Inhibition of Initiation of Protein Synthesis in Mammalian Tissue Culture Cells by L-1-Tosylamido-2-phenylethyl Chloromethyl Ketone

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SUMMARY

Incorporation of amino acids into proteins in HeLa cells, virus-transformed 3T3 mouse fibroblasts, and mouse plasmacytoma cells is inhibited after the addition of L-1-tosylamido-2-phenylethyl chloromethyl ketone, an alkylating agent and chymotrypsin-specific protease inhibitor. Addition of this drug to tissue culture cells at concentrations of 20 to 30 μg per ml results in an irreversible inhibition of the incorporation of amino acids into cellular proteins, and a rapid and complete breakdown of polyribosomes. A comparative study examining the effects of L-1-tosylamido-2-phenylethyl chloromethyl ketone and several known inhibitors of in vivo protein synthesis, with known mechanisms of action, revealed that an optimal concentration of L-1-tosylamido-2-phenylethyl chloromethyl ketone: (a) immediately and selectively inhibits initiation of protein synthesis, (b) does not significantly affect normal elongation rates, and (c) does not promote a premature release of nascent peptides. L-1-Tosylamido-2-phenylethyl chloromethyl ketone may prove to be a useful tool in investigating the initiation of protein synthesis in eukaryotic cells.

Tissue culture cells contain proteolytic enzymes which play a key role in the maturation of several DNA and RNA viruses by specific cleavage of viral precursor proteins (1, 2). Extracts from uninfected cells will convert the isolated picornavirus "polyproteins" (2) into their cleavage products (3). This cleavage process can be inhibited by a number of agents in vivo and in vitro but most effectively by L-1-tosylamido-2-phenylethyl chloromethyl ketone, a chymotrypsin-specific protease inhibitor (4). Therefore, TPCK has recently been used in studies of possible post-translational cleavage processing in several in vivo systems (3, 5-7). TPCK severely restricts the use of this drug in the investigation of the post-translational processing of newly synthesized proteins and in studies examining the role of proteolytic activities in the expression of the transformed state (8, 9). However, the results reported here suggest that TPCK may be useful in studying the process of peptide chain initiation in eukaryotic cells.

MATERIALS AND METHODS

Chemicals—Spectral quality sucrose was purchased from Mann Biomedical Co. [35S]Methionine with a specific activity of 100 Ci per mmol was obtained from Amersham-Searle. Dimethylsulfoxide was a product of Matheson Coleman and Bell. TPCK, N-2-hydroxyethylpiperezine-N'-2-ethanesulfuric acid and tris(hydroxymethyl)aminomethane were obtained from Calbiochem. Cerebroside was supplied by Sigma Chemical Co. Spectrofluor was obtained from Yorktown Research Co. and Aqualor from New England Nuclear. Joklik-modified MEM (F-13) and Dulbecco's modified MEM (H-16) were supplied by Gibco. Nonidet P-40 was provided by Shell Chemical Co.

Growth of Cells—HeLa S1 cells were grown at 37° in suspension in Joklik-modified MEM supplemented with 5% fetal calf serum. Cultures were maintained at cell densities of 2 to 4 × 10^6 cells per ml.

Plasmacytoma cells MPC-11, kindly furnished by Dr. Matthew Scharff, Albert Einstein College of Medicine, New York, were grown in suspension in Dulbecco's modified MEM supplemented with 20% horse serum. Myeloma cell cultures were maintained at a cell density of 5 to 9 × 10^6 cells per ml.

Kirsten virus-transformed 3T3 mouse fibroblast cells, a gift from Dr. T. T. August, Albert Einstein College of Medicine, New York, were grown in Dulbecco's modified MEM supplemented with 10% fetal calf serum.

Amino Acid Incorporation Studies—Cell cultures were harvested by centrifugation for 5 min at 300 × g and resuspended at a density of 4 × 10^6 cells per ml (HeLa) or 2.5 × 10^6 cells per ml (myeloma) in serum-supplemented or serum-free MEM plus 25 mM N-2-hydroxyethylpiperezine N'-2-ethanesulfuric acid, pH 7.2.

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1 The abbreviations used are: TPCK, L-1-tosylamido-2-phenylethyl chloromethyl ketone (better described as 1-chloro, 4-phenyl, 3-tosylamido-2-butenone. MEM, Eagle's minimal essential medium.
**RESULTS**

**Inhibition of Amino Acid Incorporation by TPCK**—The effect of several concentrations of TPCK on the incorporation of \[^{35}S\]methionine into HeLa cell proteins is presented in Fig. 1. HeLa cells were incubated in serum-free medium containing \[^{35}S\]methionine for 7.5 min prior to the addition of TPCK to the final concentrations of 5, 10, 20, and 30 \(\mu\)g per ml (1.4 \(\times 10^{-2}\) to 8.5 \(\times 10^{-1}\) M). Since the TPCK added was dissolved in dimethylsulfoxide at a concentration of 10 mg per ml, 0.3% dimethylsulfoxide was added to the control cells in this and subsequent experiments. It was shown previously that dimethylsulfoxide, in concentrations up to 4%, does not affect protein synthesis in HeLa cells (14). Only a 5 to 10% inhibition of methionine incorporation is observed at a concentration of 5 \(\mu\)g of TPCK per ml. At a concentration of 10 \(\mu\)g of TPCK per ml, a progressive inhibition of amino acid incorporation is observed: 75% inhibition from 10 to 20 min and 100% inhibition from 20 to 30 min. Inhibition is 75% within 5 min and complete from 10 to 20 min after the addition of TPCK to 20 \(\mu\)g per ml. A further increase in the concentration of TPCK (30 \(\mu\)g per ml) produces a complete inhibition of methionine incorporation within 5 to 7 min.

Since TPCK has been used as a protease inhibitor in several studies utilizing serum-supplemented medium, we performed an experiment to examine the effect of serum on the inhibition of amino acid incorporation by TPCK. Fig. 2 shows the effect of TPCK on amino acid incorporation in the mouse plasmacytoma cell (MPC-11) in the absence (A) and presence (B) of 20% horse serum. The incorporation of methionine is also inhibited in the presence of serum, although 1.5- to 2-fold higher concentrations of TPCK are required to obtain an inhibition comparable to that observed in the absence of serum.

The inhibition of methionine incorporation after the introduction of TPCK is not readily reversible. As shown in Fig. 3, HeLa cells treated with 30 \(\mu\)g of TPCK per ml for 10 min and then washed extensively with medium lacking TPCK show no subsequent recovery of protein synthesis by 30 min of incubation. In long-term experiments with myeloma cells, in serum-supplemented medium, we observed no recovery in protein synthesis for up to 8 hours after a 20-min treatment with 30 \(\mu\)g of TPCK per ml.

**Polyribosome Breakdown following TPCK Treatment**—Analysis, by zonal centrifugation, of cytoplasmic extracts prepared from cells incubated in the presence of 30 \(\mu\)g of TPCK per ml reveals that the observed inhibition of amino acid incorporation is accompanied by a breakdown of polyribosomes. Fig. 4 shows sucrose gradient analysis of polyribosomes from cytoplasmic extracts of HeLa cells treated with 0.3% dimethylsulfoxide for 10 min (control) or with 30 \(\mu\)g of TPCK per ml for 4 and 10 min. It is evident that, by 1 min after TPCK addition, a significant shift in 260 nm absorption from the region corresponding to large polyribosomes to the region corresponding to smaller polyribosomes has occurred. Integration of the area under the absorption profile indicates that 50% of the polyribosomes are converted to 80 S ribosomes. Essentially all of the 260 nm absorbing material is shifted to the monosome region by 10 min after TPCK addition, with no discrete peaks observed migrating ahead of the 80 S peak. Fig. 4b shows a similar effect on virus-transformed 3T3 mouse fibroblasts after treatment with 30 \(\mu\)g of TPCK per ml for 10 min. Thus, polyribosome profile analysis agrees with amino acid incorporation data suggesting that...
addition of TPCK to tissue culture cells has an immediate effect on protein synthesis.

The disappearance of polyribosomes after TPCK addition could, however, be attributed to factors other than the inhibition of protein synthesis, such as increased levels of RNase or residual effects of TPCK after cell lysis. To exclude these possibilities, the effect of TPCK on polyribosome distribution in the presence and absence of cycloheximide was investigated and compared with the effect of hypertonicity resulting from an elevated NaCl concentration. Cycloheximide inhibits the translocation of ribosomes on mRNA, resulting in stabilization of polyribosomes (15). Proper elevation of the NaCl concentration selectively inhibits the initiation of polypeptide synthesis, but has no effect on elongation and termination processes (16). HeLa cell culture suspensions at a density of 4 X 10⁶ cells per ml were treated with 0.3% dimethyl sulfoxide (control), 30 μg of TPCK per ml, 100 μm additional NaCl, 100 μg of cycloheximide per ml, or a combination of cycloheximide and TPCK. Fig. 5a shows the characteristic disappearance of polyribosomes following treatment with 30 μg of TPCK per ml. Fig. 5b shows that the addition of NaCl to a final concentration of 210 mM also results in a complete conversion of polyribosomes to 80 S ribosomes. The similarity in the kinetics of polyribosome disappearance following treatment of cells with either TPCK or hypertonic medium (data not shown) suggested to us that these inhibitors of protein synthesis may have similar mechanisms of action. Also shown in Fig. 5b is

![Fig. 2. Effects of serum on TPCK-induced inhibition of amino acid incorporation. Mouse plasmacytoma cells (MPG-11) were suspended at a density of 2.5 X 10⁶ cells per ml in Dulbecco's MEM with or without 20% horse serum. TPCK was added to the concentrations indicated below at a time 5 min after the introduction of 20 μCi of [35S]methionine per ml to serum-free cultures or 30 μCi of [35S]methionine per ml to serum-supplemented culture. 35S incorporation was determined as described for Fig. 1. A, data obtained from serum-free cultures: 0, control cells incubated with 0.3% dimethylsulfoxide; X, cells incubated with 10 μg of TPCK per ml; 0, cells incubated with 20 μg of TPCK per ml; 1, cells incubated with 30 μg of TPCK per ml. B, data obtained from serum-supplemented cultures: 0, cells incubated with 0.3% dimethylsulfoxide; X, cells incubated with 10 μg of TPCK per ml; 0, cells incubated with 20 μg of TPCK per ml; 1, cells incubated with 30 μg of TPCK per ml.

![Fig. 3. Reversibility of TPCK-induced inhibition of amino acid incorporation. Portions of HeLa cell suspensions (5 ml), at a density of 4 X 10⁶ cells per ml, were incubated at 37° for 10 min in the presence of either 0.3% dimethylsulfoxide (control cells) or 30 μg of TPCK per ml. Following incubation, the cell suspensions were pipetted into 10 volumes of crushed, frozen MEM, centrifuged for 10 min at 300 X g, washed once, and re-suspended in MEM lacking methionine. The cell suspensions were then incubated at 37° with 5 μCi of [35S]methionine per ml and incorporation of [35S]methionine into polypeptides was measured as described in Fig. 1. O---O, control cells; 0---0, cells preincubated with 30 μg of TPCK per ml.

![Fig. 4. Effects of TPCK on cellular polyribosome distribution. a, HeLa cell cultures at a density of 4 X 10⁶ cells per ml were incubated with either 0.3% dimethylsulfoxide for 10 min (---) or 30 μg of TPCK per ml for 4 min (-----) or 10 min (----). Following incubation, the cells were poured over crushed, frozen, phosphate-buffered NaCl solution and cytoplasmic extracts were prepared. Sucrose gradient analysis of cytoplasmic extracts was performed as described under "Materials and Methods." b, Kirsten virus-transformed 3T3 mouse fibroblasts grown in monolayer were also treated with either 0.3% dimethylsulfoxide (•) or 30 μg of TPCK per ml (---) for 10 min, followed by the addition of crushed, frozen, phosphate-buffered NaCl solution. Cells were immediately harvested mechanically and cytoplasmic extracts were prepared and analyzed as described for HeLa cells. Note the 5-fold shift in the ordinate scale at the beginning of the polyribosome region in both a and b.
the stabilization of polyribosomes resulting from treatment of cells with 100 μg of cycloheximide per ml. Fig. 5c reveals that preincubation of cells with cycloheximide, or the addition of cycloheximide at the same time as TPCK, prevents TPCK-induced polyribosome breakdown. These results seem to exclude the possibilities mentioned above that the observed effect of TPCK on polyribosomes is not directly related to the inhibition of protein synthesis.

Although the results of the experiments just described allow us to conclude that TPCK-induced polyribosome breakdown is a direct result of inhibition of protein synthesis, they do not permit us to make any definite statements with respect to the mechanism of inhibition resulting from TPCK treatment. On the basis of the kinetics of inhibition of amino acid incorporation following TPCK addition and the similarity between TPCK and NaCl-induced polyribosome breakdown, it was thought that TPCK inhibited protein synthesis, most probably at the level of initiation. However, the possibility that TPCK might act by promoting premature release of nascent peptides could not be excluded.

Elongation and Release of Nascent Polypeptides after TPCK Addition—To investigate the effect of TPCK on elongation and release of nascent polypeptides, pulse-chase experiments were performed to compare the effect of TPCK and other inhibitors of protein synthesis on peptide chain completion. HeLa cells were pulse-labeled for 45 s with [35S]methionine, poured onto crushed, frozen MEM containing a 1 X 10^6 excess of unlabeled methionine, centrifuged, and washed twice with the same medium. Following the final resuspension, the cell suspension was divided into five equal portions. One portion was lysed immediately, and the other portions were chased at 37° for 12 min in the presence of 0.3% dimethylsulfoxide (control cells), 30 μg of TPCK per ml, 12% dimethylsulfoxide, or 210 mM NaCl. Following the chase period, the cells were poured over 10 volumes of crushed, frozen MEM containing a 1 X 10^6 excess of unlabeled methionine, centrifuged, and washed twice with the same medium. Following the final resuspension, the cell suspension was divided into five equal portions. One portion was lysed immediately, and the other portions were chased at 37° for 12 min in the presence of 0.3% dimethylsulfoxide (control cells), 30 μg of TPCK per ml, 12% dimethylsulfoxide, or 210 mM NaCl. Following the chase period, the cells were poured over 10 volumes of crushed, frozen MEM solution, centrifuged, washed twice with the same NaCl solution, and lysed. Analysis of the labeled polypeptides by sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out as described under “Materials and Methods.” The direction of electrophoresis is from left to right. A: ○—○, cells lysed immediately; ●—●, control cells chased in the presence of 0.3% dimethylsulfoxide; △—△, cells chased in the presence of 12% dimethylsulfoxide; ◊—◊, cells chased in the presence of 210 mM NaCl. B: ●—●, cells chased in hypertonic medium; ○—○, cells chased in the presence of 12% dimethylsulfoxide; ——, cells lysed immediately.

Fig. 5. Comparative effect of TPCK, cycloheximide, and hypertonicity on polyribosome distribution. Cytoplasmic extracts from HeLa cell cultures suspended at a density of 4 X 10^4 cells per ml, treated as described below, were prepared and fractionated as described in Fig. 4. a ——, control cells incubated for 15 min with 0.3% dimethylsulfoxide; ——, cells incubated for 15 min with 30 μg of TPCK per ml. b, ——, cells incubated for 15 min with 100 μg of cycloheximide per ml; ——, cells incubated for 15 min in the presence of 210 mM NaCl. c ——, cells incubated for 15 min with 100 μg of cycloheximide per ml and then for 15 min with 30 μg of TPCK per ml. Note the 6-fold shift in the ordinate scale at the beginning of the polyribosome region in a, b, and c.

Fig. 6. Elongation of nascent polypeptides in the presence of TPCK, NaCl, and dimethylsulfoxide. HeLa cells suspended at a density of 4 X 10^4 cells per ml in MEM lacking methionine were pulse-labeled for 45 s with [35S]methionine (7.5 μCi per ml). The cells were then poured over 10 volumes of crushed, frozen MEM containing a 1 X 10^6 excess of unlabeled methionine, centrifuged, and washed twice with the same medium. Following the final resuspension, the cell suspension was divided into five equal portions. One portion was lysed immediately, and the other portions were chased at 37° for 12 min in the presence of 0.3% dimethylsulfoxide (control cells), 30 μg of TPCK per ml, 12% dimethylsulfoxide, or 210 mM NaCl. Following the chase period, the cells were poured over 10 volumes of crushed, frozen NaCl solution, centrifuged, washed twice with the same NaCl solution, and lysed. Analysis of the labeled polypeptides by sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out as described under “Materials and Methods.” The direction of electrophoresis is from left to right. A: ○—○, cells lysed immediately; ●—●, control cells chased in the presence of 0.3% dimethylsulfoxide; △—△, cells chased in the presence of 12% dimethylsulfoxide; ◊—◊, cells chased in the presence of 210 mM NaCl. B: ●—●, cells chased in hypertonic medium; ○—○, cells chased in the presence of 12% dimethylsulfoxide; ——, cells lysed immediately.

without a 12-min chase in the presence or absence of 30 μg of TPCK per ml. Comparison of the size distribution of labeled polypeptides from cells lysed immediately after pulse labeling and cells chased for 12 min clearly shows the shift of 38 counts into higher molecular weight peptides. Comparison of the polypeptide size distribution from cells chased in the presence or absence of 30 μg of TPCK per ml reveals no differences. Thus, elongation and termination of nascent polypeptides appear to proceed normally in the presence of inhibiting concentrations of TPCK, and there is no evidence of premature release of nascent polypeptides. For comparison, Fig. 6B shows the size distribution of labeled polypeptides from cells chased for 12 min at an elevated NaCl concentration and cells chased in the presence of 12% dimethylsulfoxide. Normal completion of nascent pulse-labeled peptides occurs in cells chased in hypertonic medium. However, on the basis of the differences in size distribution of labeled peptides from control and dimethylsulfoxide-treated cells, it is evident that premature release of nas-
control cells pulse-labeled for 2 min; Sample b (l--4 X 10^6 cells per ml in MEM lacking methionine were pulsed after TPCK addition, HeLa cell suspensions at a density of 1 X 10^6 cells per ml in MEM containing a 1 X 10^4-fold excess of unlabeled methionine and centrifuged. After one wash with the same medium, the cells were resuspended and incubated at 37°C for 11 min for Sample a, 9 min for Sample b, and 6 min for Sample c. Cytoplasmic extracts were prepared and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described under "Materials and Methods." Noncapsid and capsid polypeptides derived from poliovirus-infected HeLa cells were used as molecular weight markers: NCVPI (125,000); NCVPX (90,000); VP0 (40,000); VP1 (35,000); VP3 (23,000).

FIG. 7. Sequential inhibition of HeLa cell peptides with time after TPCK addition. HeLa cell suspensions at a density of 4 X 10^6 cells per ml in MEM lacking methionine were pulsed with [3S]methionine (5 μCi per ml) as follows: Sample a (O--O), control cells pulse-labeled for 2 min; Sample b (●--●), cells treated with TPCK (30 μg per ml) for 1 min and pulse-labeled for 2 min; Sample c (×), cells treated with TPCK (30 μg per ml) for 4 min and pulse-labeled for 2 min. Following pulse labeling, all samples were poured onto 10 volumes of crushed, frozen MEM containing a 1 X 10^4-fold excess of unlabeled methionine and centrifuged. After one wash with the same medium, the cells were resuspended and incubated at 37°C for 11 min for Sample a, 9 min for Sample b, and 6 min for Sample c. Cytoplasmic extracts were prepared and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described under "Materials and Methods." Noncapsid and capsid polypeptides derived from poliovirus-infected HeLa cells were used as molecular weight markers: NCVPI (125,000); NCVPX (90,000); VP0 (40,000); VP1 (35,000); VP3 (23,000).

FIG. 8. Correlation between the molecular weight and percentage of incorporation of [3S]methionine into HeLa peptides with time after TPCK addition. The data plotted in this figure were obtained from the experiments described in Fig. 7, by the following operations. The counts per min value in each fraction of the polyacrylamide gel profile of cytoplasmic extracts from cells pulse-labeled for 2 min, 1 min after TPCK addition, was divided by the counts per min value in the corresponding fraction of the gel profile of cytoplasmic extracts from control cells. This value, multiplied by 100, yielded the percentage of incorporation in that fraction. The polypeptide molecular weight corresponding to each gel fraction was then plotted against the reciprocal of the peptide molecular weight corresponding to that gel fraction.

cent polypeptides occurs in cells chased in the presence of 12% dimethylsulfoxide. These results agree with those of previous studies on the use and mechanism of action of these two protein synthesis inhibitors reported from this laboratory (14, 16).

Selective Inhibition of Peptide Chain Inhibition—The experiments described above indicate that TPCK does not promote the premature release of nascent peptides. However, a rapid and complete breakdown of polyribosomes is observed after the addition of TPCK to tissue culture cells. This suggests that TPCK may specifically interfere with the initiation of protein synthesis. Specific inhibition of initiation would lead to the sequential inhibition of labeling of proteins, depending upon their respective molecular weights. Since the average rate of protein synthesis in HeLa cells is 200 to 250 peptide bonds per min (16), the synthesis of proteins with molecular weights of up to 20,000, from a monocistronic mRNA, would be completed within 1 min, and proteins with molecular weights of up to 80,000 would be completed within 4 min. Pulse-chase experiments were performed at these times after TPCK addition, and the size distribution of labeled peptides was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (Fig. 7). Virus-specific peptides derived from poliovirus-infected HeLa cells were used as identification markers. In the 2 min pulse performed 1 min after TPCK addition, [3S]methionine incorporation into polypeptides with molecular weights under 20,000 was less than 5% of the control, whereas labeling of large polypeptides (more than 100,000) was inhibited by less than 40%. Pulse labeling following a 4-min incubation in the presence of TPCK resulted in no labeling of peptides with molecular weights below 60,000, whereas incorporation of [3S]methionine into very large polypeptides was still 50% of the control value. These results are important in several respects. The fact that no labeling of smaller molecular weight persists after the time of expected inhibition clearly shows that TPCK specifically blocks peptide chain initiation and indicates that no detectable amounts of small molecular weight peptides are derived from larger precursor peptides in uninfected HeLa cells under the conditions described. Also, the time required for complete inhibition of polypeptides of increasing molecular weight corresponds very well with the times predicted, based on published estimates of the rate of peptide chain synthesis in uninfected HeLa cells (16, 17). This fact strongly indicates that the rate of peptide chain elongation is unaffected in TPCK-treated cells.

Fig. 8 shows graphically that the percentage of incorporation of methionine into peptides, in the pulse labeling experiments just described, is related proportionally to the reciprocal of the molecular weight of the peptides. This result is predicted for inhibitors that selectively block polypeptide chain initiation. A detailed discussion of this relationship and its use in investigating post-translational processing and the specificity of inhibition of peptide chain initiation, will be presented elsewhere.2 The results of Fig. 8 demonstrate that TPCK selectively inhibits the initiation of protein synthesis under the conditions described and has little, if any, influence on the rate of elongation.

DISCUSSION

The results described in this paper show that the addition of 20 to 30 μg of the chymotrypsin-specific protease inhibitor per ml (TPCK) to cultures of HeLa, virus-transformed 3T3, or myeloma MPC-11 cells, in the presence or absence of serum, results in a rapid and irreversible inhibition of protein synthesis.

2 S.-S. Pong and G. Koch, manuscript in preparation.
At optimal concentrations of TPCK, inhibition of amino acid incorporation (Fig. 1) and polyribosome breakdown (Fig. 4) is complete within 5 to 7 min after TPCK addition. Prevention of TPCK-induced polyribosome breakdown by the addition of cycloheximide (Fig. 5) clearly rules out the possibility that the disappearance of polyribosomes after TPCK treatment is due to increased RNase levels or physical effects following cell lysis. Comparative pulse-chase experiments (Fig. 6) indicate that premature release of nascent peptides does not occur in TPCK-treated cells. Finally, size distribution analysis of peptides pulse-labeled at different times after TPCK addition, and properly chased, clearly shows that peptide chain initiation is inhibited immediately, but that incorporation of amino acids continues into preinitiated peptides at the normal rate until they are completed (Figs. 7 and 8).

TPCK has been used as an inhibitor of proteolytic activities associated with virus-transformed mammalian cells (6, 9) and with mitogen-stimulated lymphocytes (10). Although TPCK is specific in its ability to inhibit proteolytic activities, it is a strong alkylating agent especially reactive with certain sulfhydryl groups (18). It is, therefore, likely that this reagent would have other effects on cell activities. It has recently been reported that TPCK inhibits RNA synthesis (19) and that tosyl-lysyl chloromethyl ketone, a trypsin-specific protease inhibitor, inhibits DNA synthesis in eukaryotic cells (20). Since TPCK inhibits protein synthesis in a matter of minutes and RNA synthesis only after 30 min, under the conditions described in this report, we suggest that the effects of this protease inhibitor on the other cellular processes may well be a consequence of the inhibition of protein synthesis. Tosyl-lysyl chloromethyl ketone also affects protein synthesis in HeLa cells under the conditions in this report, but 5 to 10 times higher concentrations are required for a comparable degree of inhibition of amino acid incorporation (data not shown).

A reduction in amino acid incorporation and a preferential inhibition in the labeling of small molecular weight peptides following TPCK treatment of virus-infected and uninfected cells has previously been reported (5–7). Since TPCK was reported to have no effect on in vivo protein synthesis in mammalian cell-free extracts (6, 21), it was suggested that the observed relative increase in labeling of large molecular weight peptides, after the addition of TPCK, was a result of inhibition of post-translational processing and not due to inhibition of peptide chain initiation (6). Our data show that TPCK inhibits peptide chain initiation in vivo and would, therefore, preferentially inhibit the synthesis of small molecular weight peptides very early after addition to the culture medium. Thus, the apparent preferential labeling of large molecular weight peptides in cells treated with TPCK cannot be taken as unequivocal evidence for the existence of post-translational cleavage mechanism.

TPCK has been reported by several investigators to inhibit irreversibly peptide chain elongation in bacterial extracts (22, 23). However, several observations show that peptide chain initiation and not elongation is affected by TPCK in tissue culture cells. First, inhibition of amino acid incorporation and polyribosome disappearance is completed within 5 to 7 min after addition of an optimal concentration of TPCK. Second, chasing of pulse-labeled peptides proceeds normally in the presence of TPCK, with no evidence of premature release of nascent peptides. Principally, very good agreement was obtained between experimental and predicted values for sequential inhibition of peptide labeling with respect to their molecular weights at times after TPCK addition. The combined data compel us to conclude that TPCK can be used to block the initiation of protein synthesis selectively in a number of tissue culture cells.

Since peptide chain initiation is a multistep process involving many specific cellular components, one might speculate that TPCK interacts with certain factors required for in vivo peptide chain initiation. Alternatively, since TPCK effectively inhibits in vivo protein synthesis, but inhibits in vitro protein synthesis only to a limited extent, it is suggested that the inhibition of protein synthesis elicited by this reagent is membrane-mediated. This possibility is currently being investigated. In any event, TPCK, like other inhibitors of initiation of protein synthesis (14, 16, 24), may prove to be very useful in studying the mechanism of peptide chain initiation in vivo.

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S S Pong, D L Nuss and G Koch

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