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Cycloheximide-Resistant Glycosylation in L Cells Infected with *Chlamydia psittaci*

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L cells (mouse fibroblasts), uninfected and infected with the meningopneumonitis strain of *Chlamydia psittaci*, were labeled with [^{14}C]glucosamine, and their membranous organelles were separated by isopycnic equilibrium centrifugation of whole cell homogenates on discontinuous sucrose gradients. Glycosylation of host membranes continued throughout the infection. Cycloheximide almost completely inhibited glycosylation in uninfected L cells, but it only partially inhibited the process in infected host cells. Cycloheximide-resistant glycosylation of membrane fractions with [^{14}C]glucosamine increased as the infection proceeded and was probably due to the action of chlamydial enzymes. Modification of host membranes by glycosylation may play a role in the natural development of chlamydial infections.

Multiplication of *Chlamydia psittaci* in L cells is accompanied by inhibition of the synthesis of host membrane proteins and acceleration of their breakdown (6). These changes in membrane metabolism may facilitate release of infectious chlamydiae from host cells. Chlamydial infection also results in the formation of a new kind of host membrane, the inclusion vacuole membrane, which is formed by invagination of the cytoplasmic membrane around a phagocytosed chlamydial cell. As the chlamydial inclusion expands by multiplication of the chlamydial cells it contains, its enveloping membrane is enlarged by processes not requiring synthesis of new protein by the host and possibly not by the parasite (6). The purpose of this investigation was to determine the effect of infection with *C. psittaci* on glycosylation of host membranes and to assess the role of glycosylation in the expansion of the inclusion vacuole membrane.

(This investigation was presented in partial fulfillment of the requirements for the Ph.D. degree at the University of Chicago.)

MATERIALS AND METHODS

L cells (mouse fibroblasts) were grown at 37 C in either suspension or monolayer cultures in medium 199 containing 5% heat-inactivated fetal calf serum, 0.1% sodium bicarbonate, and 200 μg of streptomycin sulfate per ml (9). L cells were infected with the meningopneumonitis strain of *C. psittaci* by the procedure of Tribby and Moulder (10). The infecting dose was sufficient to produce inclusions in more than 95% of the L cells.

L-cell homogenates were fractionated on discontinuous sucrose gradients by a procedure already described (6). In brief, homogenates of 5×10^7 L cells prepared with a Dounce homogenizer were layered on discontinuous sucrose gradients consisting of five layers of 1.29, 1.23, 1.18, 1.13, and 1.08 specific gravities. The gradients were centrifuged for 17 h at 4 C in a Beckman SW27 rotor, and 0.5-ml fractions were collected from the bottoms of the tubes. Five distinct bands, representing different classes of cellular organelles, were produced, one at each interface. Band 1 was formed at the interface of the two lightest layers.

To identify the L-cell organelles and chlamydial cells present in each of the five bands, samples were dialyzed in 0.1 M tris(hydroxymethyl)aminomethane-hydrochloride buffer, pH 7.4, containing 0.25 M sucrose and 0.1% serum albumin. They were then negatively stained with 2% phosphotungstic acid and examined in an AEI-EM6B electron microscope.

L cells were labeled with D-[1-^{14}C]glucosamine (New England Nuclear Corp., Boston, Mass.). Uninfected and infected L-cell suspensions were labeled in complete growth medium containing 0.2 μCi of [^{14}C]glucosamine per ml. Labeled glucosamine was added 0, 10, and 20 h after infection, and incorporation was allowed to proceed for 10 h. Cycloheximide (5 μg/ml) was added to a portion of each L-cell suspension 2 h before addition of the labeled glucosamine. Comparable suspensions of uninfected L cells were similarly labeled. The incorporation of [^{14}C]glucosamine into glycoproteins was measured by precipitating 0.2-ml samples of each gradient fraction with cold 5% trichloroacetic acid, filtering on nitrocellulose

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filters, and counting the dried filters in a liquid scintillation counter in a toluene scintillation mixture.

RESULTS

Examination of electron micrographs made of samples from each band indicated that the composition of the five interphase bands obtained by isopycnic centrifugation of L-cell homogenates on discontinuous sucrose gradients was as follows: band 1, smooth endoplasmic reticulum, lysosomes; band 2, smooth and rough endoplasmic reticulum, ribosomes; band 3, rough endoplasmic reticulum, ribosomes; band 4, mitochondria, nuclei, polysomes, rough endoplasmic reticulum, plasma membranes, chlamydial cells; band 5, intact L cells, chlamydial cells. The distribution of L-cell organelles was similar to that described by Bosmann and Hagopian (2), whose procedure was followed closely. The location of chlamydial cells on the gradients was consistent with their previously determined densities (3, 7, 8).

The incorporation of [^{14}C]glucosamine into L-cell membranes was studied in experiments comparable to those previously done with ^{14}C-labeled amino acids (6). Glucosamine is incorporated mainly into the membrane glycoproteins of eukaryotic cells (4, 5). Figure 1 shows the distribution of glucosamine-labeled membranes from uninfected L cells on a discontinuous sucrose gradient. Bands 1, 2, and 3 contained most of the incorporated glucosamine. Cycloheximide, which inhibits L-cell protein synthesis without affecting chlamydial protein synthesis or multiplication (1), almost completely eliminated the uptake of [^{14}C]glucosamine into the membrane fractions of uninfected L cells. The mechanism of this inhibition is not known. Cycloheximide only slightly inhibits the incorporation of glucose into acid-insoluble fractions by L cells (I. I. E. Tribby, Ph.D. dissertation, The University of Chicago, Chicago, Ill., 1969).

The incorporation of glucosamine into host membranes 10 to 20 h after infection is shown in Fig. 2. Incorporation of glucosamine was similar to that of amino acids (6) in the same interval after infection. Cycloheximide strongly inhibited [^{14}C]glucosamine incorporation, as it did in uninfected L cells, but there was a small but significant cycloheximide-resistant incorporation of glucosamine into bands 1, 2, and 3.

In the 20- to 30-h period after infection, incorporation of glucosamine continued without inhibition (Fig. 3). This is in contrast to the incorporation of amino acids, which is strongly inhibited in this period (6). Incorporation into band 4 showed a marked increase over the 10- to 20-h level of uptake. The low level of cycloheximide-resistant incorporation of glucosamine seen at 20 h was greatly increased by 30 h. There was much more incorporation of [^{14}C]glucosa-

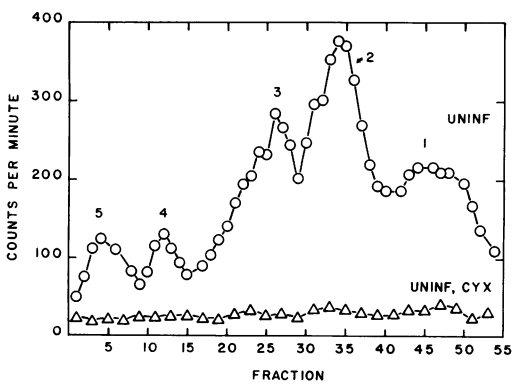

FIG. 1. *Incorporation of [^{14}C]glucosamine into glycoproteins of membranes from uninfected L cells. Uninfected L cells (UNINF) suspended in growth medium were allowed to incorporate [^{14}C]glucosamine (0.2 μCi/ml) for 10 h. To another portion of the same L-cell population was added cycloheximide (5 μg/ml) 2 h before addition of the [^{14}C]glucosamine (UNINF, CYX). At the end of the labeling period, the sucrose gradients were assayed as described in Materials and Methods. The numbers refer to the bands described in the text.*

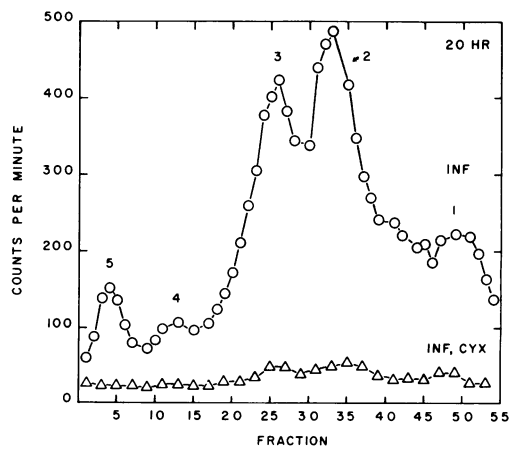

FIG. 2. *Incorporation of [^{14}C]glucosamine into glycoproteins of membranes from L cells 10 to 20 h after infection with C. psittaci. Infected L cells (INF) were suspended in growth medium to which 0.2 μCi of [^{14}C]glucosamine per ml was added for the interval 10 to 20 h after infection. Cycloheximide (5 μg/ml) was added to another portion of the same infected L-cell population 2 h before addition of the labeled glucosamine (INF, CYX).*

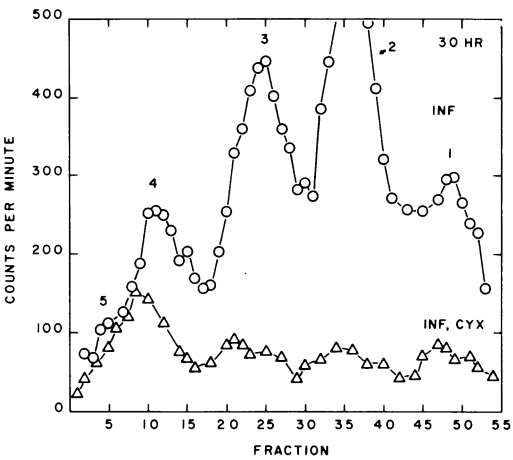

FIG. 3. *Incorporation of [^{14}C]glucosamine into glycoproteins of membranes from L cells 20 to 30 h after infection with C. psittaci. [^{14}C]glucosamine was added to infected L-cell suspensions 20 to 30 h after infection (INF). Cycloheximide (5 μg/ml) was added to another portion of the same infected L-cell population 2 h before addition of the labeled glucosamine (INF, CYX).*

mine into all bands in the presence of the inhibitor, particularly into band 4.

DISCUSSION

Although glycosylation of membranes by L-cell enzymes was not inhibited by infection with *C. psittaci*, it appears that this process, like the synthesis of new L-cell protein, is not required for the progressive enlargement of the chlamydial inclusion vacuole membrane during the developmental cycle. Cycloheximide almost completely inhibited incorporation of [^{14}C]glucosamine into the membrane glycoproteins of uninfected L cells. If it is assumed that host-catalyzed glycosylation is inhibited to the same extent in infected L cells as in uninfected ones, then such glycosylation could not have been essential for normal inclusion development.

The potentially important observation that there was a significant cycloheximide-resistant incorporation of glucosamine in L cells infected with *C. psittaci* cannot be interpreted unequivocally on the basis of these experiments. Since glucosamine incorporation was virtually abolished in uninfected L cells, the cycloheximide-resistant incorporation was probably catalyzed by chlamydial enzymes. It seems unlikely that infection with *C. psittaci* would have induced the formation of a new, host-derived, cycloheximide-resistant mechanism of glycosylation in the L cell. Further interpretation is difficult. For example, it might be concluded that the label in bands 4 and 5 represents glucosamine incorporation into chlamydial cells, whereas the ^{14}C in bands 1, 2, and 3 represents host membranes glycosylated by chlamydial enzymes. However, aggregated host glycoproteins could also account for the labeling of bands 4 and 5, and fragments of the notoriously fragile, large chlamydial cells could account for the label in the other bands. If chlamydiae glycosylate host membranes, or if they induce the host to do so by mechanisms not active in uninfected cells, then such glycosylation may be of great importance in the natural history of chlamydial infections, perhaps, for example, in modifying the structure of the inclusion membrane so that it has properties favorable to multiplication of the chlamydiae it contains.

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