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Inhibition of insulin degradation by hepatoma cells after microinjection of monoclonal antibodies to a specific cytosolic protease

(hormone/proteolysis/HepG2 cells)

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ABSTRACT **Four monoclonal antibodies were identified by their ability to bind to ^{125}I-labeled insulin covalently linked to a cytosolic insulin-degrading enzyme from human erythrocytes. All four antibodies were also found to remove more than 90% of the insulin-degrading activity from erythrocyte extracts. These antibodies were shown to be directed to different sites on the enzyme by mapping studies and by their various properties. Two antibodies recognized the insulin-degrading enzyme from rat liver; one inhibited the erythrocyte enzyme directly; and two recognized the enzyme after gel electrophoresis and transfer to nitrocellulose filters. By this latter procedure and immunoprecipitation from metabolically labeled cells, the enzyme from a variety of tissues was shown to be composed of a single polypeptide chain of apparent M_r 110,000. Finally, these monoclonal antibodies were microinjected into the cytoplasm of a human hepatoma cell line to assess the contribution of this enzyme to insulin degradation in the intact cell. In five separate experiments, preloading of cells with these monoclonal antibodies resulted in an inhibition of insulin degradation of 18–54% (average 39%) and increased the amount of ^{125}I-labeled insulin associated with the cells. In contrast, microinjection of control antibody or an extraneous monoclonal antibody had no effect on insulin degradation or on the amount of insulin associated with the cells. Moreover, the monoclonal antibodies to the insulin-degrading enzyme caused no significant inhibition of degradation of another molecule, low density lipoprotein. Thus, these results support a role for this enzyme in insulin degradation in the intact cell.**

Soon after the discovery of insulin, it became apparent that every cell that responds to this hormone also rapidly degrades it (1). This degradation has been proposed to function either in the termination of the response of the cell to insulin (2) or possibly in the generation of the signal for the insulin response (3). Also, in several cases insulin-resistance has been reported to occur as a result of excessive insulin degradation (4–8).

For these reasons, insulin degradation has been extensively studied (reviewed in ref. 2). After binding to its receptor, insulin is internalized and degraded primarily via a nonlysosomal pathway (9–13) although in some cells a lysosomal pathway also contributes substantially to the degradation of insulin (2). One enzyme, called insulin-degrading enzyme (IDE) (14), insulin protease (15, 16), or insulinase, has been implicated in this degradation since it cleaves insulin in a limited number of sites (17), which are consistent with the peptide intermediates found in cells (18, 19). Also this enzyme accounts for most of the insulin-degrading activity present in extracts of many different cell types (20–23). Finally, several agents (i.e., bacitracin and sulfhydryl modifying reagents), which inhibit the activity of this enzyme, inhibit insulin degradation in the intact cell (24–26). However, these agents are not specific for the IDE. Thus, these results must be verified with more selective reagents for the enzyme. Moreover, the properties of this IDE have been controversial. The molecular weight of the enzyme, its specificity, and its state of purity have all been disputed (27–29).

To further study this enzyme and its role in insulin degradation we, therefore, set out to produce monoclonal antibodies to the enzyme. This report describes the generation and characterization of four monoclonal antibodies to an enzyme that accounts for most of the insulin-degrading activity in human erythrocyte extracts. These antibodies have been used to show that the enzyme is present in a variety of tissues and is composed of a single polypeptide chain of M_r 110,000. Moreover, these antibodies have been microinjected into cells to assess the contribution of this enzyme to insulin degradation in the intact cell.

MATERIALS AND METHODS

Production of Monoclonal Antibodies. BALB/c mice (6 to 8 wk old) were injected three times at monthly intervals with 10–20 μg of purified human erythrocyte IDE (30) emulsified in Freund's complete adjuvant. Five and three days prior to fusion, the mouse with the highest titer was injected intravenously with 10 μg of enzyme. Its splenic lymphocytes were fused to SP2/0 myeloma cells (31) as detailed by de St. Groth and Scheidegger (32). When the hybridomas were semiconfluent, their supernatants were tested for the ability to precipitate ^{125}I-labeled insulin (^{125}I-insulin) covalently coupled to IDE (30). For this assay, 50 μl of each hybridoma supernatant were incubated in 96-well polyvinyl chloride microtiter plates coated with rabbit anti-mouse IgG at 40 μg/ml (33). After 2 hr at 24°C, wells were washed twice with phosphate-buffered saline (PBS; 20 mM sodium phosphate, 150 mM NaCl, pH 7.6) containing 0.1% bovine serum albumin (BSA) and 0.05% Tween 20. Each well was then incubated with 50 μl of IDE cross-linked to ^{125}I-insulin (about 10,000 cpm). After 2 hr at 24°C, wells were washed twice and cut apart. The radioactivity in each was then measured.

Hybridomas that were positive in the above test were cloned and used to produce ascites tumors. Immunoglobulin from ascites fluid was purified on protein A-Sepharose. Heavy and light chains were identified by the use of specific antisera (Miles) in Ouchterlony two-dimensional immunodiffusion.

Production of IDE Cross-Linked to ^{125}I-Insulin. IDE, partially purified from 400 ml of packed human erythrocytes by

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Abbreviations: IDE, insulin-degrading enzyme; LDL, low density lipoprotein; ^{125}I-insulin, ^{125}I-labeled insulin; BSA, bovine serum albumin.

ammonium sulfate fractionation, chromatography on DEAE-Sephadex and pentyl-agarose (30), was concentrated to 0.4 ml on a YM30 Centricon (Amicon). Then 0.5 ml of ^{125}I-insulin (100 μCi, 100 μCi/μg; 1 Ci = 37 GBq) was added to the IDE; and, after 1 hr at 4°C, 50 μl of 3 mM disuccinimidyl suberate was added. After an additional 40 min at 0°C, 0.1 M Tris·HCl, pH 7.6 was added; and the free ^{125}I-insulin was removed by chromatography of the reaction mixture on a Sephadex G-200 column (1.5 × 90 cm) preequilibrated with PBS containing 0.2% BSA. Radioactivity in the fractions was quantitated, and the peak of IDE, crosslinked to ^{125}I-insulin (identified by gel electrophoresis), was used for the screening assays.

Immunoblotting. Cytosol fractions from various cell lines or tissues were electrophoresed on 7.5% polyacrylamide/$NaDodSO_4$ gels (34) and transferred to nitrocellulose sheets (35). The membranes were blocked with 3% (wt/vol) BSA in PBS, pH 7.4. Filters were then incubated with monoclonal antibody (10 μg/ml) for 2 hr at 24°C, washed, and incubated with peroxidase-conjugated rabbit anti-mouse immunoglobulin (1:1000 dilution, Bio-Rad). After 2 hr at 24°C, the filters were washed, and bound immunoglobulin was visualized with the HRP Color Development Substrate from Bio-Rad, as described by the supplier.

Immunoprecipitations. Cell or organ extracts were incubated with the indicated monoclonal antibodies for 16 hr at 4°C in 200 μl of PBS containing 0.2% BSA. The monoclonal antibodies were precipitated by the addition of 50 μl of *Staphylococcus aureus* coated with anti-mouse IgG and centrifugation for 5 min at 10,000 × *g*. The supernatants were tested for their insulin-degrading activity by the trichloroacetic acid precipitation method (30).

Metabolic Labeling Studies. The human hepatoma cells HepG2 were grown in 25-cm^2 tissue culture flasks until confluent and then switched to methionine-free minimal Eagle's medium (MEM) containing 10% (vol/vol) dialyzed fetal calf sera and [^{35}S]methionine at 50 μCi/ml. After 16 hr at 37°C, the cells were washed twice with PBS and lysed with 1 ml of 1% Triton X-100 containing 50 mM Hepes, 150 mM NaCl, bacitracin at 1 mg/ml, and 1 mM phenylmethylsulfonyl fluoride. The lysate was centrifuged for 1 hr at 100,000 × *g*, precleared, and incubated with 100 nM of a pool of monoclonal antibodies (9B12, 31H7, and 26C11). These antibodies were precipitated with anti-mouse IgG-coated *S. aureus*, washed three times with 50 mM Hepes, pH 7.6, containing 0.5 M NaCl and 0.05% $NaDodSO_4$, resuspended in 0.1% $NaDodSO_4$ with 5% (vol/vol) 2-mercaptoethanol, heated for 1 min at 100°C, and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis.

Mapping Studies. The sites recognized by the various monoclonal antibodies were mapped by first incubating ^{125}I-insulin cross-linked to IDE with an excess (10^{-7} M) of one monoclonal antibody. This complex was then tested for its ability to be precipitated by a second monoclonal antibody that was adsorbed to microtiter wells coated with anti-mouse IgG as described above for the screening assay except that 4% (vol/vol) normal mouse serum was included to block the excess anti-mouse IgG sites on the wells.

Microinjection Studies. The monoclonal antibodies to IDE were loaded into HepG2 cells by the procedure of Okada and Rechsteiner (36). In brief, the HepG2 cells (≈10^7 cells in suspension) were resuspended in 0.4 ml of 0.5 M sucrose containing 10% (wt/vol) polyethylene glycol 1000 (Baker) and the indicated concentrations of antibodies. After 10 min at 37°C, the cells were diluted with a mixture of 6 ml of MEM and 4 ml of water. After an additional 2 min at 37°C, the cells were pelleted, washed with full strength MEM and plated into 24-well plates (10^6 cells per well) in MEM with 10% (vol/vol) fetal calf serum. After 16 hr at 37°C, wells were washed once with PBS, and switched to 0.5 ml of MEM containing 1% BSA and 200 pM ^{125}I-labeled insulin. After 2 hr at 37°C, the wells were quickly washed twice with ice cold PBS, and the cells were lysed by the addition of 0.05% $NaDodSO_4$ containing bacitracin at 1 mg/ml. The lysate was precipitated by 7.5% (wt/vol) trichloroacetic acid, and the precipitable and nonprecipitable radioactivity was determined.

To assess the effect of the microinjected antibodies on ^{125}I-labeled low density lipoprotein (LDL) degradation, 2.5 μg of ^{125}I-labeled LDL was substituted for ^{125}I-insulin, and degradation was measured as described (37). To assess the effect of the microinjected antibodies on the amount of IDE in the cells, HepG2 cells were prelabeled with [^{35}S]methionine (100 μCi/ml) for 3 hr, microinjected with the monoclonal antibodies as described above, and cultured for 16 hr at 37°C. The cells were then washed three times with PBS, lysed with 0.4 ml of 50 mM Hepes-buffered saline (pH 7.6) containing 0.2% Triton X-100, 2 mM EDTA, bacitracin at 1 mg/ml, and precipitated as described above except that no preclearing step was included. The immunoprecipitates were analyzed by $NaDodSO_4$ gel electrophoresis, and the labeled IDE band was identified by autoradiography, cut out, and quantitated for radioactivity.

RESULTS

Production and Characterization of Monoclonal Antibodies. Four hybridomas out of 736 tested were identified as positive for precipitation of IDE cross-linked to ^{125}I-insulin by a plate precipitation assay (33). The four different antibodies were purified from ascites tumors and found to be of the IgG class with γ1 heavy chains and κ light chains. Control experiments verified that none of the antibodies could precipitate ^{125}I-insulin alone. The ability of the different antibodies to precipitate the ^{125}I-insulin–IDE complex varied (Fig. 1*A*). Two antibodies (9B12 and 31H7) had the highest affinity and could precipitate the greatest amount of enzyme. Antibody 26C11 was slightly less potent, whereas antibody 28H1 was considerably less potent than the other two but still bound significantly more ^{125}I-insulin than normal mouse IgG.

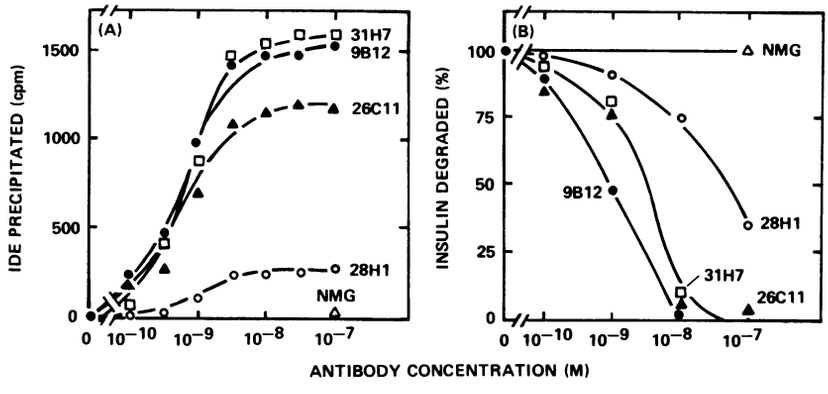

FIG. 1. Immunoprecipitation of the erythrocyte IDE by the monoclonal antibodies. (*A*) Precipitation of ^{125}I-insulin covalently linked to IDE by the monoclonal antibodies in a plate precipitation assay. (*B*) Precipitation of the insulin degrading activity of an erythrocyte extract by the monoclonal antibodies. The control value (100%) in the absence of antibody was 24%. NMG, normal mouse immunoglobulin.

Most important was the finding that the monoclonal antibodies also precipitated the insulin-degrading activity from an erythrocyte extract (Fig. 1*B*). As before, the properties of the different antibodies varied. Antibody 9B12 was the most potent, antibodies 26C11 and 31H7 were slightly less potent, and antibody 28H1 was again the weakest but still precipitated 70% of the insulin-degrading activity at 10^{-7} M. In contrast, control mouse IgG at the same concentration did not decrease this activity.

To determine how many antigenic sites on the enzyme were recognized by the antibodies, each antibody was tested for its ability to block the binding of the enzyme to the other three antibodies (Fig. 2). The IDE binding of antibodies 9B12, 26C11, and 31H7 was not inhibited by any of the other antibodies. The binding of antibody 28H1 to the enzyme was partly inhibited by antibodies 31H7 and 9B12; however, the reverse was not true, suggesting that these antibodies recognize distinct sites.

Additional differences in the properties of the antibodies were noted. Only one of the monoclonal antibodies (31H7) was found to inhibit the enzyme activity directly (Fig. 3). Two of the other antibodies were found to recognize the rat liver enzyme (9B12 and 28H1) and one of these (28H1) also recognized the mouse enzyme (Fig. 4). Further differences in the antibodies were also observed in the immunoblotting experiments described below.

Studies of the Structure of IDE with the Monoclonal Antibodies. Two of the monoclonal antibodies (26C11 and 9B12) were found to react with a single protein of M_r 110,000 in erythrocyte lysates that had been electrophoresed on gels and transferred to nitrocellulose membranes (Fig. 5, lane a). Antibody 9B12 was, therefore, used in immunoblotting experiments to examine the structure of IDE in a variety of cell types. In extracts of human lymphocytes (lane b), hepatoma cells (lane c), rat liver (lane e), and rat muscle (lane f), this antibody was found to recognize only a single protein of apparent M_r 110,000 (Fig. 5). In agreement with the precipitation studies, this antibody did not recognize the enzyme in mouse liver extracts (Fig. 5, lane d).

A pool of monoclonal antibodies (31H7, 9B12, and 26C11) was then used to precipitate IDE from extracts of the human hepatoma cell line HepG2 (38) after metabolically labeling the cells with [^{35}S]methionine. The monoclonal antibodies were found to specifically precipitate only a single polypeptide of apparent M_r 110,000 (Fig. 5, lane g). Quantitation of the radioactivity in this band indicated that about 0.01% of the radioactive methionine that was incorporated into the cell was incorporated into this protein. Pulse-labeling studies did not result in the identification of any precursor form of IDE. Similar results were obtained with ^{35}S-labeled lysates of human lymphocytes (IM-9 cells).

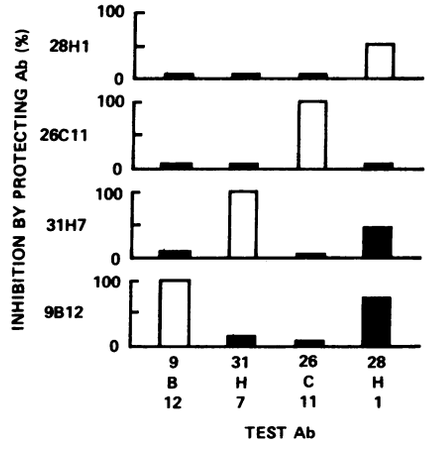

FIG. 2. Mapping the antigenic sites recognized by the monoclonal antibodies. Labeled enzyme was preincubated with the indicated protecting antibodies and then tested for binding to microtiter wells coated with the indicated test antibodies. The bars indicate the percent inhibition of IDE precipitation caused by the protecting antibody.

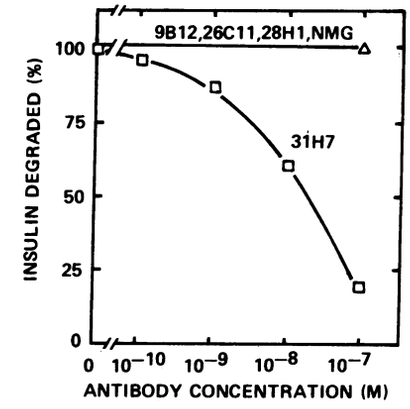

FIG. 3. Effect of the monoclonal antibodies on the insulin-degrading activity of the erythrocyte enzyme. Enzyme was incubated with the indicated concentrations of antibody and then tested for its ability to degrade ^{125}I-insulin. The control value (100%) in the absence of antibody was 23%.

Studies of the Role of IDE in Insulin Degradation in Intact Cells. To test the role of IDE in insulin degradation in intact cells, a pool of the monoclonal antibodies to IDE (antibodies 31H7, 9B12, 26C11, and 28H1) were preloaded into the cytoplasm of HepG2 cells by the procedure of Okada and Rechsteiner (36). The antibody-loaded cells were then incubated with ^{125}I-insulin and the extent of ^{125}I-insulin degradation was assessed. In five separate experiments, preloading of cells with monoclonal antibodies to IDE at 10–20 mg/ml resulted in an inhibition of insulin degradation in the intact cell of 18–54% (the average inhibition was 39%). The results of one such experiment are shown in Table 1. In cells that were preloaded with antibodies to IDE at 20 mg/ml, 17% of the cell-associated ^{125}I-insulin was degraded (Table 1). In contrast, in cells injected with either buffer, normal mouse IgG (20 mg/ml) or a monoclonal antibody to an extraneous protein 32, 32, and 28%, respectively, of the ^{125}I-insulin was degraded. The effect of the monoclonal antibodies was dose dependent since preloading the cells with antibodies to IDE at 5 mg/ml only slightly decreased insulin degradation. In addition to the effect on insulin degradation, an increase in

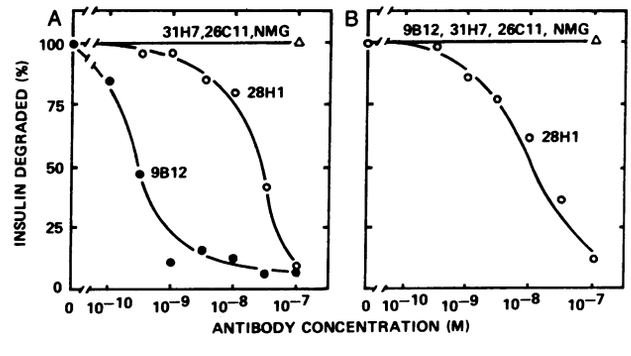

FIG. 4. Immunoprecipitation of the insulin degrading activity from rat (*A*) and mouse (*B*) liver extracts. Extracts of these tissues were adsorbed with the indicated concentrations of monoclonal antibodies, and their supernatants were tested for insulin-degrading activity. Control values (100%) were 14 and 15% for rat and mouse liver, respectively.

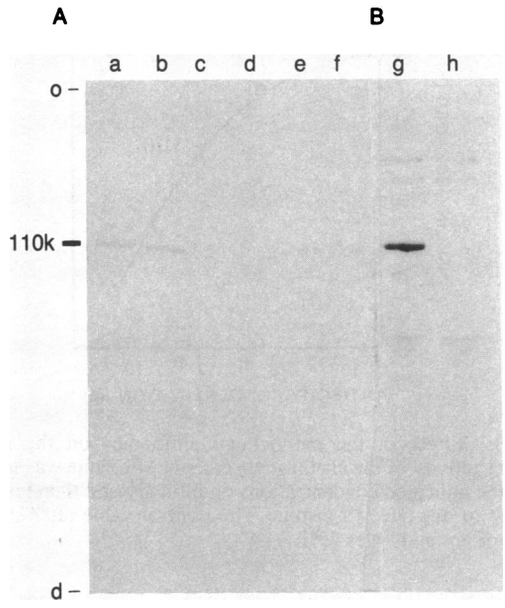

FIG. 5. Structure of the IDE in different tissues. (*A*) Immunoblotting. Extracts of human erythrocytes (lane a), hepatoma HepG2 cells (lane b), IM-9 lymphocytes (lane c), mouse liver (lane d), rat liver (lane e), and rat muscle (f) were electrophoresed on $NaDodSO_4$ gels, transferred to nitrocellulose, and tested for reactivity with antibody 9B12. (*B*) Immunoprecipitation from metabolically labeled human hepatoma cells (HepG2). Labeled extracts of HepG2 cells were precipitated with either monoclonal antibodies to IDE (lane g) or normal IgG (lane h), the precipitated material was electrophoresed, and an autoradiograph of the gel is shown. o, origin; d, dye front; 110k, M_r 110,000 protein.

the amount of ^{125}I-insulin associated with the cells was also observed with cells that had been microinjected with monoclonal antibodies to IDE at 20 mg/ml (2291 cpm vs. 1601 cpm in antibody-loaded cells and control cells, respectively) (Table 1). No such increase in ^{125}I-insulin was observed with cells that had been preloaded with control IgG (1542 cpm) or an extraneous monoclonal antibody (1592 cpm). These effects were specific to insulin since preloading of the cells with the monoclonal antibodies to IDE had no effect on the degradation and binding of ^{125}I-labeled LDL, a protein which is degraded by lysosomes (40).

To determine whether the presence of the antibodies was affecting the amount of IDE present in the cells, the cells were labeled with [^{35}S]methionine and preloaded with either control immunoglobulin or monoclonal antibodies to IDE. The amount of labeled IDE present was determined after 16 hr by immunoprecipitation and $NaDodSO_4$ gel electrophoresis. A decrease of 53% in the amount of labeled IDE present in the cells was observed by this procedure (Table 1) after microinjection of the monoclonal antibodies to IDE at 20 mg/ml. In contrast, normal mouse IgG had no effect on the amount of IDE present in cells.

DISCUSSION

The present studies were undertaken to produce monoclonal antibodies to IDE, a particular protease with specificity for insulin (2). Although this enzyme has been extensively studied (2), questions still remain on the specificity of this enzyme, its molecular weight, and its role in insulin degradation in the intact cell (27–29). The present paper describes the production of four monoclonal antibodies to this enzyme. These antibodies were detected by their ability to precipitate ^{125}I-insulin cross-linked to IDE in a plate precipitation assay (Fig. 1*A*). This cross-linking procedure has been shown to specifically label the enzyme in crude cell lysates (30), and thus this screening procedure did not require pure enzyme. Also, the utilization of the plate precipitation assay (33) greatly reduced the amount of work involved in the screening of the hybridoma supernatants. All four monoclonal antibodies that were identified as precipitating the labeled enzyme were found also to precipitate the insulin-degrading activity in erythrocyte lysates (Fig. 1*B*). These results demonstrate that the antibodies were directed against a protease in erythrocyte lysates that accounts for most of the insulin-degrading activity present in these cells. Further studies demonstrated that an immunologically related enzyme accounts for most of the insulin-degrading activity of rat and mouse liver extracts (Fig. 4).

The four monoclonal antibodies were characterized by a variety of techniques and found to have different properties. Antibody 28H1, which had the weakest affinity for the enzyme, was the only antibody that cross-reacted with IDE from rat and mouse liver (Fig. 4). Antibody 31H7 was the only antibody that inhibited the activity of IDE directly (Fig. 3), indicating that this antibody binds to a site that is required for the activity of the enzyme. Antibody 9B12 was the only antibody that recognized the rat enzyme and reacted with the enzyme after gel electrophoresis and transfer to nitrocellulose (Figs. 4 and 5). In addition to the different properties of the antibodies, they were also shown by mapping studies to bind to different sites on the enzyme since they did not inhibit the binding of each other to the enzyme (Fig. 2).

These antibodies were then utilized to examine the structure of IDE. The molecular weight of this enzyme on

Table 1. Microinjection experiments with HepG2 cells

Antibody injected*	^{125}I-insulin associated with cells,† cpm	^{125}I-insulin degradation,‡ %	^{125}I-labeled LDL degradation, %	[^{35}S]IDE present in HepG2 cells, cpm
None	1601 ± 120	32.3 ± 2.3	0.55 ± 0.02	560
Ab to IDE (20 mg/ml)	2291 ± 68	16.6 ± 0.3	0.50 ± 0.02	260
Ab to IDE (5 mg/ml)	1838 ± 93	30.3 ± 1.7	0.59 ± 0.02	n.t.
Normal mouse IgG (20 mg/ml)	1540 ± 30	32.2 ± 0.7	0.51 ± 0.01	570
Control monoclonal§ Ab (20 mg/ml)	1592 ± 81	28.4 ± 2.7	n.t.	n.t.

n.t., not tested; Ab, antibody.
*These values represent concentrations of Ab with which cells were preincubated.
†Cells, preloaded with the indicated antibodies, were incubated with ^{125}I-insulin, and cell-associated ^{125}I-labeled insulin was determined. Values given are averages of three separate dishes ± SD.
‡The percent insulin degradation was calculated from a trichloroacetic acid precipitation assay of the cell-associated insulin.
§The control monoclonal antibody was a monoclonal antibody to rat liver thiol:protein-disulfide oxidoreductase and is an IgG, with κ light chains (39).

$NaDodSO_4$ gels has been reported to be 30,000 (41), 60,000 (14, 42), and 115,000 (28). By using one of these antibodies that reacted with IDE after transfer to the nitrocellulose filter, the apparent M_r of IDE was found to be 110,000 in human erythrocytes, hepatoma cells, lymphocytes, rat liver, and rat muscle (Fig. 5). A pool of three of the monoclonal antibodies was also used to immunoprecipitate IDE from metabolically labeled human hepatoma cells, HepG2 (Fig. 5), and IM-9 cells (data not shown). Again, the antibodies were found to specifically recognize only a single protein of M_r 110,000. These results show that the enzyme in a variety of tissues consists of only a single polypeptide of M_r 110,000. This value agrees with the molecular weight found by cross-linking studies (30) and that reported by Kirschner and Goldberg (28) for the purified enzyme but differs from the molecular weight reported in several other studies (14, 23, 41, 42).

The availability of specific antibodies to IDE allowed us to directly test the role of this enzyme in insulin degradation in the intact cell. A pool of the monoclonal antibodies were preloaded into the cytoplasm of HepG2 cells by the procedure of Okada and Rechsteiner (36). In five separate experiments, the cells that were preloaded with the pool of monoclonal antibodies to IDE degraded from 18 to 54% less insulin than cells that were either injected with just buffer, normal immunoglobulins, or extraneous monoclonal antibodies. In addition, there was an increase in the amount of ^{125}I-insulin that was associated with the cells preloaded with the monoclonal antibodies to IDE (Table 1). This increase in the amount of insulin associated with the cells would be expected from a decrease in insulin degradation. In contrast to the results with insulin, no change in the degradation or binding was observed with another protein, ^{125}I-labeled LDL, which is predominantly degraded by lysosomes (40).

The ability of the antibodies to only partly inhibit insulin degradation in HepG2 can be explained in several ways. First, IDE levels in the cells were decreased by only 53% (Table 1). Thus, the intracellular concentration of antibody may not be high enough to reduce IDE levels to a greater extent. Second, it may be possible that the internalized insulin has two pathways for degradation; one mediated via IDE, and the other mediated via another mechanism. Evidence has been presented that indicates that a portion of the internalized insulin in some cells is degraded via a lysosomal pathway (12, 13, 19). However, the amount of insulin that utilizes the lysosomal pathway varies from cell type to cell type and even under different cell culturing conditions. This latter effect may in part explain the variable results we obtained in inhibiting insulin degradation in different experiments.

In summary, the present paper describes the production and characterization of four monoclonal antibodies to an enzyme that readily degrades insulin. These antibodies have been utilized to demonstrate that the enzyme in a variety of tissues is composed of a single polypeptide chain of M_r 110,000. This enzyme accounts for the majority of insulin-degrading activity in erythrocyte and liver extracts, and the structure of this enzyme appears to be fairly well conserved, since two of the antibodies were capable of recognizing both the human and rat forms of the enzyme. Furthermore, these antibodies when preloaded into the cytoplasm of cells have been shown to partly inhibit insulin degradation in the intact cell. These results are consistent, therefore, with a role for this enzyme in insulin degradation. These antibodies will be an extremely useful tool for further studies on the cellular localization of this enzyme, on the synthesis and structure of the enzyme and on the role of this enzyme in insulin-resistant states due to excessive insulin degradation.

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