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The structure of the hepatic insulin receptor and insulin binding

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Hepatocytes or hepatic plasma membranes were photoaffinity-labelled with radioiodinated $N^{\epsilon B29}$-monoazidobenzoyl-insulin. Analysis of the samples by SDS/polyacrylamide-gel electrophoresis and autoradiography revealed the insulin receptor as a predominant band of 450 kDa. When hepatic plasma membranes were first treated with clostridial collagenase and then photolabelled, the insulin receptor appeared as a predominant band of 360 kDa. This effect of collagenase treatment on the insulin receptor was due to Ca^{2+}-dependent heat-labile proteinases contaminating the preparation of collagenase, and it could be mimicked by elastase. The decrease in size of the insulin receptor to 360 kDa resulted from the loss of a receptor component that was inaccessible to photolabelling. In contrast, the size of the insulin receptor of intact cells was not affected by collagenase treatment. This suggests that the site sensitive to proteolysis was located on the cytoplasmic side of the plasma membrane. In hepatic plasma membranes that were treated with collagenase or elastase, and contained the 360 kDa form of the insulin receptor, the binding affinity for insulin was increased by up to 2-fold. These findings support the concept that a component which is either a part of, or closely associated with, the insulin receptor may regulate its affinity for insulin.

INTRODUCTION

Biosynthetic studies (Deutsch *et al.*, 1983; Ronnett *et al.*, 1984) and cDNA cloning (Ullrich *et al.*, 1985) have shown that the α and β subunits of the insulin receptor are derived from a single precursor of 190–210 kDa. In contrast, studies in which insulin receptors were labelled by either chemical cross-linking (Massague *et al.*, 1980; Crettaz *et al.*, 1984) or photoaffinity labelling (Yip & Moule, 1983; Heidenreich *et al.*, 1983*a*) and then analysed by SDS/polyacrylamide-gel electrophoresis reveal receptor structures which range in size from 450 kDa to 250 kDa. These results indicate that the $\alpha\beta$ precursor of the insulin receptor must form larger structures in the plasma membrane. Massague *et al.* (1980, 1981) and other investigators (Fujita-Yamaguchi, 1984; Boyle *et al.*, 1985) have proposed that the insulin receptor is assembled as a disulphide-linked heterodimer, $(\alpha\beta)_2$. Others have suggested that components of 40–45 kDa (Yip *et al.*, 1982; Goren *et al.*, 1983; Brossette *et al.*, 1984; Phillips *et al.*, 1986) and 85 kDa (Heidenreich *et al.*, 1983*b*; Yip & Moule, 1983) may also be associated with the insulin receptor in the plasma membrane.

Several laboratories have suggested that the appearance of heterogeneous high-M_r forms of the insulin receptor may be an artifact caused by thiol/disulphide exchange between subunits during solubilization in SDS (Endo & Elsas, 1984; Boyle *et al.*, 1985; Helmerhorst *et al.*, 1986). Consequently, the physiological significance of different structural forms of the insulin receptor is not known. However, it has been suggested that the structure of the insulin receptor may influence its affinity for insulin (Ginsberg *et al.*, 1976; Crettaz *et al.*, 1984) as well as its kinase activity (Fujita-Yamaguchi & Kathuria, 1985). In the present study, we have directly manipulated the structure of the insulin receptor by limited enzymic digestion of hepatic plasma membranes. We have then examined some kinetic parameters of insulin binding by membranes which contained discrete structural forms of the insulin receptor.

MATERIALS AND METHODS

Materials

The preparation and characterization of the photoreactive radioiodinated derivative of insulin, $N^{\epsilon B29}$-monoazidobenzoyl-insulin, have been described previously (Yip *et al.*, 1982). Bovine insulin was a gift from Connaught Laboratories (Canada). Type I clostridial collagenase [130 units (μmol/5 h)/mg] was purchased from Worthington Diagnostic systems (Freehold, NJ, U.S.A.). Endo-β-*N*-acetylglucosaminidase F was a gift from Dr. S. A. Rosenzweig (Yale University School of Medicine) (Rosenzweig *et al.*, 1984). Type IV elastase, Trypan Blue and all other enzymes and inhibitors were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Leibovitz medium (L-15), Eagle's basal medium, penicillin, streptomycin, fetal-calf serum and fungizone were purchased from GIBCO (Canada). [[^{125}I]Tyr^{A14}]-Insulin and α-aminoisobutyric acid were obtained from Amersham (Canada). Chemicals for polyacrylamide gel electrophoresis were obtained from Bio-Rad Laboratories (Richmond, CA, U.S.A.). All other chemicals were of reagent grade.

Preparation of hepatocytes and plasma membranes

Hepatocytes were isolated from male Wistar rats (Charles River; 225–275 g) by perfusion of the liver with clostridial collagenase (500 μg/ml; 15 min; 37 °C) by the method of Berry & Friend (1969), except that heparin (1.5 units/ml) was added to the pre-perfusion buffer. Exclusion of Trypan Blue by freshly isolated cells was over 94%. The sensitivity to insulin of amino acid transport in freshly isolated cells was measured by the method of Fehlmann *et al.* (1979). Insulin (100 nM)

induced a significant ($P < 0.01$) 70% increase in the uptake of α-aminoisobutyric acid. For culture, hepatocytes were plated on to Heraeus Petriperm dishes (Tekmar Co., Cincinnati, OH, U.S.A.) and incubated in L-15 medium supplemented with Hepes (18 mM), albumin (2 mg/ml), penicillin (100 units/ml), streptomycin (100 μg/ml), glucose (1.5 mg/ml), insulin (0.5 μg/ml) and 5% fetal-calf serum, and kept at 37 °C under of air/CO_2 (19:1). After 24 h the medium was replaced with fresh insulin- and serum-free L-15 and changed every 24 h. The incubation of these cells with 85 nM-insulin caused a 2.5-fold increase in the synthesis of glycogen as measured by the method of Nyfeler *et al.* (1981). Consequently, both cell preparations were responsive to insulin.

A crude plasma-membrane fraction was prepared from rat liver by homogenizing 1 g of minced tissue in 5 ml of Eagle's Basal Medium. The homogenate was centrifuged for 10 s at 8500 ***g***. The supernatant was then centrifuged for 4 min at 8500 ***g*** to yield a pellet, which was resuspended in 4 ml of Eagle's Basal Medium and centrifuged for 4 min at 8500 ***g***. The pellet was resuspended in binding buffer and used without further manipulation. Crude plasma-membrane fractions from freshly isolated or cultured hepatocytes were prepared in a similar fashion. Purified hepatic plasma membranes were prepared by the method of Ray (1970).

Enzyme treatment of membranes

Crude or purified plasma membranes (2 mg of protein) were suspended in 1 ml of 0.1 M-Hepes, pH 7.4, containing 67 mM-NaCl, 6.7 mM-KCl, 4.8 mM-$CaCl_2$,$2H_2O$ and 11 mM-glucose, and incubated with one of the following enzymes for 20 min at 37 °C: collagenase (100 μg), elastase (0.1–10 μg), chymotrypsin (0.1–10 μg) or endo-β-*N*-acetylglucosaminidase F [10 units (nmol/h)]. After incubation, the membranes were washed twice with buffer and then resuspended in fresh buffer.

Photoaffinity labelling and electrophoresis

Cells (10^6 cells/ml) or crude (100–300 μg of protein/ml) or purified (50–100 μg of protein/ml) plasma membrane were incubated with $N^{\epsilon B29}$-monoazidobenzoyl-insulin (10–50 nM) in 1 ml of Eagle's Basal Medium containing 0.16 mg of bacitracin/ml with or without an excess (10 μM) of insulin for 1 h at 18 °C. Photolysis was then carried out for 20 s with a focused light source from a 100 W high-pressure mercury lamp as previously described (Yip & Moule, 1983). Photolabelled samples were washed in Eagle's Basal Medium and then solubilized by vortex-mixing in 100 μl of 62.5 mM-Tris/HCl, pH 6.8, containing 2% SDS and 10% glycerol and keeping on ice for 30 min. Samples that were to be reduced and solubilized were suspended in 100 μl of the solubilization solution containing 0.1 M-dithiothreitol and boiled for 5 min. Electrophoresis in 3–10% and 5–10% gradient polyacrylamide slab gels and autoradiography were carried out as previously described (Yip *et al.*, 1982; Yip & Moule, 1983). Thyroglobulin (669 kDa) and its subunits (475 kDa and 280 kDa), catalase (60 kDa and 120 kDa dimer), myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase (92 kDa), bovine serum albumin (66 kDa) and ovalbumin (45 kDa) were used as M_r markers.

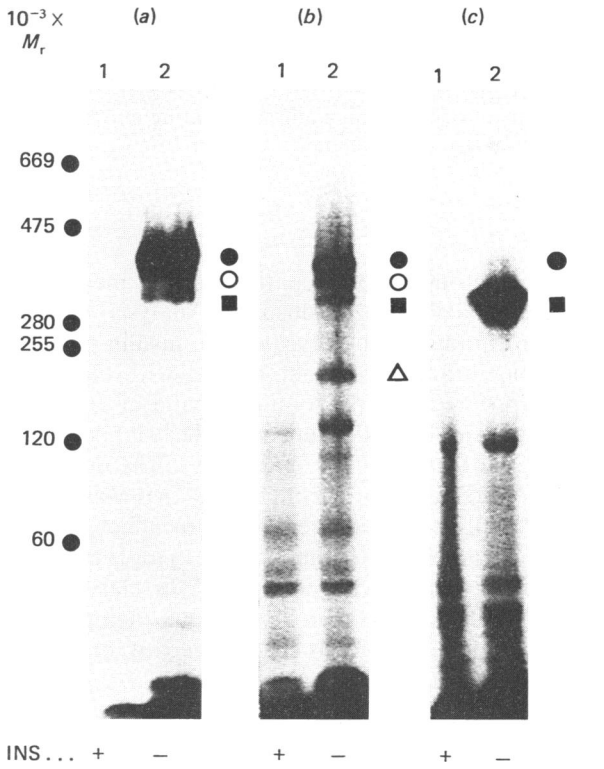

Fig. 1. Autoradiograms of the hepatic insulin receptor detected by photoaffinity labelling

Freshly isolated hepatocytes (*a*) were photoaffinity labelled, solubilized and analysed in a 3–10%-acrylamide gel as described in the text. Freshly prepared crude hepatic plasma membranes were incubated in the absence (*b*) or presence (*c*) of collagenase (100 μg/ml), washed, photolabelled, solubilized and then analysed in a 3–10% gradient gel as described in the text. Lanes marked INS (+) were obtained from samples incubated with photoprobe in the presence of excess insulin. The positions of M_r markers are indicated. The locations of the 450 kDa (●), 410 kDa (○), 360 kDa (■) and 200 kDa (△) oligomeric forms of the insulin receptor are shown.

Insulin binding and Scatchard analysis

Purified hepatic plasma membranes (12.5 μg of protein) were suspended in 0.2 ml of 50 mM-Tris/HCl, pH 7.5, containing 0.1% bovine serum albumin and 1 mM-bacitracin, and incubated with about 15 fmol of [[^{125}I]Tyr^{A14}]insulin (74 TBq/mmol) and various concentrations of unlabelled insulin for 16 h at 4 °C. Equilibrium binding was achieved under these conditions, and more than 98% of the iodinated insulin remained precipitable in 5% trichloroacetic acid at the end of the incubation. Iodoinsulin bound to the membranes was determined as follows: 50 μl of 0.4% bovine γ-globulin and 250 μl of 20% poly(ethylene glycol) in 50 mM-Tris/HCl, pH 7.5, were added to the 0.2 ml incubation mixture and vigorously vortex-mixed. After having been kept in ice for 15 min, this solution was centrifuged at 8500 ***g*** for 15 min. The supernatant was aspirated, and the tip of the Microfuge tube containing the membrane-protein pellet was cut off and its radioactivity determined.

Binding data were analysed by the method of Scatchard (1949), by using the 'Ligand' program of Munson & Rodbard (1980). Goodness of fit of a model

was analysed by this program by using the 'Runs Test' of Bennett & Franklin (1954), which predicts whether the scatter of points about a fit is likely to be due to chance. The goodness of fit of different models, as well as statistical comparisons between curves within a given model, were also assessed by the 'Ligand' program. Non-specific binding was handled as a computer-fitted parameter, and all points were weighted equally.

RESULTS

Photoaffinity labelling of freshly isolated hepatocytes resulted in the specific labelling of a predominant band of 450 kDa and minor bands of 410 kDa and 360 kDa (Fig. 1*a*). Similar results were obtained with crude hepatic plasma membranes, except that an additional specifically labelled band of 200 kDa was also observed (Fig. 1*b*). In contrast, photoaffinity labelling of crude hepatic plasma membranes which had been treated with 100 μg of collagenase/ml (20 min, 37 °C) revealed a predominant specifically labelled band of 360 kDa and no 450 kDa band (Fig. 1*c*). To see if this effect could be produced in intact insulin-responsive cells, we incubated freshly prepared hepatocytes or cultured hepatocytes with 100 μg of collagenase/ml for 20 min at 37 °C. The cells were washed and then photolabelled. In these experiments collagenase treatment did not affect the size of the insulin receptor, which appeared as a predominant band of 450 kDa. However, when crude plasma membranes were prepared from these cells and treated with collagenase, a predominant specifically labelled band of 360 kDa, but not one of 450 kDa, was obtained.

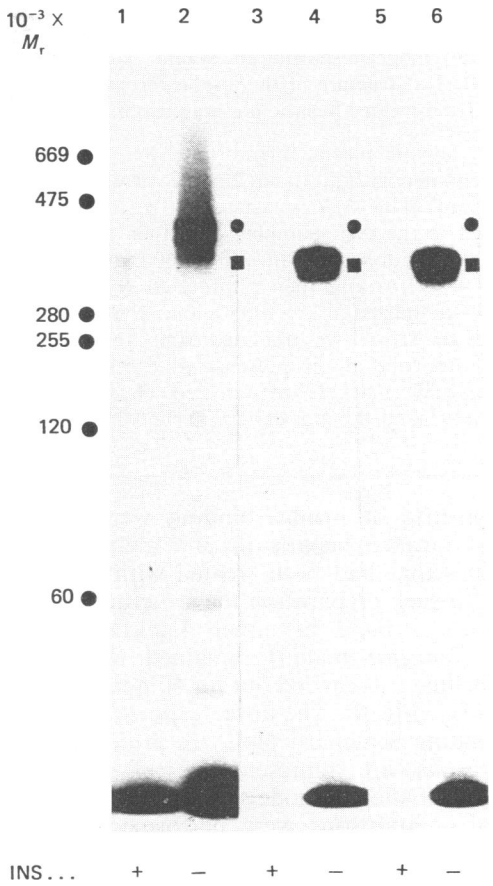

Fig. 2. Autoradiograms showing the effect of enzyme treatment on the oligomeric form of the insulin receptor in purified hepatic plasma membrane

Purified hepatic membranes were incubated in the absence (lanes 1 and 2) or presence of 100 μg of collagenase/ml (lanes 3 and 4) or 1.0 μg of elastase/ml (lanes 5 and 6) as described in the text. The samples were then washed, photoaffinity-labelled, solubilized and analysed in a 3–10% gel as described in the text. Lanes 1, 3 and 5 were obtained from samples incubated with photoprobe in the presence of excess insulin. The positions of the M_r markers are indicated. The locations of the 450 kDa (●) and 360 kDa (■) oligomeric forms of the insulin receptor are shown.

The effect of treatment with collagenase on the size of the insulin receptor in hepatic plasma membranes was found to be Ca^{2+}-dependent, heat-labile and non-diffusable. However, this effect was not inhibited by α_2-macroglobulin (1 molar equivalent), a specific inhibitor of collagenolytic enzymes (Werb *et al.*, 1974; Woolley *et al.*, 1975). These results indicate that this effect was due to proteinases contaminating the collagenase preparation and not to the collagenolytic enzymes themselves. We found that this proteinase(s) was not inhibited by benzamidine (10 mM), α_1-antitrypsin (3 molar equivalents), bacitracin (1.6 mg/ml), soya-bean trypsin inhibitor (100 μg/ml) or phenylmethanesulphonyl fluoride (2 mM).

Other enzyme preparations were examined for their effect on the insulin receptor in purified hepatic plasma membranes. The treatment of membranes with either elastase or chymotrypsin (0.1–10 μg/ml; 20 min; 37 °C) caused the appearance of oligomeric forms of the insulin receptor smaller than 450 kDa. However, treatment of membranes with 1.0 μg of elastase/ml produced a predominant band of 360 kDa which closely resembled that obtained with collagenase treatment (Fig. 2). When hepatic plasma membranes were treated with endo-β-*N*-acetylglucosaminidase F to remove carbohydrate moieties, the size of the insulin receptor was decreased from 450 kDa to 440 kDa.

The decrease in size of the insulin receptor from 450 kDa to 360 kDa produced by the treatment of hepatic plasma membranes with either collagenase or elastase might have been due to the degradation of subunits. Therefore we analysed the photolabelled subunits in control and enzyme-treated samples of purified hepatic plasma membranes. After reduction with dithiothreitol, each preparation gave rise to specifically labelled bands of 130, 115, 90 and 40 kDa (Fig. 3). To see if quantitative differences existed in the subunit composition of these preparations, we excised the photolabelled bands and determined their radioactivity. In each case the labelled bands of 130, 115, 90 and 40 kDa were present in the approximate proportions 49:26:16:8 respectively. As an alternative approach, hepatic plasma membranes were treated with collagenase or elastase after photolabelling to determine if fragments of labelled subunits were produced. Analysis of these samples after reduction revealed labelled bands of 130, 115, 90 and 40 kDa, but no labelled fragments. Consequently, the conversion of the insulin receptor from 450 kDa to 360 kDa appeared to involve the loss of a component that was inaccesible to photoaffinity labelling.

The bands of 130 kDa and 90 kDa respectively have

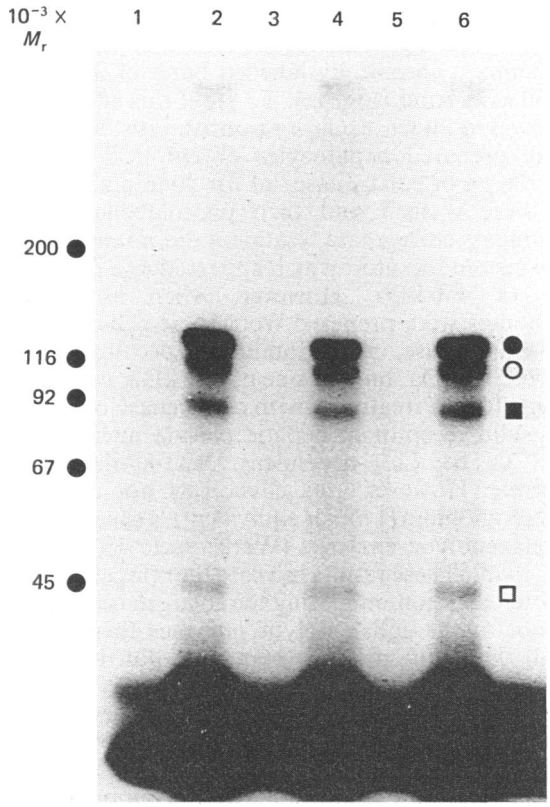

Fig. 3. Autoradiogram showing the effect of enzyme treatment on the subunits photoaffinity-labelled in the hepatic insulin receptor

Purified hepatic plasma membranes were incubated without (lanes 1 and 2) or with 100 μg of collagenase/ml (lanes 3 and 4) or 1.0 μg of elastase/ml (lanes 5 and 6) as described in the text. The samples were then washed, photolabelled, solubilized with dithiothreitol and analysed in a 5–10% gel as described in the text. Lanes 1, 3 and 5 were obtained from samples incubated with photoprobe in the presence of excess insulin. The positions of the M_r markers are indicated. The locations of subunit bands of 130 kDa (●), 115 kDa (○), 90 kDa (■) and 40 kDa (□) are shown.

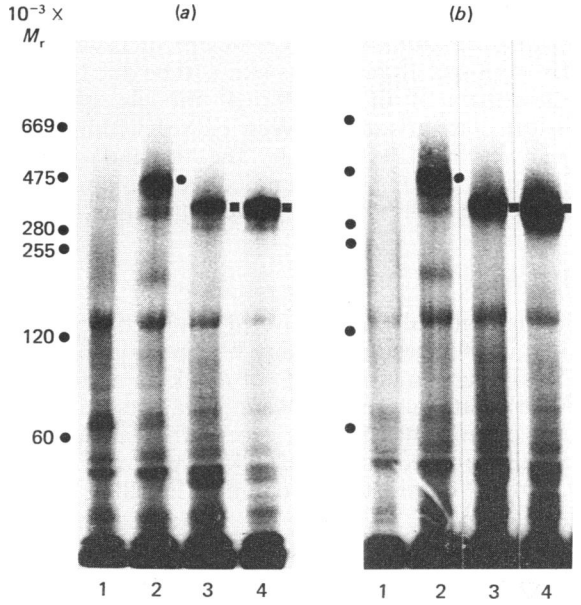

Fig. 4. Autoradiogram showing the stability of the 450 kDa and 360 kDa structure of the insulin receptor in control and enzyme-treated hepatic plasma membranes

Purified hepatic plasma membranes were prepared and then incubated without (lanes 1 and 2) or with collagenase (100 μg/ml) (lane 3) or elastase (1.0 μg/ml) (lane 4) as indicated in the text. Samples were then incubated with $N^{\epsilon B29}$-monoazidobenzoyl-insulin for 60 min at 18 °C (*a*) or 24 h at 4 °C (*b*) and then photolysed. They were then washed, solubilized in 2% SDS and analysed in a 3–10% gradient gel. Lane 1 was obtained from samples incubated with photoprobe in the presence of excess insulin. The positions of M_r markers are indicated. The locations of the specifically labelled bands of 450 (●) and 360 (■) kDa are shown.

been identified as the α and β subunits of the insulin receptor (Massague *et al.*, 1980; Yip *et al.*, 1982; Ullrich *et al.*, 1985). Previously, collagenase (Roth *et al.*, 1983) and elastase (Shia *et al.*, 1983) have been shown to degrade the β subunit in solubilized insulin receptor purified from IM-9 lymphocytes or placenta into fragments of 40–45 kDa or less. In our present study this effect of collaganase or elastase was not observed (Fig. 3). The cause for these different results is not known. However, the retention of the hepatic insulin receptor in its membrane environment may have prevented proteolysis of the β subunit. The 115 kDa band is due to proteolytic nicking of the 130 kDa α subunit (Fehlmann *et al.*, 1982; Haynes & Yip, 1985). Labelled bands of 40 kDa have been suggested to result either from endogenous degradation of the β subunit (Massague *et al.*, 1980, 1981; Fujita-Yamaguchi, 1984; Boyle *et al.*, 1985) or from the association of the insulin receptor with other components in the plasma membrane (Siegel *et al.*, 1981; Yip *et al.*, 1982; Goren *et al.*, 1983; Brossette *et al.*, 1984; Phillips *et al.*, 1986).

The kinetics of insulin binding were examined in untreated purified hepatic plasma membranes and in membranes that had been treated with collagenase or elastase. In each preparation the structure of the insulin receptor as a band of either 450 kDa (control) or 360 kDa (enzyme-treated) remained the same when photolabelling was carried out for 60 min at 18 °C or for 24 h at 4 °C (Fig. 4). The dose–response curves and the corresponding Scatchard plots are presented in Fig. 5. These results are representative of three separate experiments. Different models were fitted to the data, and statistical comparisons were performed by using the 'ligand' program. In each case, a one-site model adequately described the data. The association constant and binding capacity for insulin binding to untreated membrane were 1.0 ± 0.1 nM^{-1} and 3.1 ± 0.2 pmol/mg of membrane protein respectively. The association constant was significantly increased ($P < 0.001$) to 1.8 ± 0.1 nM^{-1} and 1.5 ± 0.1 nM^{-1} in membranes treated with collagenase or elastase respectively, whereas there was no change ($P > 0.05$) in binding capacities (2.9 ± 0.1 and 3.0 ± 0.2 pmol/mg of membrane protein respectively). The increase in binding affinity of the insulin receptor in enzyme-treated membranes was not due to changes in the glycosylation of the receptor, since the association constant for insulin binding was not significantly altered ($P > 0.05$) in membranes treated with endo-β-*N*-acetylglucosaminidase F.

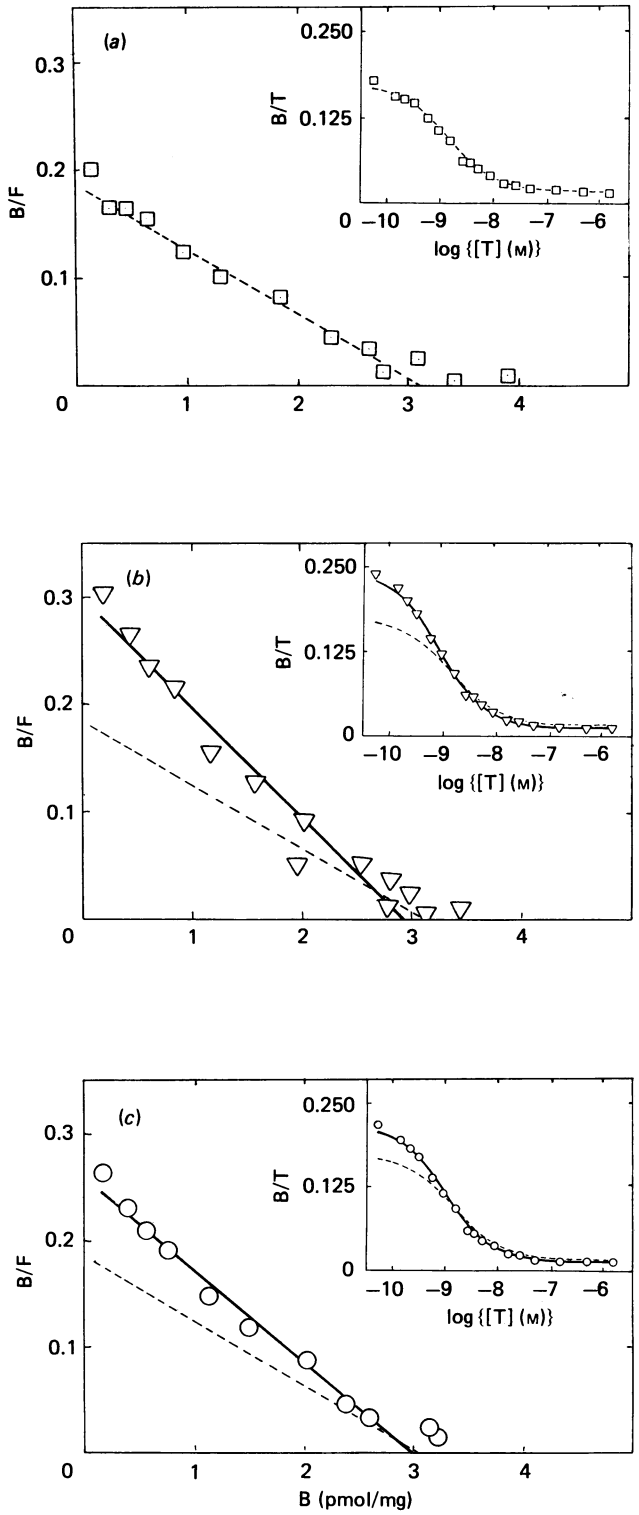

Fig. 5. Effect of enzyme treatment of hepatic membranes on insulin binding

Purified hepatic plasma membranes were incubated without (*a*) or with 100 μg of collagenase/ml (*b*) or 1.0 μg of elastase/ml (*c*) as described in the text. The incubation of membranes from each preparation with [[^{125}I]Tyr^{A14}]insulin and the analysis of the binding data were carried out as described in the text. Scatchard plots for control (□) collagenase- (▽) and elastase- (○) treated membranes are shown. The dose–response curves for each preparation are shown in the inset. Bound insulin is denoted by B, free insulin by F and total insulin by T.

DISCUSSION

The insulin receptor photolabelled in intact cells or plasma membranes appeared predominantly as a band of 450 kDa, but in membranes treated with collagenase it appeared predominantly as a band of 360 kDa (Fig. 1). This change in the receptor's apparent size evidently resulted from the loss of a component inaccessible to labelling, since no change in the photolabelling of subunits was observed (Fig. 3). These results are consistent with the demonstration by Roth *et al.* (1983) that the 450 kDa insulin receptor purified from IM9 lymphocytes was converted into a 300 kDa form by treatment with collagenase. We have found further that this effect could be mimicked by elastase (Figs. 2 and 4) and was due to a contaminating proteinase(s) rather than the collagenolytic enzymes themselves.

In hepatic plasma membranes that had been treated with collagenase or elastase and contained the 360 kDa form of the insulin receptor, the association constant of the high-affinity binding site for insulin was increased by 80% and 50% respectively (Figs. 4 and 5). The mechanism(s) responsible for this change in affinity is not known. The increased affinity for insulin in membranes treated with enzymes may have been due to changes in the insulin-binding sites that were not related to the change in size of the receptor. Alternatively, the change in affinity may have been due to the removal of a component from the receptor, thus affecting the binding of insulin. This alternative explanation is consistent with the increase in the affinity for insulin in these membranes being accompanied by the conversion of the receptor's size from 450 kDa to 360 kDa (Fig. 4).

The relationship between receptor size and binding affinity has also been observed by others. Thus Schweitzer *et al.* (1980) found that insulin binding was increased by 20% in liver plasma membranes treated with dithiothreitol. Crettaz *et al.* (1984) demonstrated that insulin binding by Fao hepatoma cells was increased when large oligomeric forms of the insulin receptor were converted into smaller forms by dithiothreitol. However, in both these cases the effect of dithiothreitol on insulin binding may have been due to the reduction of disulphide bonds in the ligand-binding region of the α subunit (Clark & Harrison, 1982, 1983; Ullrich *et al.*, 1985), rather than to changes in receptor size.

Other investigators have suggested that the affinity of the insulin receptor may be influenced by components of the receptor which do not bind insulin. For example, Harmon *et al.* (1980, 1981) observed that the binding of insulin was increased in hepatic plasma membranes that had been irradiated with high-energy electrons. They concluded that the 450 kDa insulin receptor contained a component that did not bind insulin, but could regulate insulin binding. Maturo & Hollenberg (1978) demonstrated that the affinity of the purified insulin receptor was altered by its association with uncharacterized membrane glycoproteins which could not bind insulin themselves. Ginsberg *et al.* (1976) and Krupp & Livingston (1979) suggested that the affinity of the insulin-binding subunit of the receptor was altered by its association with other receptor components. In the current study, we observed both the loss of an unlabelled component from the 450 kDa receptor and an increased affinity for insulin in hepatic plasma membranes that were treated with enzymes. Consequently, our observations are consistent

with the proposal that a component of the receptor, which does not bind insulin itself, may affect insulin binding. It is therefore possible that the increased affinity for insulin that we observed was due to the loss of a component which 'regulates' insulin binding, or alternatively to an indirect effect of removing a non-insulin-binding component associated with the receptor.

Our finding that the insulin receptors of plasma membranes, but not of intact cells, were altered from 450 kDa to 360 kDa by collagenase treatment (Fig. 1) suggests that the access of the proteinase(s) to a site on the cytoplasmic side of the plasma membrane was necessary for this effect to occur. This raises the possibility that a constituent on the cytoplasmic side of the plasma membrane is involved in the structural organization of the insulin receptor. It is possible that the component lost from the 450 kDa insulin receptor and the constituent susceptible to proteolysis on the intracellular side of the plasma membrane were the same entity.

The loss of an unlabelled component from the receptor in this study is consistent with previous demonstrations that neither chemical cross-linking nor photoaffinity labelling necessarily detects all of the subunits associated with the insulin receptor. Thus, although chemical cross-linking (Massague *et al.*, 1980, 1981; Goren *et al.*, 1983; Baron & Sonksen, 1983; Endo & Elsas, 1984) or photoaffinity-labelling techniques (Yip *et al.*, 1978; Jacobs *et al.*, 1979; Fehlmann *et al.*, 1982; Berhanu *et al.*, 1982; Yip *et al.*, 1982; Heidenreich *et al.*, 1983*a*) label the α subunit strongly, the β subunit and the 40 kDa band are labelled poorly or not at all. An exception to this is the ready labelling of the α and β subunits and the 40 kDa bands of the receptor by the $N^{\epsilon B29}$-monoazidobenzoyl-insulin photoprobe (Fig. 3) (Yip *et al.*, 1982; Yip & Moule, 1983). However, the inability of these methods to detect consistently the β subunit and 40 kDa bands demonstrates that the 450 kDa form of the photolabelled insulin receptor can obtain subunits that are not readily labelled.

The conversion of the insulin receptor from 450 kDa to 360 kDa may have been due to the loss of an unlabelled α or β subunit. Alternatively, it may have resulted from the loss of some other components associated with the receptor in the plasma membrane. Of interest in this regard is the labelling of an additional, uncharacterized, component in the adipocyte insulin receptor. A component of 80–85 kDa was labelled in adipocytes by the $N^{\alpha B1}$-monoazidobenzoyl-insulin photoprobe (Yip & Moule, 1983) and by chemical cross-linking (Massague & Czech, 1982) after these cells were treated with dithiothreitol. Wang *et al.* (1982) also observed a receptor component of 85 kDa in adipocytes photolabelled by insulin modified on the $N^{\alpha B1}$ phenylalanine position.

Utilizing a combined mixture of $N^{\alpha B1}$- and $N^{\epsilon B29}$-monoazidobenzoyl-insulin photoprobes, Hofmann *et al.* (1981) specifically labelled an additional, uncharacterized, component of 72 kDa in the insulin receptor of H4 hepatoma cells. In hepatoma cells, Kasuga *et al.* (1982) also observed that a component of 68 kDa was immunoprecipitated with the insulin receptor. Using liver plasma membranes, Baron & Sonksen (1983) demonstrated a 395 kDa and a 330 kDa form of the hepatic insulin receptor; they concluded that the 330 kDa form represented the loss of an unlabelled component of 65 kDa. These observations suggest that components in addition to the α and β subunits may be associated with insulin receptors in the plasma membrane. An interaction of the insulin receptor with other components in the plasma membrane may be explained by, and is consistent with, the 'mobile receptor' model of insulin action (Hollenberg, 1979, 1982). On the other hand, receptor components in addition to the α and β subunits have not been observed in the preparations of insulin receptor of 300–350 kDa which have been purified by affinity chromatography (Fujita-Yamaguchi, 1984; Boyle *et al.*, 1985). It is possible that components associated with the insulin receptor in the plasma membrane are lost during the process of purification (Siegel *et al.*, 1981).

In conclusion, we have demonstrated that the affinity for insulin was significantly increased in hepatic plasma membranes in which the size of the insulin receptor had been changed from 450 kDa to 360 kDa by treatment with enzymes. However, no differences could be detected in the photolabelling of subunits in these two forms of the insulin receptor. This suggests that the effect on insulin binding may have been due to the loss of a component that was associated with the insulin receptor but was inaccessible to labelling. This unlabelled component may be a receptor component that does not bind insulin but which regulates the binding of insulin.

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