA markers. (Lane e) Noncomplexed ^{32}P-labeled gRNAs separated on a native 4% polyacrylamide gel and visualized by autoradiography. (Lane f) Four RNPs, marked G1–G4, form with exogenous 5′-end-labeled gRNA upon addition of *T. brucei* mitochondrial extract. (Lane g) Same as lane e. (Lane h) Gel shift of ^{32}P-labeled gRNAs with *T. brucei* nuclear extract. (Lane i) Gel shift of a 56-nt nonspecific RNA transcript synthesized by T7 run-off transcription of pBluescript DNA (Stratagene) with *T. brucei* mitochondrial lysate. (Lanes j and k) Gel shifts of synthetic uniformly labeled *T. brucei* gRNA gA6–14 (22) in the absence (lane j) and presence (lane k) of *T. brucei* mitochondrial lysate.

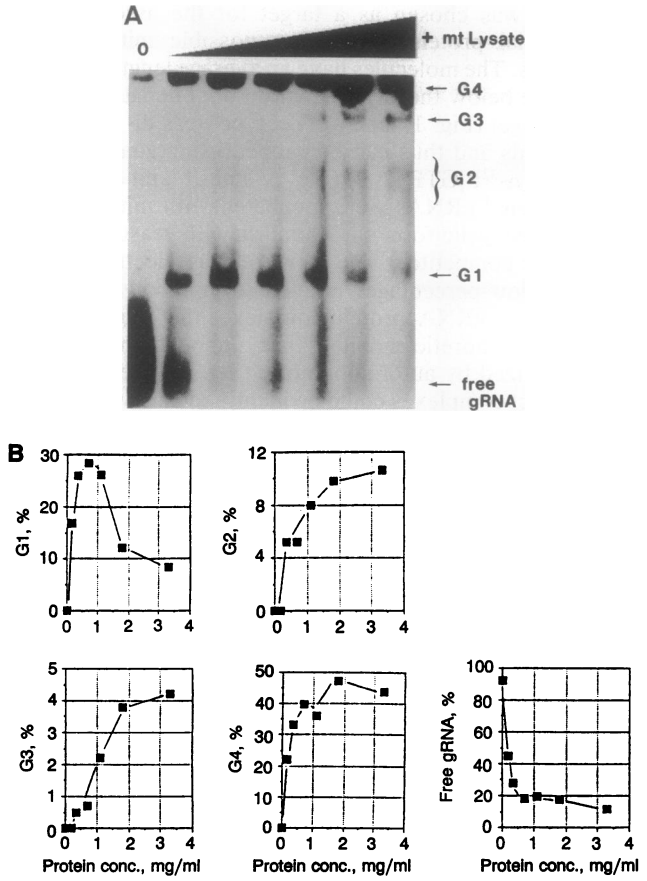

FIG. 2. gRNA–RNP complex binding curves. Optimal binding conditions for gRNA–RNP complex formation were determined by using a fixed amount of ^{32}P-labeled gRNA (2×10^4 cpm, 0.7 μg/ml) and increased amounts of mitochondrial extract. (*A*) Autoradiogram of a mitochondrial extract titration experiment using total mitochondrial protein at 0.18–3.3 mg/ml (left to right). The specific RNP complexes (G1–G4) as well as unbound ^{32}P-labeled gRNA are marked by arrows. (*B*) Graphic representation of densitometric analysis of the autoradiogram with results expressed as percentage of total signal in all complexes present in each complex; the percentage of free gRNA probe at each mitochondrial-extract concentration is also shown.

molecules into complexes, indicating that under these conditions the reaction was limiting for input gRNA. In most cases ≈5–10% of input gRNA remained uncomplexed, and under these conditions the amount of bound gRNA molecules increased with increased concentration of mitochondrial extract in a manner that fits theoretical saturation curves (Fig. 2*B*). At saturation levels of extract, ≈50% of the input ^{32}P-labeled gRNA was assembled in complex G4, 25–30% in G1, 10% in G2, and <5% in G3. Only complex G1 reached a saturation level at lower extract concentrations from where it declined upon further lysate addition. Because no gRNA molecules were concomitantly released, this might indicate that G1 is a precursor complex that can be titrated away to form a higher-order complex. Although we were recently able to resolve the complexes by isokinetic density centrifugation on glycerol gradients (H. H. Shu, personal communication), we could not resolve them on sucrose gradients. Either their kinetic stability was too low or more likely they dissociated at a high rate under these conditions. Gel-mobility-shift assays are not sensitive to these phenomena because diffusion effects are lowered due to the "caging effect" of the gel matrix, thus favoring reassociation (30, 31).

The Specificity of the gRNA–RNP Complex Formation. The specificity of the gRNA–RNP complex formation was tested in competition experiments by using homologous and heterologous RNA competitors. A 2.5- to 5.0-fold molar excess of nonradioactive preparations of *T. brucei* gRNA molecules or total mtRNA (≈50- to 100-fold excess on a mass basis) could completely abolish the signals for all four complexes (Fig. 3*A*). In contrast, poly(A) (Fig. 3*B*) and yeast tRNAs (data not shown) had no effect on the formation of G1 and G4, even at RNA concentrations up to 1.0 mg/ml (≈10^3-fold molar excess). Complexes G2 and G3 behaved similarly up to ≈100-fold molar excess (0.1 mg/ml) of either of the two competitor RNAs but declined significantly at higher concentrations, which may imply nonspecific interference with RNA–RNA interactions.

All four complexes were absolutely sensitive to heparin addition (2.5 mg/ml) after the initial incubation, a method commonly used to quench nonspecific binding (15) (data not shown).

Requirements for Complex Formation and Thermal Stability. Our initial salt conditions for complex formation contained 30 mM K^+ and 2 mM Mg^{2+}. Because mono- and divalent cations can have a pronounced effect on the assembly and function of RNP particles (32), we were interested

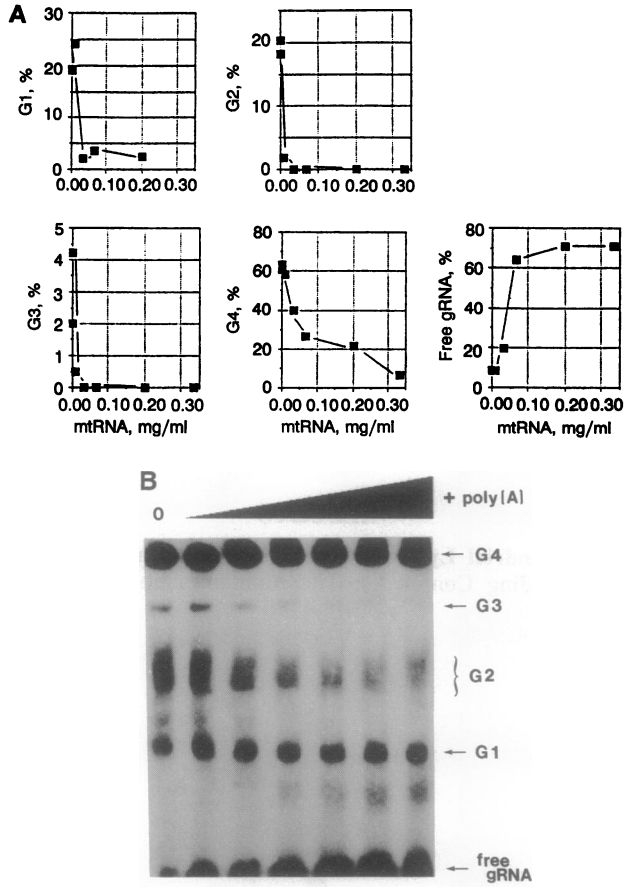

FIG. 3. Competition of gRNA–RNP complex formation. (*A*) Binding reactions were done with *T. brucei* mitochondrial protein at 0.9 mg/ml and ^{32}P-labeled gRNA at 0.7 μg/ml (2×10^4 cpm) in the presence of unlabeled total mtRNA (0–0.33 mg/ml). The different graphs (G1–G4) show the percentages of complex formation as a function of increased amounts of competitor RNA. Identical curves were obtained with purified unlabeled *T. brucei* gRNA. (*B*) Gel-mobility-shift analysis to test the ability of unlabeled poly(A) as a nonspecific competitor. Binding reactions were done with *T. brucei* mitochondrial extract at 0.7 mg/ml and ^{32}P-labeled gRNA at 0.5 μg/ml (1.6×10^4 cpm) in the presence of poly(A) at 0–0.7 μg/ml (0–1300-fold molar excess) (left to right). The specific RNP complexes (G1–G4), as well as unbound ^{32}P-labeled gRNA, are marked by arrows.

whether association of the four different gRNA–RNP complexes would depend on the presence of these ions. Complex formation was independently monitored at $MgCl_2$ concentrations from 0 to 50 mM and KCl concentrations from 0 to 150 mM. The mitochondrial extracts used for this series of experiments were prepared without KCl. In both cases, even at zero (additional) cation concentration we found complex formation. Thus, complex assembly is either independent of Mg^{2+} or K^+ or, more likely, the concentrations of these cations in the mitochondrial lysates were already sufficient to promote an initial complex assembly. The addition of KCl up to a concentration of 5 mM gave a small, but significant, stimulation of complex formation, which remained unchanged up to 30 mM. Higher concentrations resulted in a reduction and finally >50% inhibition of formation of all four complexes at 150 mM KCl (data not shown). The Mg^{2+} dependency for the four RNPs varied in its sensitivity. Formation of G1 was sensitive to Mg^{2+}; concentrations up to 5 mM Mg^{2+} reduced its signal to ≈50% of the initial value, but higher concentrations did not completely diminish its formation. Formation of G4 was initially stimulated by 20% at 2.5 mM Mg^{2+}, but higher concentrations reduced the signal drastically, although also not to zero. Formation of both G2 and G3 was stimulated by Mg^{2+} up to ≈25 mM for G2 and 15 mM for G3 (data not shown).

All four complexes formed with mitochondrial lysates essentially depleted of ATP. Addition of exogenous ATP to the reaction mixture slightly stimulated complex formation (1.5-fold) at concentrations from 0.5 to 3.0 mM. An increase up to 5 mM ATP showed no further stimulus (data not shown).

The four RNP particles presumably are the result of specific gRNA–protein, secondary protein–protein, and gRNA–mRNA interactions. In line with this assumption, pretreatment of the mitochondrial extract with 0.2% (wt/vol) SDS essentially eliminated all complexes (Fig. 4*D*). Digestion of the lysate with proteinase K (50 μg/ml for 45 min) also interfered with assembly of the RNP particles (Fig. 4*B*). Only a resistant "core" complex with an electrophoretic mobility slightly faster than complex G1 could form. This core complex, however, was sensitive to a preincubation of the mitochondrial extract with micrococcal nuclease (85 units/ml for 20 min at room temperature) (Fig. 4*C*).

Complex formation at temperatures up to 75°C was examined. An incubation temperature of 27°C was initially chosen because this is the optimal temperature for procyclic forms when growing in the insect (Tsetse fly) midgut. The formation of G1 and G3 was stimulated by ≈30% at 37°C or 42°C. In contrast, formation of G2 was reduced at all temperatures >27°C. At 55°C, complex assembly was totally abolished for G2 and G3, and complex G1 showed a slightly increased electrophoretic mobility, identical to the result of the abovementioned proteinase K experiment (Fig. 4*A*). None of the complexes were detected at 65°C or higher. The characteristics of G4 were difficult to quantitate in this set of experiments; the inability of G4 to even enter a low-percentage polyacrylamide gel interfered with the appearance of aggregates and other denaturation products on top of the gels.

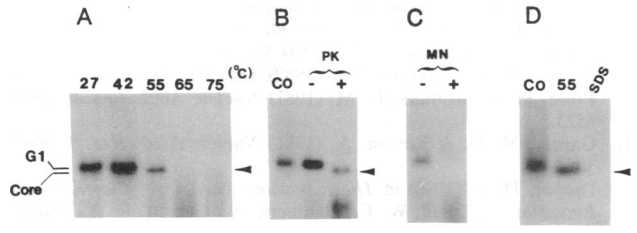

FIG. 4. Effect of various treatments on G1 complex formation. Effects of temperature (*A*), proteinase K (PK) (*B*), micrococcal nuclease (MN) (*C*), and SDS treatment (*D*). Note that the core complex (arrowhead) with a slightly greater electrophoretic mobility than complex G1 appears upon treatment at 55°C and with proteinase K but is sensitive to micrococcal nuclease digestion. CO, control sample treated under standard binding conditions (^{32}P-labeled gRNA input RNA at 0.5–0.7 μg/ml and mitochondrial protein at 0.5–0.9 mg/ml).

Kinetics of Complex Formation. To analyze the time dependence of complex assembly, ^{32}P-labeled gRNA was incubated with mitochondrial lysate under standard conditions for various times, and aliquots were tested for the four different RNP particles. Complexes G1 and G4 assembled very rapidly. Within the first 2 min of incubation, both complexes reached maximal levels, and no significant additional accumulation was visible on further incubation (Fig. 5). This independent behavior of the extent of complex assembly from the reaction time was seen at very low lysate concentrations, and thereby the reaction was judged to be at equilibrium. Longer incubation times decreased both signals by ≈10% (G4) to 30% (G1), indicative of an instability at prolonged reaction times. Complexes G2 and G3 could also be detected early; however, both complexes continued to accumulate, reaching maximum at 20 min (G2) or 30 min (G3), respectively. From that point they declined, although no concomitant increase in either of the two other complexes appeared. Therefore, the decrease must result from complex disassembly rather than from conversion into a different RNP complex.

DISCUSSION

We have shown that mitochondrial extracts from *T. brucei* bind exogenously added gRNAs and form four distinct, stable RNP complexes. The complexes are specific for gRNAs because homologous, but not heterologous, RNA blocks their formation. Complex assembly occurs at low monovalent cation concentration without concomitant ATP hydrolysis. The association reaction is extremely rapid (within 1–2 min at 27°C), similar to the assembly of the editosome complex of the apolipoprotein B system (33), implying high concentrations of reactants or, more likely, avid interactions. The lower proportion of the G2–G3 complexes that form with homogeneous synthetic gRNA compared with total gRNA may be from a larger pool of endogenous components, including mRNAs and proteins, that can react with the heterogeneous gRNAs. The greater diversity of G2 complex with the heterogeneous gRNAs may reflect different gRNA

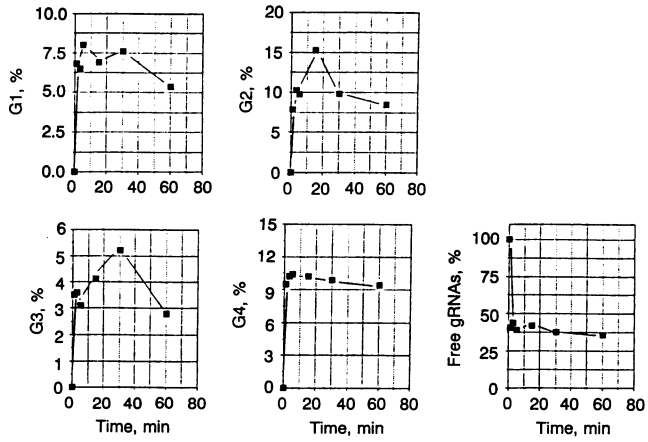

FIG. 5. Kinetic of complex formation. Binding reactions were done with *T. brucei* mitochondrial protein at 0.9 mg/ml and ^{32}P-labeled gRNA at 0.7 μg/ml (2×10^4 cpm). Incubation was done at 27°C for 1 min up to 60 min. Complexes were separated on native polyacrylamide gels and quantitated by densitometry.

secondary structures and/or higher order structures of the RNP complexes.

Several lines of evidence suggest that the binding partners are mitochondrial proteins and RNA. Complex formation is eliminated by SDS, incubation at 65°C, and pretreatment of the mitochondrial extract with proteinase K. A temperature (55°C) resistant core complex is resistant to proteinase K digestion but is sensitive to micrococcal nuclease or heating at 65°C. This core may simply be the gRNA–mRNA duplex. Its minimal difference in electrophoretic mobility in native polyacrylamide gels from that of complex G1 suggests that the molecular composition of these two complexes is not very different. Thus, G1 may have one or a few proteins associated with the gRNA or the core gRNA–mRNA. G2–G4 may be the result of binding of additional proteins and possibly RNAs other than gRNA and mRNA.

The molecular complexity of the four RNP complexes cannot be unequivocally deduced from their electrophoretic behavior. However, it is tempting to interpret the decrease in electrophoretic mobility from G1 to G4 as an increase in molecular complexity, perhaps analogous to spliceosome or ribosome assembly. A scenario can be envisioned wherein a specific association occurs between RNA and one or more proteins to initially form G1, and subsequent binding of additional components would form G2, G3, and G4, although not necessarily in that order. The rapidly saturatable formation of G1 at low concentrations of mitochondrial extract and diminishment in the amount of this complex at higher extract concentrations is consistent with it being an early assembly precursor; additional studies are needed to test this hypothesis. We cannot rule out the possibility that G4 represents nonspecific associations, trapped material, or aggregate complexes that enter the gel. However, the competition data imply that some, but not all, signal in G4 is the result of specific associations.

The dissociation of all four RNP complexes in the presence of the polyanion heparin indicates a major contribution of electrostatic interactions to the binding between the protein components and the negatively charged gRNAs and pre-mRNA molecules. The assembly of an identical macromolecular complex from a large set of different gRNA sequences and various pre-mRNAs might be achieved by a defined number of ionic contacts where a three-dimensional array of positive charges on the proteins matches the conformation and arrangement of negative charges on the gRNA and/or mRNA phosphate backbones. The specificity could, in part, be based on the surface recognition of a specific secondary-structure motif, other forms of sequence-independent interactions (e.g., hydrophobic) (34, 35) or conserved features of gRNA, such as the 3′ uridine tail, 5′ di- or triphosphate, or conserved sequence motifs [e.g., RYAYA (6)].

Complex assembly at ATP concentration below 1 μM implies a lack of ATP dependence or the use of sequestured ATP. The 1.5-fold stimulation of complex formation by ATP addition might reflect some energy-requiring steps that may involve refolding or protein phosphorylation. We cannot exclude the possibility that additional proteins and RNA components are also present but left undetected in this type of analysis. Furthermore, it is important to emphasize that we have no experimental evidence to connect the gRNA–RNP particles directly to the kRNA editing process. However, the only known biochemical function of the mitochondrial gRNA moiety is its participation in this process. This makes it very plausible to suggest that gRNAs function in association with mitochondrial proteins and that perhaps the whole editing process is catalyzed by a multicomponent RNP complex. Such a complex, called the editosome (17–19), may have several functions vital to kRNA editing. These functions include spatial alignment of RNA reactants in the catalytic core, retention of the 5′-mRNA portion released by the first transesterification step, and controlling the second transesterification that religates the mRNA.

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