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Conjugation of poly-L-lysine to albumin and horseradish peroxidase: A novel method of enhancing the cellular uptake of proteins

(membrane transport of macromolecules/cationized enzyme/pinocytosis)

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ABSTRACT **The carbodiimide-catalyzed conjugation of a 6700 molecular weight fragment of poly-L-lysine to radiolabeled human serum albumin or to horseradish peroxidase enhances the membrane transport of each protein into cultured mouse fibroblasts approximately 11- and 200-fold, respectively. At least 50% of the peroxidase activity remained after conjugation. Trypsinization and carbamylation of the two conjugates demonstrates that the enhancement of their cellular uptake is related to their poly-L-lysine content. Simple addition to the medium of comparable amounts of free poly-L-lysine has no effect on the transport of either native protein. Addition of poly-L-ornithine (molecular weight 200,000) at 3–30 μg/ml, a condition known to cause enhancement of ^{125}I-labeled human serum albumin uptake by mouse sarcoma cells, has no visible effect on the cellular uptake of native horseradish peroxidase. The intracellular localization of the enzyme–poly-L-lysine conjugate can be demonstrated cytochemically by either light or transmission electron microscopy. A concentration of conjugate that increases the uptake more than 200-fold does not cause any detectable morphological change suggestive of cell toxicity. Furthermore, because poly-L-lysine is an excellent substrate for intracellular proteolytic enzymes, it can be expected to be broken down and reutilized in the cell.**

The transport of protein at the surface of mammalian cells was described 10 years ago as a process of considerable potential biological significance (1). Examples have since been found in which transport is preceded by a specific protein–receptor interaction (2–4). The alteration of such interactions and the concomitant reduction of protein transport have been associated in several instances with specific genetic diseases. The molecular changes responsible for the decreases in protein transport were found to reside either in the surface receptor (5) or in the protein itself (4, 6). It has become increasingly important therefore to develop procedures that will enhance cellular penetration of macromolecules for the purpose either of introducing molecules that are not ordinarily transported to a significant extent or of restoring a normal transport when a receptor-mediated mechanism is defective.

Previous work from this laboratory demonstrated that addition of positively charged proteins or polyamino acids to a medium containing radiolabeled albumin enhanced the cellular penetration of albumin into sarcoma S 180 monolayers (1, 7, 8) and suspension cultures (9). This observation was used by others to increase the cellular penetration of interferon (10, 11), viral nucleic acids (12), diphtheria toxin (13), and other biologically active macromolecules into various avian and mammalian cells. The procedure was effective also in increasing infection of plant cell protoplasts with tobacco mosaic virus RNA (14). An analysis of the phenomenon revealed that poly-L-ornithine [poly(Orn)], poly-D-lysine, and poly-L-arginine [poly(Arg)] were the most effective enhancers (8), that poly-D-lysine was more effective than poly-L-lysine [poly(Lys)] (9), and that their effects increased with their molecular size (15). However, there was a considerable scatter in the magnitude of enhancement measured in different experiments and in different cell lines under comparable conditions. Furthermore, cytochemical experiments aimed at revealing intracellular activity of horseradish peroxidase failed to demonstrate any effect of poly(Orn) and poly-D-lysine on the intracellular accumulation of horseradish peroxidase. In this paper we report that the cellular transport of human serum albumin and horseradish proxidase is dramatically increased when the proteins in question are covalently linked to a small fragment of poly(Lys).

MATERIALS AND METHODS

Crystalline human serum albumin, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, and 3,3′-diaminobenzidine were purchased from Sigma Chemical Co., St. Louis, MO; poly(Lys) hydrobromide (molecular weight, 6700) and poly(Orn) hydrobromide (molecular weight, 200,000) were purchased from Pilot Chemicals, Watertown, MA; horseradish peroxidase was from Worthington Biochemical Corporation, Freehold, NJ, and had a purity number (R. Z.) of 3.03. Chloramine-T was purchased from Eastman Kodak, Rochester, NY. The tissue culture products and trypsin solution (0.25%) were purchased from Grand Island Biological Co., Grand Island, NY. L929 cells were purchased from the American Type Culture Collection, Rockville, MD. The rabbit anti-human serum albumin antibody was a gift from Lawrence Levine, Brandeis University, Waltham, MA.

Cells and Culture Medium. L929 mouse fibroblasts were grown as monolayers in Leighton tubes with a flat surface of 13.5 cm^2 (Bellco Glass, Inc., Vineland, NJ). The growth medium was Eagle's minimal essential medium supplemented with 10% fetal calf serum, and penicillin (50 units/ml) and streptomycin (50 μg/ml). The cells were fed every other day and on the day before the experiment.

Modifications of Human Serum Albumin and Horseradish Peroxidase and Their Conjugates. *Iodination.* Crystalline human serum albumin was radioiodinated with Na^{125}I by the chloramine-T procedure (16). After purification, the product (^{125}I-HSA) contained less than 3% free ^{125}I and had a specific activity of approximately 50 μCi/mg. The ^{125}I-HSA was diluted

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Abbreviations: poly(Orn), poly-L-ornithine; poly(Arg), poly-L-arginine; poly(Lys), poly-L-lysine; ^{125}I-HSA, ^{125}I-labeled human serum albumin; P_i/NaCl, phosphate-buffered saline; ^{125}I-HSA-poly(Lys), poly(Lys) conjugate of ^{125}I-HSA; HRP-poly(Lys), poly(Lys) conjugate of horseradish peroxidase.

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with unlabeled albumin to give the protein concentrations desired for each experiment.

Conjugation of ^{125}I-HSA with poly(Lys). ^{125}I-HSA, poly(Lys), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide were dissolved (20 mg each) in 0.8 ml of water. The solution was incubated for 3 hr at 25° with occasional shaking and then loaded onto a Sephadex G-100 column that had been equilibrated with 0.01 M phosphate-buffered saline (P_i/NaCl), pH 7.0. The column was eluted with P_i/NaCl, and protein fractions emerging at or near the void volume were collected, pooled, concentrated to 1.0 ml, and diluted with water to 10.0 ml. Unreacted ^{125}I-HSA was removed by passing the low-salt solution through a DEAE-Sephadex column. A standard radioimmunoassay for human serum albumin showed that the purified conjugate [^{125}I-HSA-poly(Lys)] had lost more than 99% of its albumin antigenicity and thus contained less than 1% unreacted albumin. Polyacrylamide disc gel electrophoresis at pH 4.5 confirmed the absence of unmodified albumin: the normal albumin band was replaced by a set of bands including some faster moving ones. The complexity of the electrophoretogram suggested that some poly(Lys)-induced crosslinking between albumin molecules might have occurred.

Conjugation of horseradish peroxidase to poly(Lys). The same procedure was used to prepare the horseradish peroxidase–poly(Lys) conjugate [HRP-poly(Lys)]. The enzyme showed two close bands on polyacrylamide gel electrophoresis at pH 4.5. The Sephadex G-100 column elution profile of the reaction mixture showed two incompletely separated peaks that were collected separately. Native horseradish peroxidase added to the column eluted with the second peak. The first peak was rechromatographed and used as HRP-poly(Lys) conjugate. With an assay using *o*-dianisidine as an electron acceptor (17) in which the rate of color development at 460 nm was measured as expression of enzymatic activity, it was found that conjugation decreased the enzymatic activity of horseradish peroxidase by about 50%. Polyacrylamide gel electrophoresis showed total disappearance of the two bands of native horseradish peroxidase molecules.

Trypsinization of ^{125}I-HSA-poly(Lys) and HRP-poly(Lys). In several experiments the poly(Lys) content of the conjugates was decreased by brief trypsinization. An aliquot (0.3 ml) of ^{125}I-HSA-poly(Lys) (1.5 mg/ml) or HRP-poly(Lys) (0.85 mg/ml) was mixed with 30 μl of 0.25% trypsin solution and incubated for 5 min at 37°. The enzymatic reaction was stopped by dilution of the 0.33 ml reaction mixture with 8.0 ml of Eagle's minimal essential medium containing 5% fetal calf serum (in the case of albumin) or by 500-fold dilution with serum-free medium (in the case of the peroxidase). The concentrations used in the experiments were 54 μg/ml for HSA-poly(Lys) and 1.5 μg/ml for HRP-poly(Lys).

Carbamylation of HSA-poly(Lys). In several experiments the positive charge contributed by poly(Lys) was abolished by carbamylation. To 1 ml of ^{125}I-HSA-poly(Lys) (1.5 mg/ml in P_i/NaCl, pH 7.0) was added 16 mg of KCNO and the mixture was incubated at 25° for 24 hr. After extensive dialysis against P_i/NaCl, more than 90% of the total protein and of the ^{125}I radioactivity was recovered.

Measurement of Cellular Uptake of ^{125}I-HSA and ^{125}I-HSA-poly(Lys). Confluent monolayers of L929 fibroblasts were incubated in serum-free growth medium containing ^{125}I-HSA or ^{125}I-HSA-Poly(Lys) at 50 μg/ml for 1 and 60 min at 37°. The monolayers were then washed twice with Earle's balanced salt solution at neutral pH and detached from the culture flask by brief exposure to trypsin. The cells were washed twice in 5 ml of balanced salt solution, once in 5 ml of heparin (5 mg/ml in balanced salt solution) and twice more in 5 ml of balanced salt solution. The final cell pellet was dissolved in 1M NaOH. The protein content of this cell extract was determined by the method of Lowry *et al.* (18); its radioactivity was measured in a γ-scintillation counter and expressed as μg of ^{125}I-HSA per mg of cell protein. The amount of cell-associated radioactivity measured at 1 min, which corresponds to surface adsorption, was subtracted from the 60-min value to yield the amount of net uptake.

Measurement of Cellular Uptake of Horseradish Peroxidase and HRP-poly(Lys). The incubation and washing procedures were as described for ^{125}I-HSA uptake, except that the peroxidase and HRP-poly(Lys) concentrations in the medium were 100 μg/ml. The final cell pellet was lysed in 0.05% Triton X-100. The cell extract was used to measure peroxidase activity in the *o*-dianisidine colorimetric assay (17) to give an estimate of the amount of peroxidase taken up by the monolayer.

Cytochemical Demonstration of Horseradish Peroxidase and HRP-poly(Lys). *By light microscopy.* Sparse monolayers of L-929 fibroblasts were grown on glass coverslips in Leighton tubes and incubated in the presence of horseradish peroxidase or HRP-poly(Lys) at concentrations ranging from 1.5 to 150.0 μg/ml. The washing procedure was modified as follows. After incubation, the coverslips were washed twice in balanced salt solution, transferred to clean Leighton tubes, incubated for 4 min at 37° in heparin (5 mg/ml in balanced salt solution), rinsed three times in balanced salt solution, and fixed for 20 min at 25° in 2.5% glutaraldehyde/0.1 M cacodylate buffer, pH 7.4. After five rinses the cells were stained (19) for 10 min with diaminobenzidine (0.5 mg/ml in 0.05 M Tris buffer, pH 7.6/0.01% H_2O_2). After four rinses, the monolayers were fixed for 60 min in 2% O_5O_4 in 0.1 M cacodylate buffer (pH 7.4) at room temperature. The monolayers were rinsed four more times, dehydrated, and mounted on glass slides.

By transmission electron microscopy. Sparse monolayers were grown on carbon-coated glass coverslips in Leighton tubes. The monolayers were processed as described above until completion of the post-osmium rinses. They were then stained *en bloc* overnight at 4° with 1% aqueous uranyl acetate, rinsed abundantly in distilled water, dehydrated, and embedded in Epon. Filled Epon capsules were inverted on top of the monolayers and cured. The hardened capsules were broken away from the coverslip, ripping off the cell-laden carbon film. Thin sections cut parallel to the plane of growth were observed without further staining in a Philips 300 electron microscope.

RESULTS

Uptake of ^{125}I-HSA and ^{125}I-HSA-poly(Lys). Table 1 summarizes the results of five experiments comparing the net cellular uptake of ^{125}I-HSA and ^{125}I-HSA-poly(Lys). The mean (± SEM) uptake of ^{125}I-HSA after 1 hr was 0.506 ± 0.077 μg/mg of protein; that of ^{125}I-HSA-poly(Lys) was 5.62 ± 0.62 μg/mg. This 11-fold enhancement is highly significant ($P < 0.0005$). When added to ^{125}I-HSA-containing medium at concentration roughly comparable to that introduced with the conjugate (10 μg/ml), free poly(Lys) caused no enhancement of ^{125}I-HSA uptake. Unlabeled HSA-poly(Lys) prepared in the same way as the labeled conjugate and added to the ^{125}I-HSA-containing medium at the same final concentration (50 μg/ml) had no effect. Two modifications aimed at reducing the positive charge of the poly(Lys) conjugate markedly reduced the uptake of ^{125}I-HSA-poly(Lys). Brief trypsinization cut the uptake by half, and carbamylation of the α- and ε-amino groups of the conjugate totally abolished enhancement and brought

Table 1. Uptake of ^{125}I-HSA and its poly(Lys) conjugate by L-929 mouse fibroblasts during a 1-hr exposure

Form of ^{125}I-HSA (50 μg/ml)	Additions,* μg/ml	Cell-associated ^{125}I-HSA, μg/mg cell protein† Exp. no. 1	2	3	4	5
HSA-poly(Lys)	—	5.2	5.9	5.0	6.7	5.3
HSA	—	0.41	0.60	0.44	0.59	0.49
HSA	poly(Lys), 10	0.41	—	—	—	—
HSA	HSA-poly(Lys), 50	—	0.77	—	—	0.72
HSA-poly(Lys)	5% FCS	—	6.2	—	—	—
HSA-poly(Lys) (Tryp)‡	5% FCS	—	3.2	—	—	—
HSA-poly(Lys) (Carb)§	—	—	—	0.51	—	—
HSA-CDI¶	—	—	—	—	0.80	—

* FCS, fetal calf serum.
† The amount of ^{125}I-HSA measured after 1 min of incubation was subtracted from all 60-min measurements.
‡ Briefly trypsinized before use; concentration in the medium was 54 μg/ml.
§ Conjugate was carbamylated before use.
¶ Treated with carbodiimide reagent as for conjugation but in the absence of poly(Lys).

the uptake down to control values. Treatment of ^{125}I-HSA alone with the carbodiimide reagent did not significantly alter its cellular uptake.

Uptake of Horseradish Peroxidase and HRP-poly(Lys). The enzymatic activity of horseradish peroxidase was 80 times higher in the extract of cells exposed to HRP-poly(Lys) than in cells exposed to the unmodified enzyme (ΔA460/min, 0.159 and 0.002, respectively). No endogenous peroxidase activity was detected in extracts of control cells. Because the conjugation procedure decreases the enzymatic activity to 50% of the initial value, it is inferred that the 80-fold difference in activity corresponds to a 160-fold increase in enzyme protein—i.e., a 160-fold enhancement of protein uptake (see *Note Added in Proof*).

To ascertain that the peroxidase activity measured in the cell extract corresponded to real cellular uptake, monolayers exposed to the identical experimental conditions were processed for cytochemical staining and cellular localization of peroxidase reaction products. All cells contained strongly stained and coarsely granular reaction product localized predominantly in paranuclear areas of the cells but seen also in more peripheral locations (Fig. 1*A*). The cell surface and the intercellular areas of the coverslip were remarkably free of peroxidase stain. In contrast, cells exposed to unconjugated peroxidase at 15 μg/ml showed no trace of peroxidase reaction product and could not be distinguished from control cultures. To obtain a roughly quantitative estimate of the difference in the cellular uptake of the two forms of peroxidase, the concentrations of peroxidase and HRP-poly(Lys) were chosen so as to differ by a factor of 100. Cells exposed for 60 min to HRP-poly(Lys) at 1.5 μg/ml (Fig. 1*B*) showed a distinct, coarse, and granular accumulation of reaction product, localized mostly in the perinuclear areas of the cell. In contrast, cells exposed for 60 min to unconjugated peroxidase at 150 μg/mg showed only faint, fine granular, and hardly perceptible reaction product visible in only very few cells (Fig. 1*C*). Because the enzymatic activity in the conjugate is only 50% of the initial activity, it would appear that the cellular uptake of the conjugated enzyme protein differs from the cellular uptake of the native enzyme protein by a factor greater than 200.

When HRP-poly(Lys) was exposed briefly to trypsin in order to decrease its poly(Lys) content, the enhancement of HRP-poly(Lys) uptake was abolished (Fig. 1*D*). The same trypsin treatment of HRP-poly(Lys) did not decrease its enzymatic activity *in vitro* and it therefore can be concluded that the difference (Fig. 1 *B* and *D*) reflects the failure of trypsin-treated HRP-poly(Lys) to penetrate the cells.

The intracellular localization of the peroxidase reaction product seen in Fig. 1 was confirmed by electron microscopy (Fig. 2).

Numerous attempts were made to determine cytochemically whether addition of free basic polyamino acids to the incubation medium would enhance the cellular uptake of horseradish peroxidase. The lowest concentration of peroxidase required to give minimal detectable staining after 1 hr of incubation at 37° was 0.1 mg/ml. Monolayers were incubated with peroxidase at 0.1, 0.3, 0.6, and 1.0 mg/ml for 3, 15, 30, and 60 min in the presence or absence of poly(Orn) at 3, 10, and 30 μg/ml. Such conditions were known from previous experiments to cause marked enhancement of ^{125}I-HSA uptake (15). The monolayers were stained for peroxidase, coded for blind examination, and ranked by six examiners according to the amount of visible peroxidase reaction product. The observers were able to detect a time- and concentration-dependent increase of peroxidase uptake and their ranking of slides according to these two parameters was highly consistent. However, they were unable to detect any effect of basic polyamino acids on peroxidase accumulation. Poly(Orn) had no inhibitory effect on the enzymatic activity of horseradish peroxidase *in vitro* when tested at a concentration as high as 1 mg/ml and at a poly(Orn)/peroxidase weight ratio as high as 10^4. These negative results demonstrate that a covalent linkage of the basic polyamino acid to the enzyme is required to enhance the cellular uptake of peroxidase in cytochemically visible fashion.

DISCUSSION

Two different proteins conjugated covalently in the same manner to a fragment of poly(Lys) of molecular weight 6700 show marked increases in their ability to penetrate mammalian fibroblasts grown in culture. That this increase is related to the positive charges of the poly(Lys) fragment is best demonstrated by the fact that chemical masking of these charges by carbamylation totally abolishes the effect (see Table 1, Exp. 3). This conclusion is also supported by the data obtained with mildly trypsinized HRP-poly(Lys). As shown in Fig. 1*D*, trypsin-treated HRP-poly(Lys) is not taken up by fibroblasts in detectable amounts, and it was taken up to a lesser extent than the intact HSA-poly(Lys) (Table 1, Exp. 2). It can be assumed that further trypsinization of the latter conjugate would have further decreased the enhancement of uptake due to conjugation.

It has been shown that aggregation of proteins can itself increase cellular penetration. For instance, ferritin is taken up more readily by sarcoma monolayers in aggregated than in

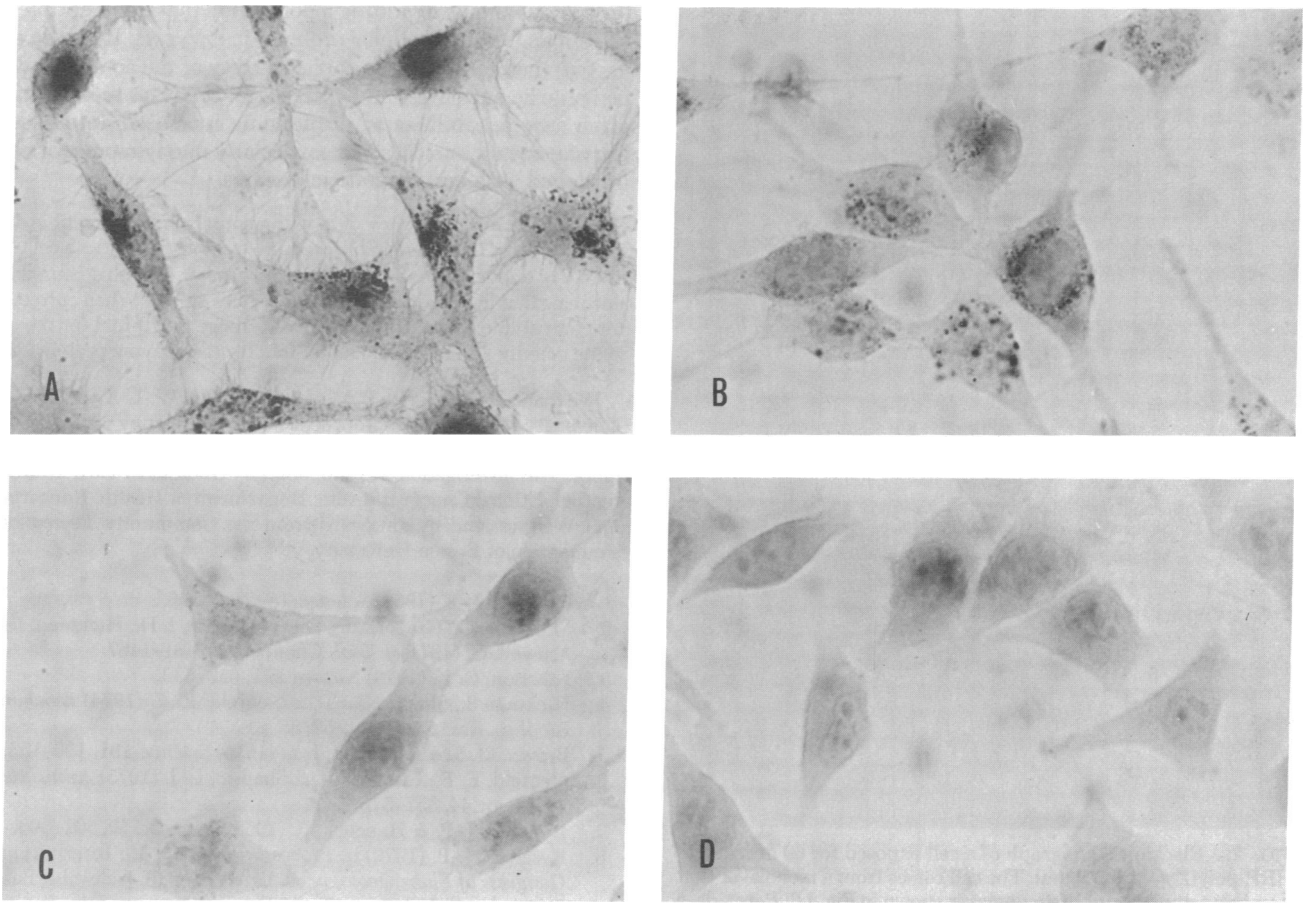

FIG. 1. Monolayer of L-929 mouse fibroblasts exposed for 60 min at 37° to (*A*) HRP-poly(Lys) at 15 μg/ml. (*B*) HRP-poly(Lys) at 1.5 μg/ml. (*C*) Unconjugated horseradish peroxidase at 150 μg/ml. (*D*) HRP-poly(Lys) that had been briefly trypsinized before use, at 1.5 μg/ml. Cells in *A* and *B* show distinct intracellular staining of peroxidase reaction product. Staining is only faint in cells exposed to a higher concentration of unconjugated peroxidase (*C*). Control monolayers as well as cells exposed to unconjugated peroxidase at 15 μg/ml showed no discernible staining and were undistinguishable from the cells in *D*. (×1575.)

soluble form (20). The possibility that intermolecular bonding of the albumin occurring during conjugation might contribute to the enhancement described in Table 1 can be ruled out on the ground that carbamylation, which abolishes enhancement, cannot be expected to break intermolecular bonds and influence the state of albumin aggregation. Large aggregates of ferritin (20) and polynucleotides (21) share the property of enhancing the cellular uptake of ^{125}I-HSA present in the experimental medium, an observation that suggests that large albumin aggregates formed during the conjugation procedure might play a role in enhancing the cellular uptake of ^{125}I-HSA. There are two reasons to exclude such an effect as a factor in the data of Table 1. One is that we showed that ^{125}I-HSA-poly(Lys) contains less than 1% unreacted ^{125}I-HSA. The other is that (as shown by Exps. 2 and 4) the addition of unlabeled HSA-poly(Lys) to ^{125}I-HSA does not significantly influence its uptake. The possibility that enhancement of ^{125}I-HSA-poly(Lys) uptake could be due to some other protein modification caused by the carbodiimide reagent such as the transformation of carboxyl to *N*-acyl urea groups (22) is excluded by the fact that carbodiimide-treated ^{125}I-HSA was not taken up differently than the untreated protein (Exp. 4).

It is apparent also from our data that enhancement requires covalent attachment of poly(Lys) to the two proteins considered in this paper. Indeed, free poly(Lys) of the same molecular weight as that used for conjugation fails to enhance the uptake of ^{125}I-HSA (Table 1, Exp. 1), and poly(Orn) of high molecular weight fails to enhance the uptake of horseradish peroxidase in cytochemical experiments. The lack of effect of poly(Lys) is consistent with previous data that showed that poly(Lys) of low molecular weight had little or no effect on the cellular uptake of ^{125}I-HSA (15).

The amount of cell-associated ^{125}I-HSA measured after 1 min of incubation was generally $\frac{1}{10}$ of the amount measured at 60 min. Such 1-min values are expressions of surface binding, and the subtraction of these values from all measurements rules out the possibility that surface adsorption would contribute significantly to the data of Table 1. Furthermore, one can infer from the intracellular localization of HRP-poly(Lys) (Figs. 1 and 2) that HSA-poly(Lys) is also intracellular.

The addition of the same poly(Lys) fragment causes strikingly greater enhancement of peroxidase uptake than of albumin uptake. We assume that this difference is related to the fact that the two proteins have different isoelectric points (7.2 and 4.7, respectively). The negative charges remaining on the albumin molecule may diminish the effect of poly(Lys) additions, and it can be postulated that a more quantitative substitution of carboxyl groups caused by a more complete conjugation reaction would increase the magnitude of albumin uptake to a level more comparable to that of peroxidase uptake.

The cytochemical data obtained with HRP-poly(Lys) are of particular interest. On the one hand, they show that the covalent linkage of a 6700 molecular weight poly(Lys) fragment does

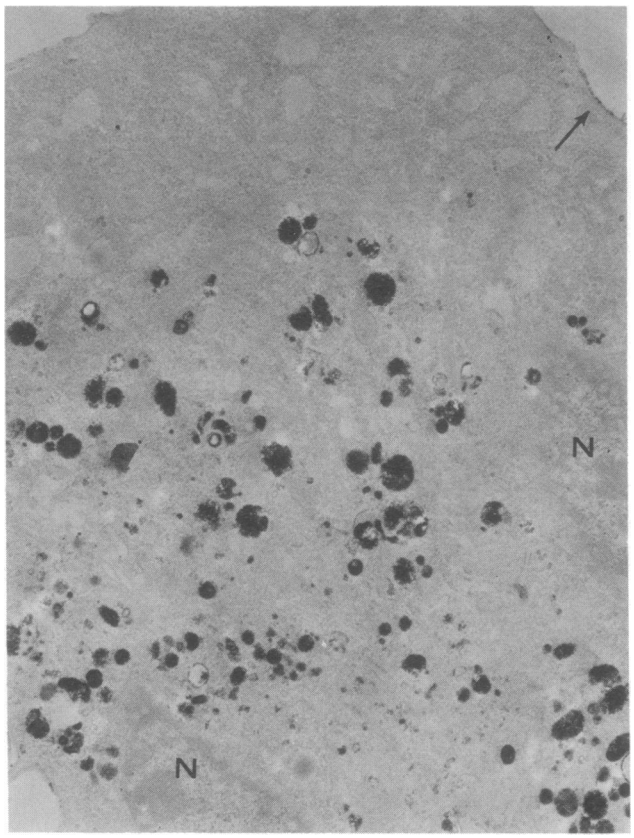

FIG. 2. Electron micrograph of a cell exposed for 60 min at 37° to HRP-poly(Lys) at 1.5 μg/ml. The cell comes from a monolayer that was an exact duplicate of the monolayer shown in Fig. 1*B*. Peroxidase was localized by using the method of Graham and Karnovsky (19). Dark-stained peroxidase reaction product is seen in numerous pinocytotic vesicles and vacuoles, some of which are in close proximity to the nucleus, demonstrating a deep intracellular localization of the conjugated enzyme. The section cuts two parts of the nucleus (N). The arrow points to peroxidase reaction product on one area of the cell surface. (×10,481.) (Photograph courtesy of Frederick B. Merk and Paul W.-L. Kwan.)

not confer cellular toxicity on the protein. On the other hand, they demonstrate that the measured enhancement corresponds to a true cellular uptake of the enzyme. Most importantly, they show that the conjugation procedure does not abolish the enzymatic activity of the peroxidase. It is likely that the loss of activity measured in our experiments can be reduced by modifying the conjugation reaction or the length of the poly-(Lys) fragment. It can be expected that, to some extent, the relative enhancement of uptake and relative loss of activity of an enzyme can be balanced to suit specific needs. The favorable ratio of enhancement to enzyme activity described in this paper is due, we believe, to the fact that a large number of positive charges—at least 30 with poly(Lys)·HBr, molecular weight 6700—can be added to the horseradish peroxidase molecule at the cost of modifying only one exposed carboxyl group on the enzyme. A procedure conjugating a diamine to proteins has been used to cationize ferritin (23, 24) and low density lipoprotein and to enhance the cellular uptake of the latter by human fibroblasts (25). It has been reported, however, that this procedure modifies 70% of the carboxyl groups of the lipoprotein (25). Such a drastic modification can be expected to destroy the biological activity of most functional proteins. The poly(Lys) conjugation method described in this paper has the further advantage of adding a polycation that is susceptible to trypsin and to intracellular proteolytic enzymes. It can be expected, therefore, that the carrier moiety of the conjugate will be nontoxic once ingested by the target cell. This method may offer new possibilities of introducing increased amounts of active enzymes into cells and particularly into lysosomes of cells burdened with certain genetic diseases.

Note Added in Proof. New data with another preparation of HRP-poly(Lys) show that comparable exposure to 15 μg/ml causes a cellular uptake of active enzyme that is 4.7-fold greater than that caused by unconjugated horseradish peroxidase at 1500 μg/ml. When corrected for comparable concentrations, the difference is 470-fold for the enzyme activity and more than 1000-fold for the enzyme protein (26).

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1. Ryser, H. J.-P. (1968) *Science* **159,** 390–396.
2. Morell, A. G., Gregoriadis, G., Scheinberg, I. H., Hickman, I. & Ashwell, G. (1971) *J. Biol. Chem.* **246,** 1461–1467.
3. Nicolson, G. L. (1974) *Nature* **251,** 628–630.
4. Hickman S., Shapiro, L. T. & Neufeld, E. F. (1974) *Biochem. Biophys. Res. Commun.* **57,** 55–61.
5. Brown, M. S. & Goldstein, J. L. (1976) *Science* **191,** 150–154.
6. Neufeld, E. F., Lim, T. W. & Shapiro, L. J. (1975) *Annu. Rev. Biochem.* **44,** 357–376.
7. Ryser, H. J.-P. & Hancock, R. (1965) *Science* **150,** 501–503.
8. Ryser, H. J.-P. (1970) in *Proceedings of the 4th International Congress of Pharmacology,* ed. Eigenmann, R. (Schwabe, Basel, Switzerland), Vol. 3, pp. 96–132.
9. Ryser, H. J.-P. (1968) *Bull. Swiss Acad. Med. Sci.* **24,** 363–385.
10. Tillis, J. G. (1967) *Proc. Soc. Exp. Biol. Med.* **125,** 996–999.
11. Pitha, J. & Pitha, P. M. (1971) *J. Gen. Virol.* **24,** 385–389.
12. Koch, G., Quintrell, N. & Bishop, J. M. (1966) *Biochem. Biophys. Res. Commun.* **24,** 304–309.
13. Moehring, J. M. & Moehring, T. J. (1968) *J. Exp. Med.* **127,** 541–554.
14. Takebe, I. & Otsuki, Y. (1969) *Proc. Natl. Acad. Sci. USA* **64,** 843–848.
15. Ryser, H. J.-P. (1967) *Nature* **215,** 934–936.
16. Sonoda, S. & Schlamowitz, M. (1970) *Immunochemistry* **7,** 885–898.
17. Worthington Biochemical Corporation (1972) *Worthington Enzyme Manual* (Worthington Biochemical Corp., Freehold, NJ), p. 43–45.
18. Lowry, O. H., Rosebrough, N. S., Farr, A. L. & Randall, R. I. (1951) *J. Biol. Chem.* **193,** 265–275.
19. Graham, R. C. & Karnovsky, M. J. (1966) *J. Histochem. Cytochem.* **14,** 291–302.
20. Petitpierre-Gabathuler, M.-P. & Ryser, H. J.-P. (1975) *J. Cell Sci.* **19,** 141–156.
21. Ryser, H. J.-P., Termini, T. E. & Barnes, P. R. (1976) *J. Cell. Physiol.* **87,** 221–228.
22. Franzblau, C., Gallop, P. M. & Seifens, S. (1963) *Biopolymers* **1,** 79–97.
23. Danon, D., Goldstein, L., Marikovsky, Y. & Shutelsky, E. (1972) *J. Ultrastruct. Res.* **38,** 500–512.
24. Grinnell, F., Tobleman, M. Q. & Hackenbrock, C. R. (1975) *J. Cell Biol.* **66,** 470–479.
25. Basu, S. K., Anderson, G. W., Goldstein, J.-L. & Brown, M.S. (1977) *J. Cell Biol.* **74,** 119–135.
26. Ryser, H. J.-P., Shen, W.-C. & Merk, F. B. (1978) *Life Sciences* **22,** 1253–1260.