THE MECHANISM OF FORMATION OF INHIBITOR-INDUCED RIBOSOME HELICES IN *ENTAMOEBA INVADENS*

THANIT KUSAMRARN, PRASERT SOBHON, and
GORDON B. BAILEY

From the Department of Biochemistry, Faculty of Science, Mahidol University, Bangkok, Thailand. Dr. Bailey's present address is St. Louis University/Chiang Mai University Anemia and Malnutrition Research Center, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand.

ABSTRACT

Helices and aggregates of helices (chromatoid bodies) composed of ribosome-like particles appear in cysts and slow-growing trophozoites of *Entamoeba invadens*. We found that similar helix aggregates were formed abundantly in actively growing *E. invadens* trophozoites treated with a variety of direct or indirect inhibitors of protein synthesis. The inhibitor-induced helices appeared cytochemically and ultrastructurally identical to those seen in cysts.

Numerous single helices and small arrays occurred randomly distributed throughout the trophozoite cytoplasm within 15 min after treatment with NaF, which rapidly and completely stopped all nucleic acid and protein synthesis. Cycloheximide (CH), which inhibited protein synthesis as effectively as NaF, stimulated aggregate formation more slowly, and only after a delay of 30–60 min. CH temporarily blocked NaF-stimulated aggregate formation. Aggregation was slowest with actinomycin-D, which strongly inhibited RNA synthesis but depressed protein synthesis only slowly. These results suggested that release of ribosomes from mRNA was required for aggregation.

Inhibition by CH was reversible, and aggregates disappeared from CH-treated amebas shortly after they were transferred to inhibitor-free growth medium. There was no evidence that helices assembled about a structural organizer within the cell or that the process involved metabolic activity. It was concluded that the inhibitor-induced helices were composed of mature, normally functional ribosomes and that helix formation was a spontaneous and reversible consequence of the accumulation within the cell of free monosomes (or subunits) which were prevented from binding to mRNA.

Considerable evidence has accumulated which indicates that the aggregated ribonucleoprotein helices (chromatoid bodies) found in cysts of *Entamoeba* are composed of ribosomal particles (18, 14, 15). There are, however, uncertainties about the precise structural and functional nature of the unit particles within the helices and about the total composition of the arrays of helices.

The ultrastructure of chromatoid bodies has been scrutinized in some detail by electron microscopy and by optical diffraction analysis of the images in electron micrographs. The diffraction studies of Lake and Slayter (15) indicated that the basic helix assembly unit contained two subunits with dimensions very similar to those of the large and small ribosome particles of eucaryotic orga-

nisms. However, Barker and Swales (3) reported that the sedimentation constants of monosomes and ribosome subunits from helix-containing cysts were slightly greater than those of the comparable particles from trophozoites, and that cyst rRNA was of higher molecular weight than trophozoite rRNA. They suggested that ribosomes in helices may represent immature precursor particles synthesized during encystation.

There have been several reports that chromatoid bodies contain extraribosomal components. In addition to the large and small ribosome subunits, the diffraction analyses of Lake and Slayter (15) suggested the existence of a third particle, approximately one-third the size of the small ribosome subunit. Other authors have postulated the existence of a core material within the helices (19), of microfilamentous organizers of helix assembly (12), as well as the localization of cellular zinc (17), a tryptophan-rich protein (8), and a tubulin-like protein (11) in the aggregated structure. However, the evidence for all of these extraribosomal components is indirect, and the existence of none has been confirmed.

Single helices and helix aggregates are often observed in trophozoites of *Entamoeba*. They have ultrastructural and diffraction properties identical to those of the arrays in cysts (4). We have noted in the literature and in our own studies a correspondence between the appearance of aggregates in trophozoites and periods of reduced metabolic activity. For example, in one report (1) the number of aggregates was seen to increase with culture age, and in another (22), slow-growing trophozoites were found to contain more helical arrays than rapidly growing cells. In our own work we have observed that particles with the staining properties of chromatoid bodies frequently appear in trophozoites which have entered stationary growth phase and disappear when the amebas are transferred to fresh growth medium. Of course, in cysts, which probably represent the metabolic ebb in the *Entamoeba* life cycle, virtually all the ribosomes are aggregated (2, 13).

These observations suggested a simple mechanism to explain ribosome aggregation in *Entamoeba*, namely that mature, polysome-bound ribosomes, when rendered free and inactive by a reduction in protein synthesis, interact spontaneously to form helices and arrays of helices. Although not wholly consistent with other proposals to explain the structure and function of helices, this hypothesis could be tested easily, since it predicted that inhibitors of protein synthesis which caused the accumulation of free ribosomes should stimulate the formation of helices in actively growing trophozoites. This prediction was confirmed as described in this report.[1] Further, we describe studies of the mechanism of inhibitor-stimulated ribosome aggregation in *E. invadens*. In the following paper we compare some of the physical and biological properties of ribosomes derived from polysomes and from helices in this organism.

MATERIALS AND METHODS

Cultures

Entamoeba invadens, strain IP-1, was used for these studies. Trophozoites were cultured in Diamond's Trypticase Panmede Serum (TPS) axenic growth medium (10) by techniques described previously (24). Encystation was stimulated by the procedure of Rengpien and Bailey (21). Unless otherwise specified, isotopes and inhibitors used were added during the preparation of media.

Visualization of Ribosome Aggregates

LIGHT MICROSCOPY: Ribosome aggregates were revealed by staining with toluidine blue after glutaraldehyde fixation. Cells were collected by centrifugation at 500 *g* for 5 min and resuspended in a small volume of the supernatant culture medium. They were fixed for 10 min at room temperature with an equal volume of 5% glutaraldehyde in 10 mM potassium phosphate, pH 7.0, plus 0.3 M NaCl. The fixed cells were collected by centrifugation, washed twice with 25% aqueous ethanol, and resuspended in a small volume of the same solvent. A drop of suspended cells was dried on a microscope slide at 50°C, then stained for 90 s with 0.03% toluidine blue in 25% ethanol. Excess stain was removed by soaking in 95% ethanol until the ribosome aggregates could be observed clearly. Before viewing, the stained cells were air dried and covered with xylene.

ELECTRON MICROSCOPY: Amebas were collected and resuspended in culture medium as described above. They were then fixed for 30 min at room temperature with an equal volume of 5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.0, and postfixed for 10 min in Dalton's chrome-osmium mixture (9). After dehydration the cells were embedded in Araldite, sectioned, and stained with uranyl acetate for 1 h followed by lead citrate for 10 min. Sections were viewed in a Hitachi model 11-C electron microscope.

RNase treatment

Unstained slides, smeared with glutaraldehyde-fixed cells, were incubated in an aqueous solution of bovine

[1] A preliminary report of this observation has appeared in Bailey, G. B., T. Kusamrarn, and W. P. Callahan. 1972. *Fed. Proc.* **31**:236.

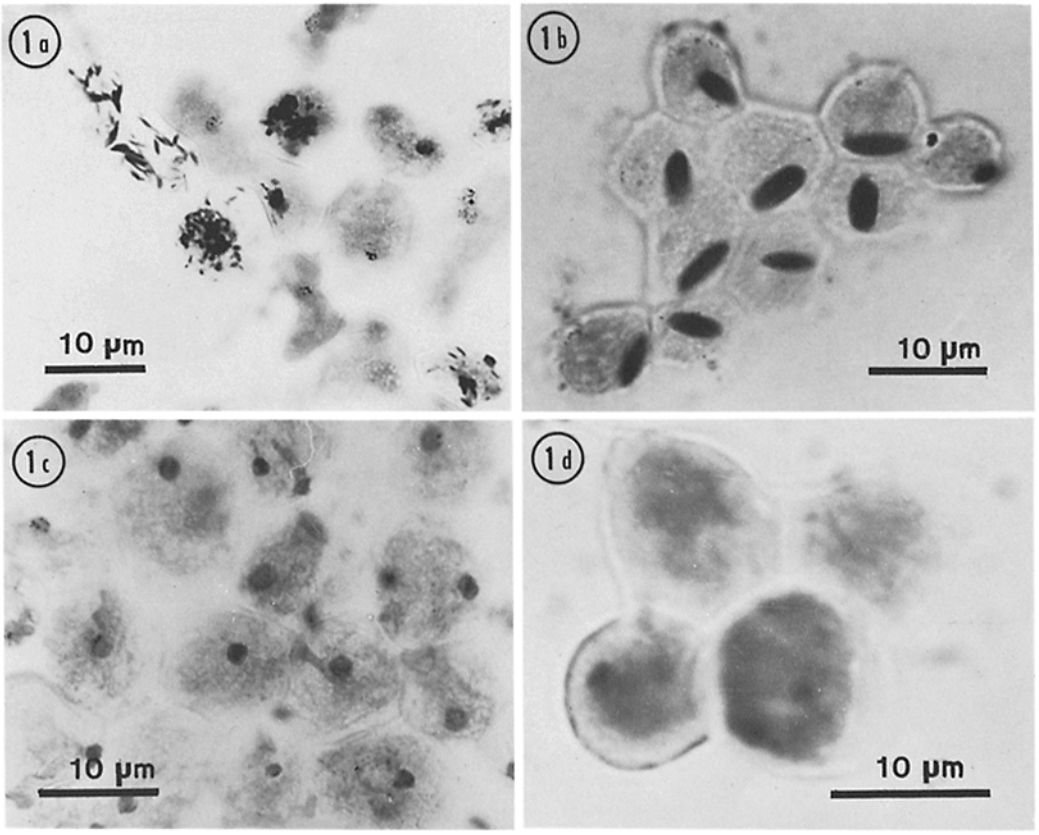


FIGURE 1 The appearance of aggregates in *E. invadens* trophozoites treated for 3 h with CH (*a*), and of the naturally occurring ribosomal aggregate (chromatoid body) in *E. invadens* IP-1 cysts (*b*). The appearance of CH-treated trophozoites (*c*) and of cysts (*d*) after treatment with RNase (1 mg/ml, 4 h). The trophozoites in (*c*) were stained more intensely than those in (*a*) to reveal the nuclei, which were not affected by RNase. (*a*) × 1,300; (*b*), (*c*) × 1,500; (*d*) × 2,000.

pancreatic RNase (1 mg/ml) (Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N.Y.) for 4 h at 37°C. Control slides were incubated in water.

Radioisotope Incorporation

In vivo incorporation of L-[^{3}H]leucine and [^{32}P]inorganic phosphate by trophozoites was measured in encystation medium, which contained lower concentrations of nonradioactive leucine and phosphate than TSP growth medium. Growth-phase trophozoites were collected by centrifugation, washed once, and then resuspended in encystation medium at a concentration of approximately 2×10^6 amebas/ml. 0.5-ml aliquots were pipetted into tubes containing an equal volume of medium plus L-[^{3}H]leucine (5 μCi/ml) (New England Nuclear, Boston, Mass.) or [^{32}P]phosphate (3 μCi/ml) (Atomic Energy Commission of Thailand) and inhibitor where appropriate. Incubation was carried out at 25°C. Incorporation was stopped by addition of 2 ml of cold 0.9% NaCl containing 3 mg/ml nonradioactive precursor. The cells were washed once with this solution and then lysed with 0.5 ml of 0.1% sodium dodecyl sulfate. After 30 min in ice, macromolecules were precipitated with 1 ml of cold 10% trichloroacetic acid (TCA)[2] and collected on Whatman GF/A filters. The filters were washed with cold 5% TCA, dried, and counted in toluene-based scintillation fluid.

RESULTS

Preliminary Observations

TPS medium supports only vegetative growth of *E. invadens* IP-1 (24). With an inoculum size of 2×10^4 amebas/ml, trophozoite growth reached stationary phase in approximately 1 wk. During the period of most rapid multiplication (2nd to 5th

[2] *Abbreviations used in this paper:* ACT-D, actinomycin-D; CH, cycloheximide; TCA, trichloroacetic acid.

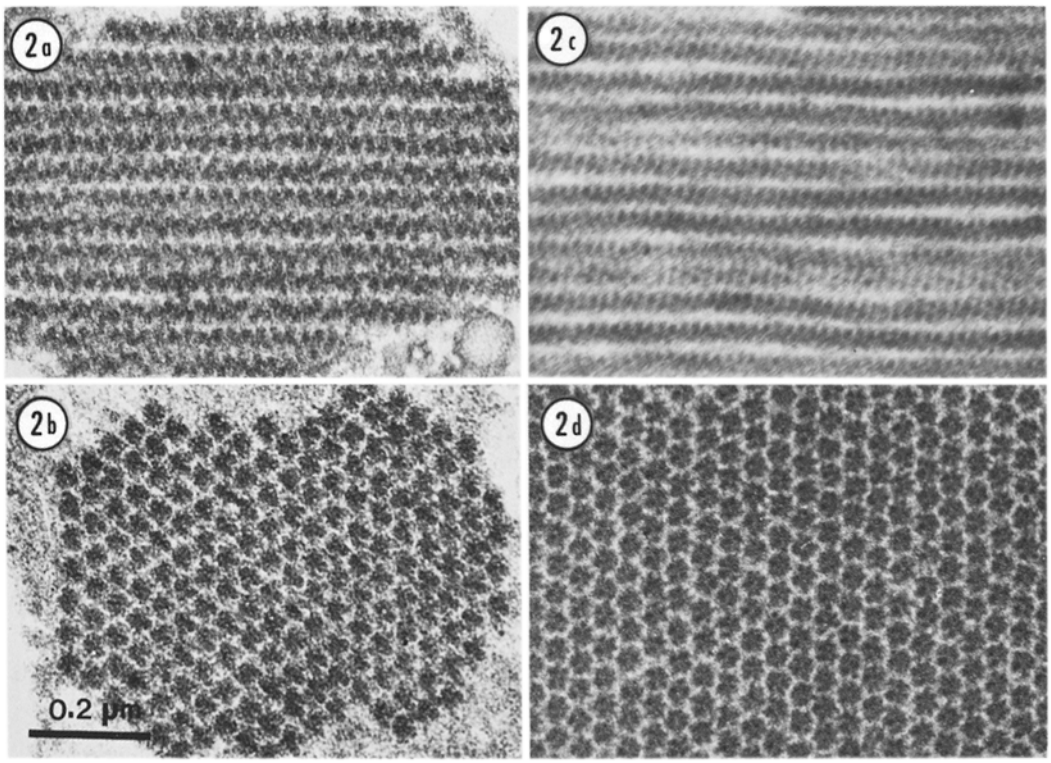


FIGURE 2 Longitudinal and cross-sectional views of portions of aggregated helices in CH-treated trophozoites (*a*, *b*) and in cysts (*c*, *d*) of *E. invadens* IP-1. Aggregates from both sources had an estimated interaxial distance between helices of 450 Å and a helix turn interval of approximately 200 Å. × 82,000.

day) cells containing aggregates were rarely visible. However, a day or more after the amebas ceased dividing, many of them contained numerous small arrays.

When growing trophozoites were transferred to encystation medium, mass encystation occurred within 24–48 h. Small, randomly distributed arrays appeared within these cells as cyst wall synthesis was being completed. Nearly every completed cyst contained a large single chromatoid body (Fig. 1 *b*).

Incorporation of exogenous amino acids could not be detected in either stationary-phase trophozoites or cysts, suggesting, as would be suspected, that protein synthesis was greatly reduced or had ceased at these stages.

These observations led us to propose that aggregate formation may be related to the cessation of protein synthesis and may involve inactive potentially functional ribosomes. The proposal was tested by treating actively growing trophozoites with inhibitors which directly or indirectly block protein synthesis.

Inhibitor-Stimulated Ribosome Aggregation

The following inhibitors were added separately to growth-phase trophozoite cultures for a test period of 3 h: cycloheximide (CH), 100 μg/ml; NaF, 50 mM; puromycin, 50 μg/ml; actinomycin-D (ACT-D), 100 μg/ml; and the clinical amebicides emetine, 100 μg/ml, and metranidazole (Flagyl, May and Baker, Ltd., Dagenham, England), 10 μg/ml. Each of these inhibitors stimulated the appearance of abundant stainable particles in the cells, although the response was weaker with puromycin and occurred in the fewest cells with ACT-D. We established in a separate experiment that the inhibitors which induced massive aggregate formation considerably depressed [^{3}H]leucine incorporation in the same time period. Moreover, when trophozoites were cooled by the technique used to stimulate ribosome crystallization in chicken embryos (5), profuse aggregation also took place. Although not checked, presum-

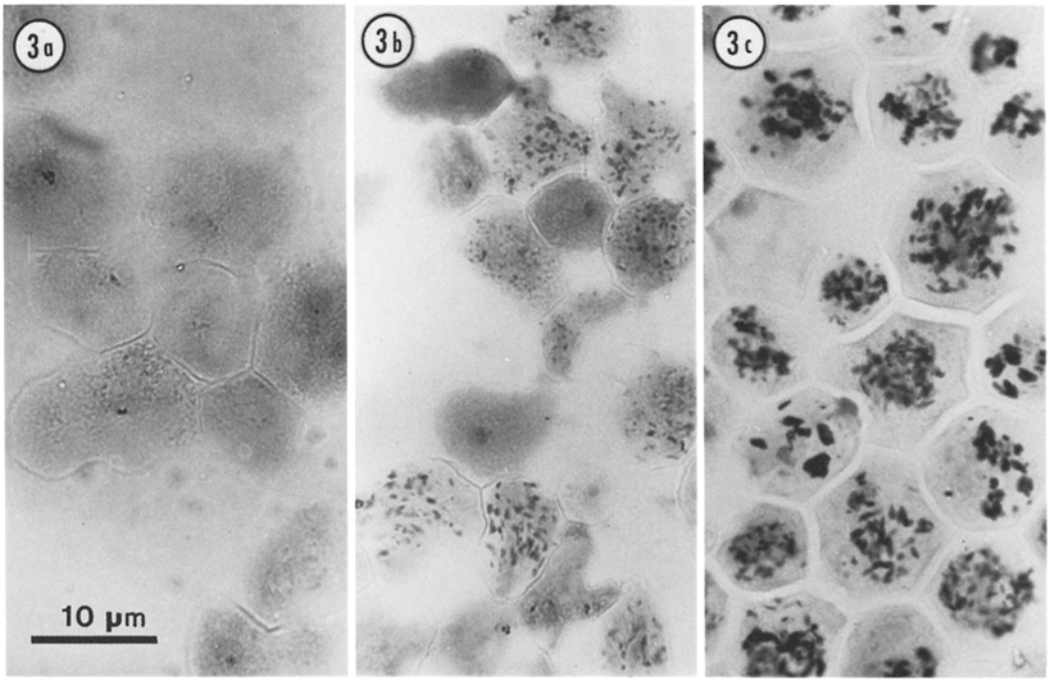


FIGURE 3 Light micrographs showing the time course of aggregate formation in *E. invadens* IP-1 trophozoites treated with 50 mM NAF. The inhibitor was added to a culture of growing trophozoites. Samples were taken at 0 (*a*), 15 (*b*), and 120 (*c*) min, fixed in glutaraldehyde, and stained with toluidine blue. Cells were destained sufficiently to bleach the nuclei. × 1,500.

ably macromolecular synthesis ceased in the chilled amebas, as it does in the chicken (20).

The typical appearance of inhibitor-treated trophozoites is shown in Fig. 1 *a*. It typifies, as well, the appearance of aggregates in cooled or stationary-phase trophozoites. Cysts with prominent chromatoid bodies are shown in Fig. 1 *b* for comparison. It was estimated that in many trophozoites the total volume of the accumulated aggregates after extended inhibitor treatment was equal to or greater than the size of the single-cyst chromatoid body. Arrays in inhibitor-treated trophozoites usually remained fragmented and distributed throughout the cell. However, in several experiments in which amebas were treated with a lower concentration of CH (10 μg/ml) for as long as 7 days, many cells ultimately contained a single aggregate similar in gross shape and size to the cyst chromatoid body.

RNase treatment (Fig. 1 *c*, *d*) destroyed the staining properties of the aggregates both in inhibited trophozoites and in cysts, an indication that the particles in the vegetative cells were of the same ribonucleoprotein composition as cyst chromatoids.

Electron micrographs (Fig. 2) revealed the close ultrastructural similarity of inhibitor-induced arrays to those in cysts. When viewed at the same magnification, no differences in helix diameter, interhelix radius, or helix turn period could be detected. Comparison of arrays seen in longitudinal and cross sections showed the ordering of helices to be the same in both trophozoites and cysts. There was no evidence that helices formed at specific sites in the cell or about microfilaments, as has been suggested by others (22, 12, 19).

Light and Electron Microscope Observation of the Course of Formation of Helix Aggregates in Inhibitor-Treated Trophozoites

Typical light and electron micrographs of growth-phase trophozoites before treatment are shown in Figs. 3 *a* and 4 *a*. Light staining by toluidine blue was seen throughout the cells in light micrographs. Nuclei were vague and no aggregates could be seen. Aggregates were also not detected in the electron micrographs, although in some cells single helices were observed.

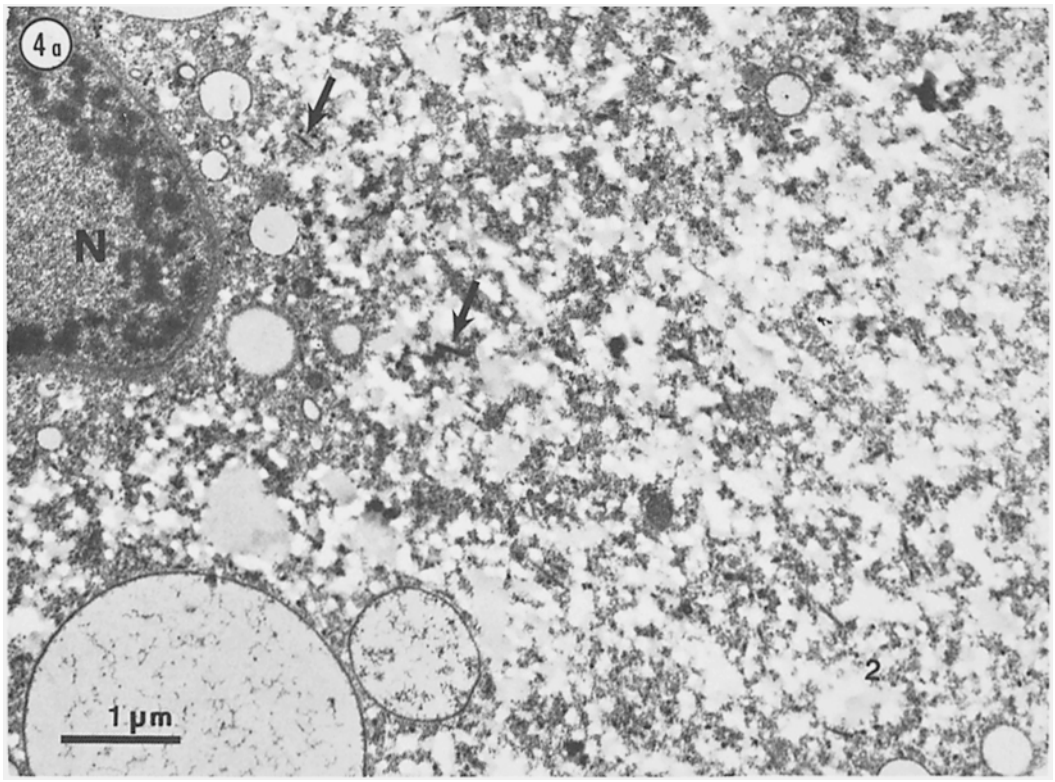


FIGURE 4 Electron micrographs of the time course of aggregate formation in the cells shown in Fig. 4. (*a*) 0 min. A few short, single helices are visible (arrows). A portion of the trophozoite nucleus can also be seen (*N*). (*b*) 15 min; (*c*) 120 min. (*a*) × 16,500; (*b*) × 15,400; (*c*) × 17,400.

After treatment with NaF for 15 min, the first small particles could be detected by light microscopy (Fig. 3 *b*). The diffuse staining of the cytoplasm by toluidine blue was less evident in cells containing particles than in trophozoites in which aggregation had not yet occurred. This suggested coalescence of the diffuse material into the structure of the aggregates. Electron micrographs of these cells (Fig. 4 *b*) revealed many free helices and the beginning of arrays of helices distributed randomly throughout the cytoplasm of nearly all cells. The arrays became fewer but larger, and free helices were less evident after longer periods of treatment (Figs. 3 *c* and 4 *c*).

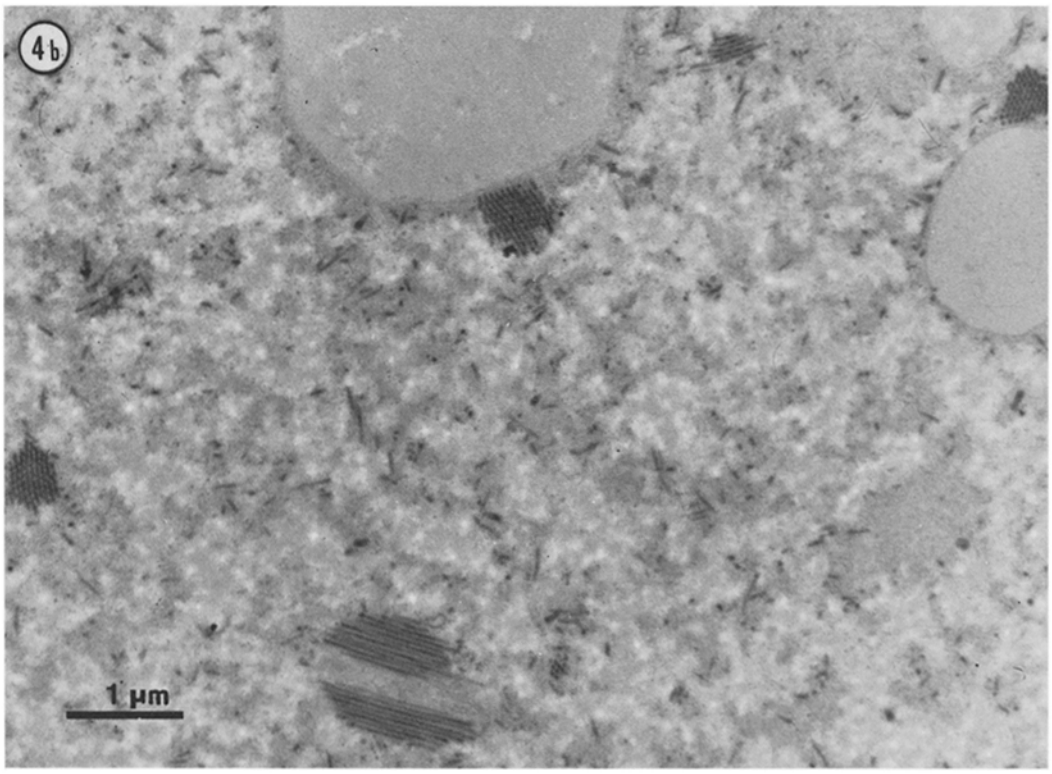
The Absence of Macromolecular Synthesis during NaF-Stimulated Aggregation

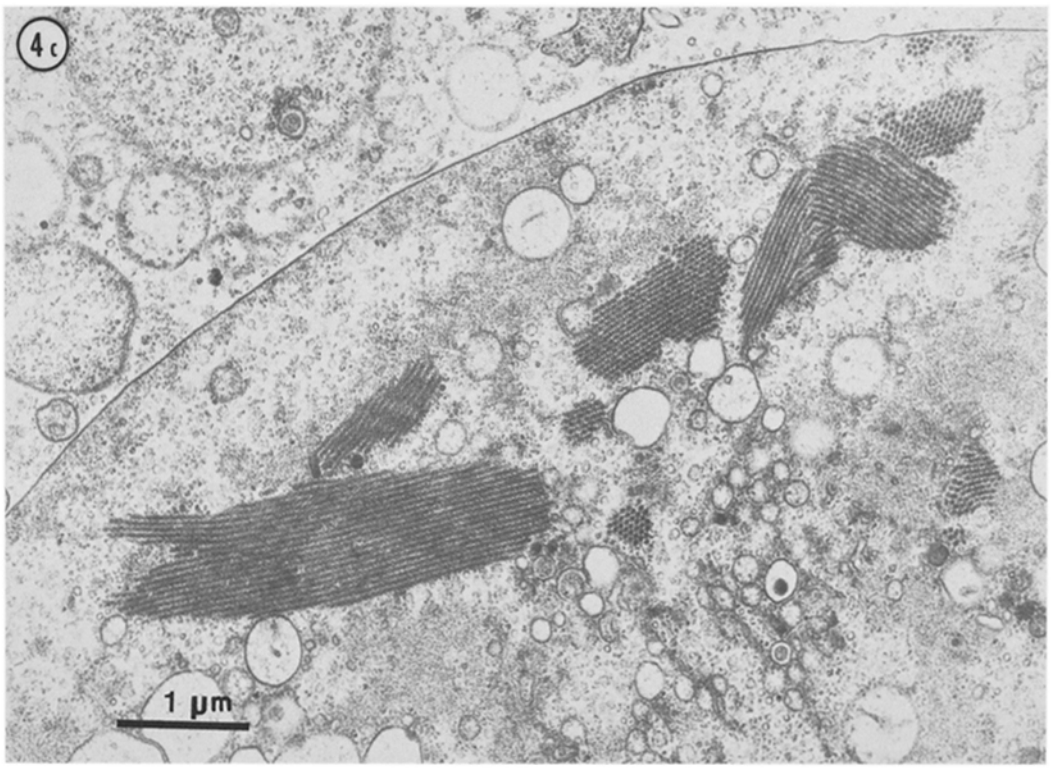
Seng[3] has shown that exogenous [^{32}P]inorganic phosphate is incorporated into DNA, RNA, and protein in growing trophozoites of *E. invadens*. We found that 50 mM NaF completely blocked [^{32}P]phosphate incorporation (data not shown), indicating that all of these macromolecular processes were stopped while aggregation was occurring. Trophozoites treated with NaF soon become inactive and began to lyse, suggesting a general metabolic breakdown caused by this broadly acting inhibitor. It would appear, therefore, that inhibitor-stimulated aggregation not only required no macromolecular synthesis, but was probably not an energy-dependent process.

Comparison of the Kinetics of Protein Synthesis Inhibition and of Aggregate Stimulation by NaF, CH, and ACT-D

NaF, CH, and ACT-D each inhibit protein synthesis by a different mechanism. NaF inhibits initiation of mRNA translation (16). However, elongation and termination are not prevented, and free ribosomes accumulate as they complete translation. CH blocks all stages of translation and

[3] L. Seng. Manuscript in preparation.


4b
1 µm


4c
1 µm

stops ribosome movement along and release from mRNA (16). The duration of the "frozen" polysome state is probably not indefinite, and eventually disassembly should occur yielding mRNA-free ribosomes prevented from reinitiating protein synthesis. At appropriate concentrations, ACT-D effectively inhibits transcription in *E. invadens* while at the same time having only a small effect on translation (see Results, below). In the presence of such concentrations of this drug, protein synthesis should decrease and free ribosomes should accumulate only as the supply of translatable mRNA becomes limiting.

We had proposed that the formation of helices and arrays of helices in *E. invadens* was a consequence of the accumulation of free ribosomes in the cell. If so, the rate of aggregate formation in trophozoites treated separately with each of the above inhibitors might be predictably different. If it is assumed that translatable mRNA survives longer than CH-frozen polysomes in *E. invadens*, the relative rates at which free ribosomes, hence ribosome aggregates, would form in treated cells should be in the order NaF > CH > ACT-D.

The kinetics of in vivo protein synthesis inhibition and aggregation were measured simultaneously in the same cultures. The incorporation of [^{3}H]leucine by control and inhibited trophozoites is shown in Fig. 5. Both NaF and CH at the concentrations employed completely stopped protein synthesis within 15 min or less. ACT-D caused much slower inhibition, although a difference from the control was evident after 3–5 h. The incorporation of [^{3}H]uridine by trophozoites has been shown by other workers in our laboratory[3] to be inhibited 80% within 3 h by the same concentration of ACT-D. Therefore, the slow inhibition of protein synthesis by ACT-D more probably reflected a gradual breakdown of mRNA than inefficient inhibition of transcription.

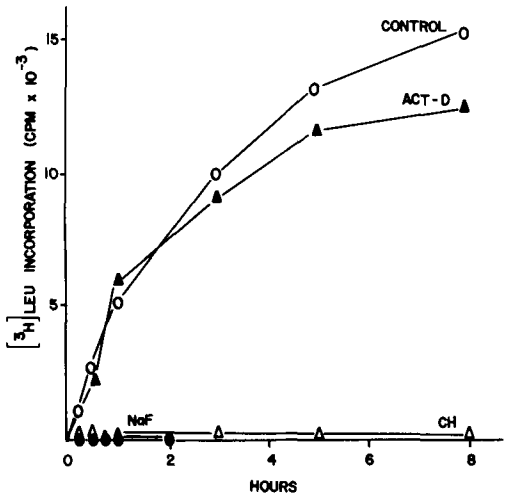


FIGURE 5 Inhibition of in vivo incorporation of [^{3}H]-leucine in *E. invadens* trophozoites by NaP (50 mM), CH (100 μg/ml), and ACT-D (100 μg/ml). At 0 h, amebas (5.8 × 10^5 cells/ml) were placed in encystation medium containing 10 μCi/ml [^{3}H]leucine. All procedures are described in Materials and Methods.

The rate of aggregation in the presence of each inhibitor is shown in Fig. 6. This was expressed as the percentage of trophozoites containing cytoplasmic inclusions detectable by light microscopy in toluidine blue-stained preparations. The increase in total aggregate content within individual cells also reflects the rate of aggregation but is difficult to quantitate. However, the latter change, at least until most cells contained visible arrays, appeared to proceed in proportion to the increase in the number of cells containing aggregates.

The rate of aggregation was markedly different for each inhibitor and followed the predicted order. Numerous small aggregates were present in most of the trophozoites after treatment with NaF for 15 min. Within 1 h virtually all the cells contained particles. With continued incubation the number of aggregates per cell decreased, but their size increased, indicating continued formation and coalescence of helices. Aggregates were not visible in CH-treated amebas until after 30 min. Thereafter, the percentage of cells containing particles increased steadily and aggregate size and number per cell changed as in the NaF-treated trophozoites.

As predicted from its slow effect on protein synthesis, ACT-D stimulated aggregate formation much more slowly than either NaF or CH. It should be noted, however, that after 3 h, 15% of the cells contained aggregates, whereas in the same cultures total [^{3}H]leucine incorporation was only negligibly reduced at this time. This disparity probably reflected differences in the metabolic activity of individual cells in the culture. Inactive cells would be expected to contain reduced levels of translatable mRNA compared to active amebas. Inhibition of these cells would have an insignificant effect on the amount of protein synthesis measured in the total population, but would be apparent when individual trophozoites were scanned for the presence of aggregates. This explanation is supported by two related observations. First, *E.*

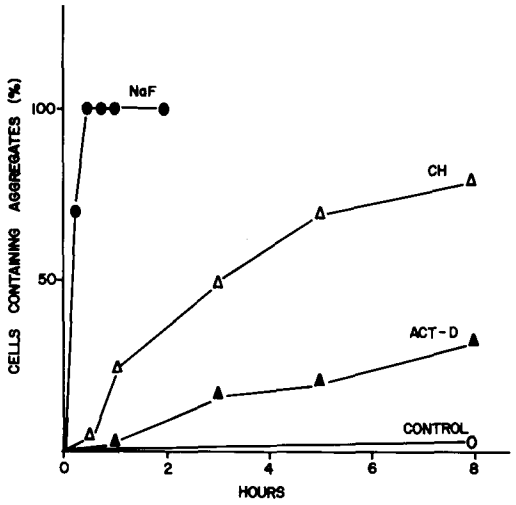


FIGURE 6 Kinetics of ribosome aggregate formation in *E. invadens* trophozoites treated with NaF, CH, and ACT-D. Samples were taken from the cultures described in Fig. 5. Cells were fixed and stained with toluidine blue as described in Materials and Methods. 100 cells were observed on each slide to determine the percent containing ribosome aggregates.

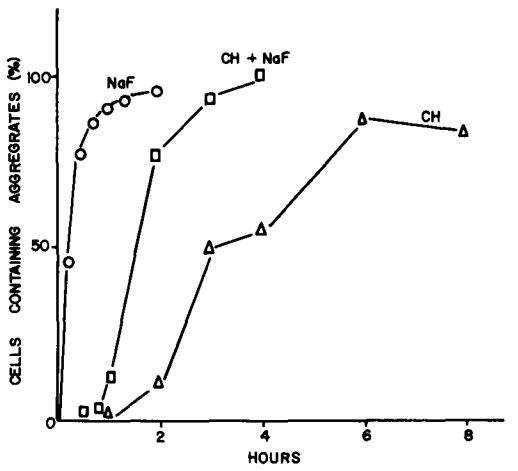


FIGURE 7 Retardation of NaF-stimulated ribosome aggregation by CH. CH (100 μg/ml) was added to one portion of a growing trophozoite culture in TPS growth medium 15 min before the addition of NaF (50 mM) at 0 h. CH and NaF at these concentrations were added singly to other portions of the same culture at 0 h. The kinetics of ribosome aggregation were followed as described in Fig. 6.

invadens IP-1 does not multiply exponentially in TPS growth medium, which indicates a decreased rate or cessation of growth (metabolic activity) by a portion of the cells, even during the most active growth phase. Second, aggregation can be stimulated by inhibitors more rapidly in trophozoites entering the stationary-growth phase than in cells in young cultures. This indicates an increased sensitivity to inhibitors as growth (metabolism) slows.

CH stopped protein synthesis as rapidly as NaF (Fig. 5), but stimulated aggregate formation more slowly, and only after a lag of 30 min. We suspected that this lag reflected the time required for the breakdown of CH-frozen polysomes. If so, CH might be expected to retard the onset of aggregation stimulated by NaF. That this was the case is shown in Fig. 7. Aggregates were not evident for 45 min in cultures receiving CH before NaF, whereas almost all the cells treated with NaF alone contained aggregates at this time. Interestingly, the rate of aggregate accumulation was faster in the doubly inhibited culture than in an identical culture inhibited with CH alone. Possibly NaF hastened the disassembly of CH-frozen polysomes or prevented rebinding of some ribosomes to mRNA, which may have occurred (without peptide synthesis) in the presence of CH alone.

The Reversibility of Aggregate Formation

By the time a majority of the amebas in the presence of NaF or ACT-D contained aggregates, inhibition could not be reversed and the cells died. However, trophozoites treated with CH recommenced vegetative growth when the drug was removed even after long incubation times. To determine speed of reversibility of aggregate formation, growing trophozoites were incubated with CH as described in Fig. 6. After 4 h, 70% of the cells contained many aggregates. The amebas were then washed several times with and transferred to fresh growth medium. Within 90 min no aggregates were visible by light microscopy in the cells. Thereafter, the trophozoites commenced to multiply. Thus, disassembly of arrays occurred rapidly when a resumption of protein synthesis was allowed.

DISCUSSION

There are several reasons to believe that the helices and aggregates of helices formed in inhibitor-treated trophozoites were composed of mature, mRNA-free ribosomes. They had cytochemical properties expected of RNA-containing structures and appeared ultrastructurally identical to the known ribosomal arrays seen in *E. invadens* cysts.

Helices were generated by some inhibitors which are believed to act specifically on the translation mechanism. This indicated that cessation of translation was a prelude to aggregation and implicated once-functional ribosomes as the units of assembly.

NaF stimulated aggregation more rapidly than CH, and this rate was retarded in the presence of CH. In other eucaryotes NaF causes rapid polysome breakdown, whereas CH holds polysomes intact, at least temporarily. That the same is true in *Entamoeba* was confirmed by sucrose gradient analysis of ribosome profiles from inhibited cells (13). Thus, not only was cessation of translation essential for aggregate formation, but release of functional ribosomes from mRNA also appeared to be a prerequisite. This strongly suggests that polysome-derived ribosomes were the structural units of the helices formed.

All of our results are consistent with the hypothesis that helix assembly is a spontaneous consequence of the accumulation in the ameba cytoplasm of large numbers of free but normally functional monosomes (or subunits) which are prevented from engaging in the translation cycle. Aggregation occurred rapidly in the absence of macromolecular synthesis and arrays disappeared soon after inhibition was relieved. Thus, the formation of helices seemed to depend on no special metabolic activity and was reversible. Ribosome aggregation, therefore, may be imagined as an equilibrium process, controlled by the availability of translatable mRNA. When messenger is plentiful, ribosomes are preferentially utilized for translation. When mRNA is lacking, or ribosomes are prevented from binding to it, free particles accumulate and interact with one another to form symmetrical helices. As the numbers of helices increase, these in turn interact specifically to form arrays.

Although it cannot be firmly concluded on the basis of our results that the helices which formed in the inhibitor-treated trophozoites are identical to those occurring naturally in metabolically inactive trophozoites and cysts, it is likely that they are. Clearly, the mechanism we have proposed can explain the natural occurrence of ribosome helices, since the mRNA content of the amebas at the growth-cycle or life-cycle stages when aggregates appear can very reasonably be expected to be less than that required to occupy the available ribosomes. We find no reason to relate ribosome aggregation in *Entamoeba* specifically to the process of cell differentiation or to specific stages of the cell cycle, as it may be in other organisms (23, 5).

There is, nevertheless, some analogy between the proposed mechanism of ribosome helix formation in *Entamoeba* and, for example, ribosome crystallization in hypothermic chick embryos. In both systems aggregates appear to form from mature, inactive monosomes (or subunits) free of mRNA (20). Lowered temperature is required for crystallization of chick ribosomes, but in neither system does there seem to be any specific metabolic process necessary for aggregation, once ribosomes are released from messenger. Stable tetramers isolated from crystal-containing chick cells or generated in vitro consist solely of ribosome subunits (20, 6). Specific binding apparently occurs between adjacent large subunits (7). As mentioned in the introductory paragraphs, a variety of extraribosomal components have been suggested as constituents of the chromatoid bodies of *Entamoeba*. If such additional components exist and are required for natural helix assembly or aggregation, they also must be contained within the structure of the ribosome arrays formed in the presence of inhibitors. The latter arrays required no macromolecular and probably no energy synthesis. Thus, if filaments, proteins, or other particles were included in the aggregates, they must have been present in some form throughout the cytoplasm before helix induction. In this case, there arises the question of the cellular role of these components in the absence of helix formation.

We thank Dr. Douglas C. Barker for helpful comments during the course of this work. We gratefully acknowledge the assistance of Miss Prapee Sethara and Miss Ubonrat Vanasantakul in conducting the electron microscopy, and the technical help of Mr. Surathep Trakulboon.

This work was supported by funds from the Rockefeller Foundation, The National Research Council of Thailand, and The Faculty of Graduate Studies, Mahidol University.

Received for publication 20 November 1974, and in revised form 18 February 1975.

REFERENCES

1. BARKER, D. C., and K. DEUTSCH. 1958. The chromatoid body of *Entamoeba invadens*. *Exp. Cell. Res.* **15**:604–610.
2. BARKER, D. C., and G. J. SVIHLA. 1964. Localization of cytoplasmic nucleic acid during growth and

encystment of *Entamoeba invadens*. *J. Cell. Biol.* **20**:389–398.

3. BARKER, D. C., and L. S. SWALES. 1972 *a*. Comparison of trophozoite helical polysomes with cyst ribosomogen microcrystals in axenic *Entamoeba* sp. *Cell Diff.* **1**:307–315.
4. BARKER, D. C., and L. S. SWALES. 1972 *b*. Characteristics of ribosomes during differentiation of trophozoites to cysts in axenic *Entamoeba* sp. *Cell Diff.* **1**:297–306.
5. BYERS, B. 1967. Structure and formation of ribosome crystals in hypothermic chick embryo cells. *J. Mol. Biol.* **26**:155–167.
6. BYERS, B. 1971. Chick embryo ribosome crystals: analysis of binding and functional activity *in vitro*. *Proc. Natl. Acad. Sci. U. S. A.* **68**:440–444.
7. CAREY, N. H., J. R. W. HOBBS, and E. A. COOK. 1972. The structure of ribosomes as indicated by studies on tetramers from hypothermic chick embryos. *Biochem. J.* **130**:871–877.
8. CZETO, A. R., R. S. MORGAN, and G.K. STROTHER. 1973. The ultraviolet absorption spectra of chromatoid bodies of *Entamoeba invadens in situ*. An optical titration. *Exp. Cell. Res.* **78**:349–350.
9. DALTON, A. J. 1955. A chrome-osmium fixative for electron microscopy. *Anat. Rec.* **121**:281.
10. DIAMOND, L. S. 1968. Techniques of axenic cultivation of *Entamoeba histolytica* Schaudinn, 1903 and *E. histolytica*-like amoebae, *J. Parasitol.* **54**:1047–1056.
11. KELLER, P. M., N. H. MORGAN, R. S. MORGAN, and A. R. CZETO. 1973. Tubulin in cysts of *Entamoeba invadens*. *J. Cell. Biol.* **59**(2, Pt. 2): 165 a. (Abstr.).
12. KRESS, Y., M. WITTNER, and R. M. ROSENBAUM. 1971. Site of cytoplasmic ribonucleoprotein-filament assembly in relation to helical body formation in axenic trophozoites of *Entamoeba histolytica*. *J. Cell. Biol.* **49**:773–784.
13. KUSAMRARN, T. K., K. VINIJCHAIKUL, and G. B. BAILEY. 1975. Comparison of the structure and function of polysomal and helical ribosomes from *Entamoeba invadens*. *J. Cell. Biol.* **65**:540–548.
14. LAKE, J. A., and H. S. SLAYTER. 1970. Three dimensional structure of the chromatoid body of *Entamoeba invadens*. *Nature* (*Lond.*). **227**:1032–1037.
15. LAKE, J. A., and H. S. SLAYTER. 1972. Three dimensional structure of the chromatoid body helix of *Entamoeba invadens*. *J. Mol. Biol.* **66**:271–282.
16. LIN, S. Y., R. MOSTELLER, and B. HARDESTY. 1966. The mechanism of sodium fluoride and cycloheximide inhibition of hemoglobin synthesis in the cell free reticulocyte system. *J. Mol. Biol.* **21**:51–57.
17. MORGAN, R. S., and R.F. SATILLARO, 1972. Zinc in *Entamoeba invadens*. *Science* (*Wash. D.C.*). **176**:929–930.
18. MORGAN, R. S., H. S. SLAYTER, and D. L. WELLER. 1968. Isolation of ribosomes from cysts of *Entamoeba invadens*. *J. Cell. Biol.* **36**:45–51.
19. MORGAN, R. S., and B. G. UZMAN. 1966. Nature of the packing of ribosomes within chromatoid bodies. *Science* (*Wash. D.C.*). **152**:214–216.
20. MORIMOTO, T., F. BLOBEL, and D. D. SABATINI. 1972. Ribosome crystallization in chicken embryos. I. Isolation, characterization, and *in vitro* activity of ribosome tetramers. *J. Cell. Biol.* **52**:338–354.
21. RENGPIEN, S., and G. B. BAILEY. 1975. Differentiation of *Entamoeba*. A new medium and optimal conditions for axenic encystation of *E. invadens*. *J. Parasitol.* In press.
22. ROSENBAUM, R. M., and M. J. WITTNER. 1970. Ultrastructure of bacterized and axenic trophozoites of *Entamoeba histolytica* with particular reference to helical bodies. *J. Cell. Biol.* **45**:367–382.
23. TADDEI, C. 1972. Ribosome arrangement during oogenesis of *Lacerta sicula* RAF. *Exp. Cell Res.* **70**:285–292.
24. THEPSUPARUNGSIKUL, V., L. SENG, and G. B. BAILEY. 1971. Differentiation of *Entamoeba*: encystation of *Entamoeba invadens* in monoxenic and axenic cultures. *J. Parasitol.* **57**:1288–1292.