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Comparative Studies on Human Placental Insulin and Basic Somatomedin Receptors

G.D. Armstrong, M.D. Hollenberg, B. Bhaumick, and R.M. Bala

Endocrine Research Group, Department of Pharmacology and Therapeutics, University of Calgary, Calgary, Alberta, Canada T2N 4N1 (G.D.A., M.D.H.), and Department of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0X0 (B.B., R.M.B.)

The disuccinimidyl suberate, affinity-labeling procedure, and proteolytic mapping techniques have been employed to characterize further the human placental receptors for insulin and basic somatomedin. Electrophoretic analysis of the basic somatomedin receptor, selectively crosslinked to ^{125}I basic somatomedin in the presence of excess native insulin revealed, under reducing conditions, major labeled constituents of 270–280 and 125–140 kd, substantiating our previous work employing a photoaffinity labeling reagent. Affinity labeling also demonstrated the presence of less intensely labeled components with apparent molecular weights of 40 and 45 kd but failed to reveal a distinct 90- to 100-kd species observed in parallel experiments with insulin. In the absence of β-mercaptoethanol, all components specifically labeled with ^{125}I basic somatomedin migrated in the 300- to 400-kd range. In comparison, selective affinity labeling of the insulin receptor in the presence of excess native basic somatomedin revealed components, upon electrophoresis under reducing conditions, with apparent molecular weights of 270–280, 125–140, 90–100, and 40 kd. The major insulin-labeled component (125–140 kd) comigrated with the major constituent (125–140 kd) selectively labeled with basic somatomedin. When digestion was performed prior to solubilization, chymotryptic and tryptic proteolysis of the membrane-localized selectively labeled insulin, and basic somatomedin receptors yielded quite similar gel electrophoretic maps. However, when digestion was done subsequent to solubilization, chymotryptic and tryptic proteolysis of selectively labeled insulin and basic somatomedin receptors solubilized in SDS yielded similar but not identical gel electrophoretic maps. We conclude that the receptors for basic somatomedin and insulin are highly homologous structures with respect to their disulfide crosslinked composition, and with respect to the size of the major components detected by selective affinity-labeling procedures. Nevertheless, the detection of electrophoretically distinct labeled receptor components upon analysis of specifically labeled intact or proteolytically digested receptors points to subtle differences between the polypeptide compositions of the two receptors.

Abbreviations: BSA, bovine serum albumin; BSM, basic somatomedin; DSS, disuccinimidyl suberate; IGF-I, -II, insulinlike growth factors I and II: KRP, Krebs-Ringer phosphate; MSA, multiplication-stimulating activity; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; SDS-PAGE, SDS polyacrylamide gel electrophoresis; SM-A, C, somatomedin A and C.

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The somatomedins (SMs) comprise a group of growth hormone-dependent polypeptides that can be purified from human serum [1,2]. The SMs have the common ability to stimulate cartilage proteoglycan synthesis and to mimic the action of insulin in various tissues [3]. The SMs can be classified into two main groups according to their isoelectric points. The "basic" group includes somatomedin-C (SM-C) [4], insulinlike growth factor-I (IGF-I) [5], and basic somatomedin (BSM) [6]. It is possible that the terms basic somatomedin, IGF-I, and somatomedin-C may all represent the same polypeptide; all three of these preparations cross-react with similar affinities in both radioimmunoassay (anti-BSM antibody) and radioreceptor assay systems. Nonetheless, until peptide sequence data become available for BSM and SM-C for comparison with that reported for IGF-I, the exact chemical relationship between the "basic" group of somatomedins remains somewhat unresolved. The "acidic-neutral" group includes insulinlike growth factor II (IGF-II) [5] and somatomedin-A (SM-A) [7]; multiplication-stimulating activity (MSA), a polypeptide isolated from the culture medium of buffalo rat liver cells [8–11] appears to be related to IGF-II.

A number of tissues contain specific membrane-localized binding sites that are comparatively selective for insulin or for the SMs [12–18]. However, the specific binding sites, suggestive of distinct receptors for each of the insulinlike polypeptides, display cross specificity, such that, at sufficiently high concentrations, the SMs can compete for the insulin receptor and vice versa.

In view of the structural relatedness of the several insulinlike ligands, we have begun to focus our attention on the structural properties of the receptors for insulin and BSM found in the particulate membrane fraction of the human placenta. We recently reported on some of the physical properties of Triton X-100–solubilized, placental BSM receptors, and we described the close relationship between this receptor and the one for insulin [19]. In the present communciation we have extended our preliminary work to investigate further the relationship between the affinity-labeled BSM and insulin receptors by proteolytic mapping (trypsin, chymotrypsin) methodology.

MATERIALS AND METHODS

Iodination of BSM and Insulin

BSM was purified in our laboratory as described earlier [6]. Insulin was a gift from Eli Lilly. Both peptides were iodinated by a modification of the chloramine T method [20–22] to specific activities of 100–150 μCi/μg (1 Ci $-$ 3.7 $\times$ 10^{10} becquerels).

Preparation of Placental Membrane

A "microsomal" membrane fraction was prepared from term human placenta as described previously [23,24] with the exception that 200 mg phenylmethylsulphonyl fluoride (PMSF; dissolved in 10 ml of dimethylsulfoxide) was added to every 1,000 ml of placenta homogenization buffer just prior to blending. Optimal results were obtained with preparations made from fresh, rather than fresh-frozen, tissue.

Affinity Labeling With Disuccinimidyl Suberate

Particulate membrane preparations (2–4 mg protein per ml) were incubated with ^{125}I-labeled insulin or BSM in the presence or absence of excess unlabeled insulin or BSM (as described in the figure legends) in 0.5 ml of 50mM Tris-HCl buffer (pH 7.2) containing 0.5% BSA. Equilibration was allowed to proceed at 4°C overnight for BSM, and at room temperature for 1 hr for insulin. The membranes were then collected by centrifugation at 37,000g for 20 min at 4°C, and resuspended in 1 ml of ice-cold Krebs-Ringer phosphate (KRP) buffer (pH 7.4). Crosslinking was achieved by a modification of the method previously described [25]. Disuccinimidyl suberate (DSS) dissolved in dimethyl sulfoxide (concentration, 100 mM) was added to the washed membrane suspension to give a final concentration of 1 mM. The reaction mixture was incubated for 15 min at 0°C, and was quenched by the addition of excess (10 volumes) 25 mM Tris-HC1 buffer. Crosslinked membranes were washed two times by centrifugation at 37,000g for 20 min. The final pellet was resuspended in 0.5 ml 50 mM Tris-HCl buffer. The crosslinked particulate membranes were subjected to tryptic or chymotryptic proteolysis either before or after solubilization in the SDS sample buffer of Laemmli [26] prior to sodium dodecylsulfate (SDS) polyacrylamide gel electrophoresis (SDS-PAGE).

Proteolytic Mapping

Fifty μl aliquots of the affinity-labeled, particulate membranes in 50 mM Tris-HCl buffer (pH 7.4) were incubated with 2.5 μg trypsin (10 μl) or 10 μg αchymotrypsin (10 μl) for 15 min at room temperature. Proteolysis was terminated by heating the samples for 10 min in a boiling water bath after the addition of 60 μl of twofold concentrated SDS sample buffer and 10 μl β-mercaptoethanol. Alternately, 50 μl of affinity-labeled, particulate membranes were dissolved in 50 μl of twofold concentrated SDS sample buffer and heated for 2 min in a boiling water bath as described by Clevelend et al [27]. The samples were transferred to a 37°C water bath and incubated for 30 min with 25 μg trypsin (10 μl) or 10 μg chymotrypsin (10 μl). Proteolysis was terminated by the addition of 10 μl β-mercaptoethanol and heating the samples in a boiling water bath for 8 min.

Electrophoretic Analysis and Autoradiography

Affinity-labeled membranes were analysed in SDS-containing polyacrylamide gels in the presence of the reducing reagent β-mercaptoethanol (5–10% v/v). Linear gradient resolving gels (5–15%) or 7.5% gels were employed as described in the figure legends. After electrophoresis, gels were stained in 0.1% (w/v) Coomassie brilliant blue in methanol:acetic acid:water (50:10:40, v/v) and were destained with methanol:acetic acid:water (5:10:85, v/v). The gels were dried under vacuum and exposed at −70°C to Kodak X-Omat R x-ray film using Dupont Cronex Lightening plus intensifying screens. Molecular weights were estimated using human erythrocyte ghost markers. [28].

RESULTS

In our initial characterization of the BSM receptor, we employed a photoaffinity-labeling technique to crosslink ^{125}I-BSM to the human placental BSM receptor [29].

To complement and extend these observations, we have more recently turned to the DSS affinity-labeling procedure [19] so that our results may be compared with those of others [30, 31]. When placental membranes were affinity-labeled with ^{125}I-insulin in the presence of excess native BSM, SDS-PAGE of β-mercaptoethanol-reduced samples revealed labeled protein species of 280, 130, 100, and 40 kd (Fig. 1, lane A). The intensity of labeling of all the ^{125}I-insulin protein species was drastically reduced when the membranes were affinity-labeled in the presence of excess native insulin (Fig. 1, lane B). When the placental membranes were affinity-labeled with ^{125}I-BSM in the presence of excess native insulin, labeled protein species of 280, 130, 40, and 45 kd were observed (Fig. 1, lane C). Excess native BSM drastically reduced the intensity of labeling of the ^{125}I-BSM labeled proteins (Fig. 1, lane D). Upon SDS-PAGE in the absence of sulfhydryl reducing reagents, all of the insulin and BSM affinity-labeled proteins migrated as species with molecular weights greater than 300 kd (data not shown). Therefore, the BSM receptor would appear to exist in placental membranes as a sulfhydryl-linked, oligomeric structure analogous to the insulin receptor (the type I receptor [32]).

In a separate experiment using a different preparation of placental membranes and a 7.5% acrylamide gel (Fig. 2), the protein species affinity-labeled with ^{125}I-insulin exhibited molecular weights of 270, 140, 130, and 100 kd (lane A). With ^{125}I-BSM, affinity-labeled species of 270 and 140 kd were detected (lane B). Affinity-labeling of these bands was blocked with excess amounts of the appropriate native ligand (data not shown). The absolute values of the molecular weights for the labeled protein species estimated from the calibration curves (standard proteins were simultaneously analysed in each experiment) varied somewhat when identical samples were subjected to electrophoresis in separate experiments. For example, there was a variation of about 8% in the molecular weight estimated for the so-called α-subunit [31,32] of the insulin receptor (130 $\pm$ 10 kd; mean $\pm$ SEM for five experiments). A labeled species of this molecular weight was consistently and intensely crosslink-labeled using either ^{125}I-BSM or ^{125}I-insulin; in separate experiments there was always comigration of the major 130 $\pm$ 10 kd BSM-labeled and insulin-labeled constituents. Importantly, we were unable (Figs. 1,2) to detect a ^{125}I-BSM-labeled constituent of 90 kilodaltons, that correspond to the so-called β-subunit of the insulin receptor [31,32]. The observed variations in the molecular weights of the affinity-labeled proteins and in the number of labeled proteins observed in the 30- to 50-kd range may have been due to 1) the use of different preparations of placental membranes; 2)the length of time that samples were stored frozen before electrophoresis; 3) differences in the resolving powers of the 5–15% gradient versus the 7.5% acrylamide gels; and 4) different exposure times of the autoradiograms. Although variations in the intensity of affinity labeling of the higher molecular weight species (270–280 kd) were observed, these proteins were always found to be present. These larger species may represent crosslinked receptor oligomers or receptors crosslinked to other membrane proteins which were in close proximity to the receptors at the time of cross linking. Alternately, labeling of the 270- 280-kd species may reflect affinity labeling by insulin or BSM of the 225-kd MSA receptor [33,34]. The exact nature of these proteins remains to be elucidated.

In our previous report [19], chymotryptic peptide mapping demonstrated a high degree of structural homology between solubilized insulin and BSM receptors. As an extension of our earlier studies, we have subjected membrane localized as well as

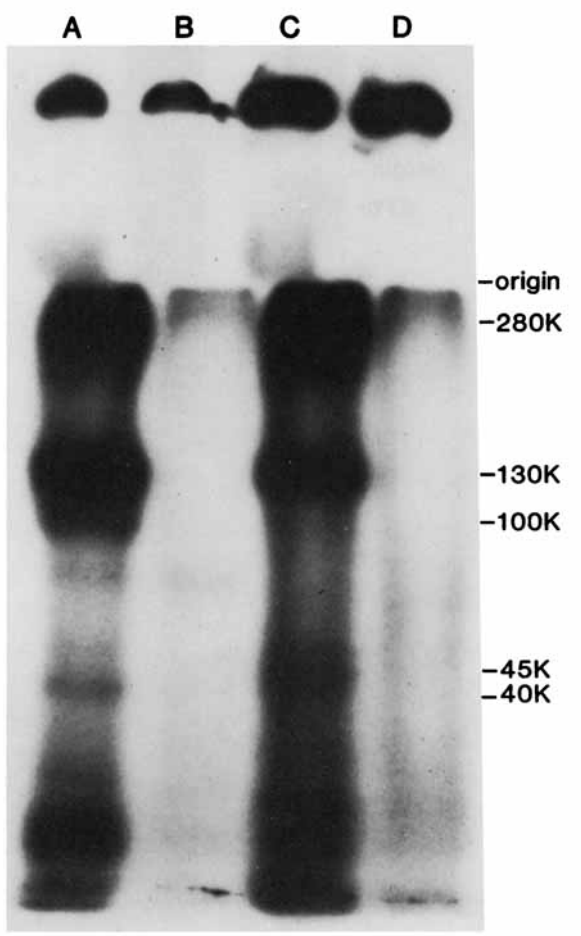

Fig. 1. Electrophoretic analysis of DSS affinity-labeled insulin and BSM receptors. The receptors in particulate placenta membranes were crosslink-labeled as outlined in the text with ^{125}I-labeled insulin or ^{125}I-labeled BSM using disuccinimidyl suberate (DSS). A) Receptor labeled with ^{125}I-insulin (1–2 pmole/ml, 10^6 cpm/pmole in the presence of excess (10 μg) native BSM. B) Receptor labeled with ^{125}I-insulin in the presence of excess (10 μg) native insulin. C) Receptor labeled with ^{125}I-BSM (4–10 pmole/ml, 10^6 cpm/pmole) in the presence of excess (1 μg) native insulin. D) Receptor labeled with ^{125}I-BSM in the presence of excess (10 μg) native BSM. The molecular weights of the affinity-labeled proteins shown to the right of the figure were determined using red blood cell marker proteins [28]. SDS polyacrylamide gel electrophoresis was performed as described previously [19] using a 5–15% linear gradient separating gel.

SDS solubilized, affinity-labeled insulin and BSM receptors to tryptic and chymotryptic proteolysis and peptide mapping. Treatment of the affinity-labeled insulin receptor with 40 μg/ml of trypsin prior to solubilization produced peptides with molecular weights of slightly less than 120 kd and of 110, 72, and 20 kd (Fig. 3). Virtually all of the 130-kd insulin subunit was degraded by treatment with trypsin. Trypsin proteolysis of the affinity-labeled BSM receptor prior to solubilization yielded peptides with molecular weights of slightly less than 120 kd and of 110, 76, and 24 kd. Trypsin treatment of the membrane-localized BSM receptor did not completely degrade the 130-kd protein analogous to the insulin α-subunit. Chymotryptic proteolysis

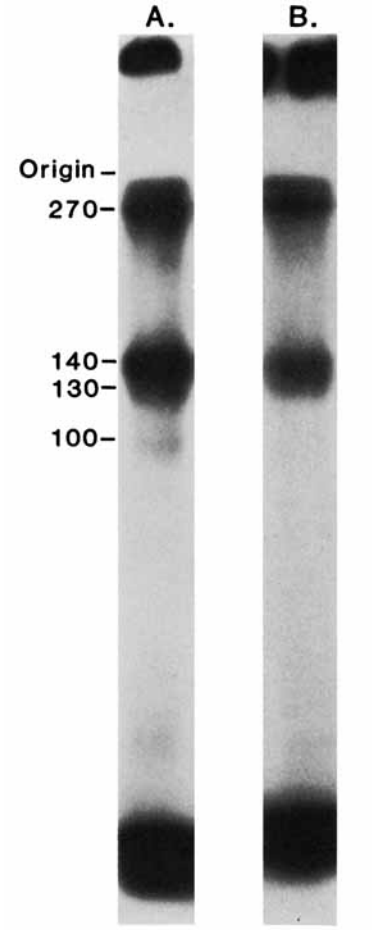

Fig. 2. Electrophoretic analysis of DSS affinity-labeled insulin and BSM receptors on 7.5% SDS polyacrylamide gels. Insulin and BSM receptors were affinity-labeled as outlined in the text and in the legend to Figure 1. A) Receptor labeled with ^{125}I-insulin; B) Receptor labeled with ^{125}I-BSM. The molecular weights of the affinity-labeled proteins shown to the left of the figure were determined using red blood cell marker proteins [28]. SDS polyacrylamide gel electrophoresis was performed in the presence of 20 mM dithiothreitol as described previously [19] using a 7.5% separating gel.

of the affinity-labeled insulin receptor prior to solubilization produced peptides with molecular weights of 120, 94, 45, and 20 kd; similar treatment of the crosslink-labeled BSM receptor produced 120-, 100-, 47-, and 26-kd peptides (Fig. 3). Moreover, chymotryptic cleavage of the insulin receptor completely degraded the 130-kd α-subunit but not the 130-kd protein that was affinity-labeled with BSM.

The previous peptide mapping studies [19,30] evaluated only the membrane-localized, affinity-crosslinked insulin and BSM receptors. Thus proteolysis was directed only at receptor domains which are external to the membrane lipid bilayer. We decided to extend our previous studies by subjecting the affinity-labeled receptors to proteolytic mapping subsequent to solubilization in the SDS sample buffer of Laemmli [26]. Treatment of solubilized insulin receptor with 400 μg/ml of trypsin produced peptides with molecular weights of 92, 44, and 28 kd (Fig. 4). The insulin α-subunit,

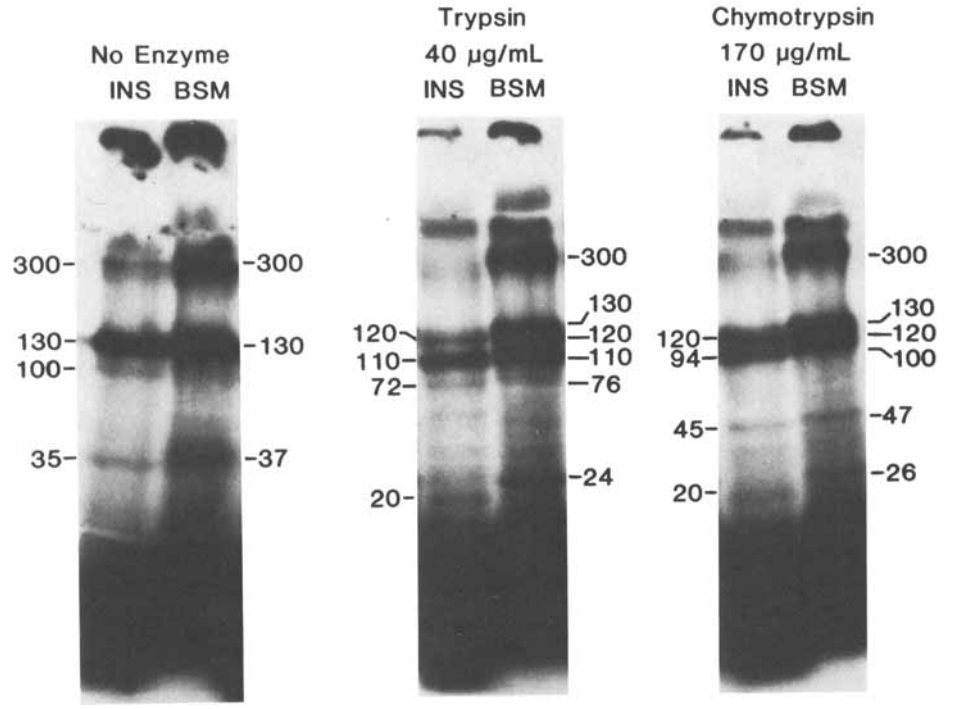

Fig. 3. Electrophoretic analysis of DSS affinity-labeled insulin and BSM receptors subjected to tryptic or chymotryptic proteolysis in the particulate membranes. Insulin receptor was affinity-labeled with ^{125}I-insulin in the presence of excess (10 μg) native BSM using DSS as outlined in the text and in the legend to Figure 1. BSM receptor was affinity-labeled with ^{125}I-BSM in the presence of excess (10 μg) native insulin. Fifty μl aliquots of the affinity-labeled particulate membranes in 50 mM Tris-HCl (pH 7.2) were incubated at room temperature for 15 min with 10 μl Tris-HCl buffer (control) or 2.5 μg trypsin (in 10 μl) or 10 μg αchymotrypsin (in 10 μl). Proteolysis was terminated by heating the samples for 10 min in a boiling water bath after the addition of 60 μl of twofold concentrated SDS sample buffer of Laemmli [26], and 10 μl β-mercaptoethanol. SDS polyacrylamide gel electrophoresis was performed as described previously [19] using 5–20% linear gradient separating gels. The molecular weights of the peptides were determined using red blood cell marker proteins [28].

which migrated with a molecular weight of 125 kd on this gel was not completely degraded. Similar treatment of the solubilized BSM receptor yielded peptides with molecular weights of 100, 95, 72, and 49 kd as well as the 125-kd protein. Treatment of the solubilized insulin receptor with 170 μg/ml of chymotrypsin produced peptides with molecular weights of 92, 60, 44, and 27 kd. Chymotrypsin almost completely degraded the insulin α-subunit. Similar treatment of the solubilized BSM receptor produced peptides with molecular weights of 100, 50, 36, and 28 kd, but the 125-kd protein was not completely degraded. Peptide mapping of the SDS-solubilized insulin and BSM receptors revealed them to be highly homologous structures, but differences were noted that were not evident in experiments with nonsolubilized receptor preparations—notably, the 72-kd peptide, produced by trypsin and not found in the insulin receptor. Also, the 60-kd insulin peptide produced by chymotrypsin treatment of the solubilized insulin receptor was not found in experiments with the solubilized affinity-labeled BSM receptor.

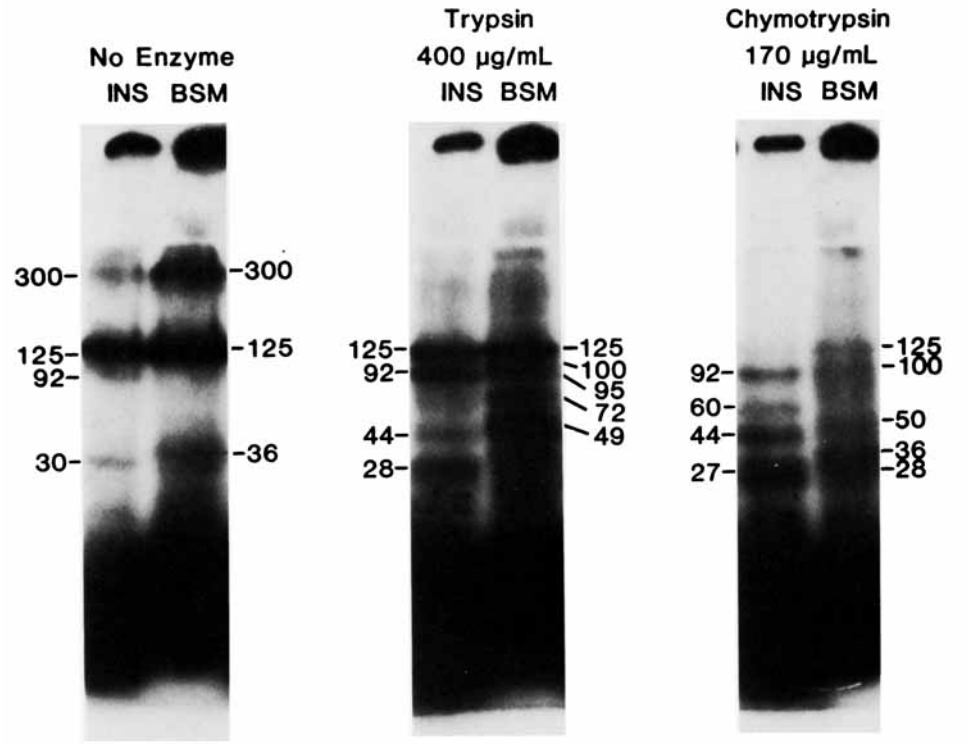

Fig. 4. Electrophoretic analysis of DSS affinity-labeled insulin and BSM receptors subjected to tryptic or chymotryptic proteolysis in SDS sample buffer. Insulin receptor was affinity-labeled with ^{125}I-insulin in the presence of excess (10 μg) native BSM using DSS as outlined in the text and in the legend to Figure 1. BSM receptor was affinity-labeled with ^{125}I-BSM in the presence of excess (10 μg) native insulin. Fifty μl aliquots of the affinity-labeled particulate membranes were heated for 2 min in a boiling water bath in the presence of 50 μl of twofold concentrated SDS sample buffer of Laemmli [26] as described by Cleveland et al [27]. The samples were chilled on ice, then placed into a 37°C water bath. The samples were incubated for 30 min with 10 μl 50 mM Tris-HCl buffer (control) or 25 μg trypsin (in 10 μl) or 10 μg chymotrypsin (in 10 μl). The reactions were terminated by the addition of 10 μl β-mercaptoethanol followed by heating for an additional 8 min in a boiling water bath. The samples were subjected to SDS polyacrylamide gel electrophoresis as described previously [19] using 5–20% linear gradient separating gels. The molecular weights of the peptides were determined using red blood cell marker proteins [28].

DISCUSSION

Our previous work [19,29] has pointed out the remarkable structural and hydrodynamic similarities between the receptors for insulin and BSM. It appears that the BSM receptor, like the insulin (type I) receptor [32] is a disulphide-linked oligomeric structure that in solution (Triton X-100) exhibits a molecular weight of about 409,000 [19]. Like the insulin receptor, the crosslink-labeled BSM receptor behaves as a high molecular weight species (MW > 300,000) upon electrophoresis in nonreducing polyacrylamide gels, but upon reduction with thiol yields a major crosslink-labeled substituent with a molecular weight of 125 to 140 kd (akin to the α-subunit of the insulin receptor). It may be of importance that under stringent conditions of specific crosslink labeling of the BSM receptor (ie, in the presence of about

160 nM insulin), we were unable in reducing gels to detect a 90- to 100-kd species analogous to the so-called α-subunit of the insulin receptor, that in control experiments with insulin was readily detected (Figs. 1,2). This result should sound a cautionary note in terms of accepting, without further proof, an $(\alpha\beta)_2$ structure for the BSM receptor, simply by analogy with results for the insulin receptor [31–33].

Concurrent work by Chernausek et al [30] using somatomedin-C for crosslink-labeling of the placental receptor can be taken as independent evidence for the structural homologies that appear to exist between the receptors for insulin and for the "basic" somatomedins. Although differences in proteolysis conditions and in electrophoretic analysis make it difficult to compare our present and previous [19] peptide mapping results directly with the work of Chernausek et al, it is clear from all studies that tryptic and chymotryptic cleavage of the membrane-localized, affinity-labeled BSM (or SM-C) receptor yields fragments with molecular weights similar to those resulting from cleavage of the membrane-associated crosslink-labeled insulin receptor. Interestingly, the most convincing differences between the cleavage maps for the insulin and BSM receptors came from our results in which the receptors were solubilized prior to proteolysis. The distinct 72-kd tryptic BSM receptor species and the 60-kd chymotryptic insulin receptor species may reflect differences in the hydrophobic receptor regions (possibly intramembranous) that become accessible to the enzymes only in the presence of detergent.

To date, the comparative peptide maps of the affinity-labeled receptors for BSM and insulin have provided information that is, of necessity, restricted primarily to domains in the proximity of the ligand-binding site. Despite the marked similarities between the BSM and insulin receptors suggested by the proteolytic map analyses, it is now evident that there is only limited immunological cross reactivity between the BSM and insulin receptors [29,34]. Speculation has just begun as to the locations of the distinct receptor regions. Thus, there will be great interest in evaluating receptor regions distant from the ligand-binding domains, so as to determine if the unique biological actions of insulin and BSM can be attributed to unique receptor sequences that are localized in the lipid bilayer.

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REFERENCES

1. Daughaday WH, Hall K, Raben M, Salmon WD, Van den Brande JL, Van Wyk JJ: Nature (Lond) 235:107, 1972.
2. Phillips LS, Vassilopoulou-Sellin R: N Engl J Med 302:371, 438, 1980.
3. Van Wyk JJ, Underwood LE, Baseman JB, Hintz RL, Clemmons, DR, Marshall RN: In Luft R, Hall K (eds): "Advances in Metabolic Disorders," Vol 8. New York: Academic Press, 1975, pp 127–150.
4. Svoboda ME, Van Wyk JJ, Kapper DG, Fellow RE, Gissom FE, Schlneter RJ: Biochemistry 19:790, 1980.
5. Rinderknecht E, Humble, RE: Proc Natl Acad Sci USA 73:2365, 1976.
6. Bala RM, Bhaumick B: Can J Biochem 57:1289, 1979.

7. Fryklund L, Uthne K, Sievertsson H: Biochem Biophys Res Commun 61:957, 1974.
8. Dulak NC, Temin HM: J Cell Physiol 81:153, 1973.
9. Dulak NC, Temin HM: J Cell Physiol 81:161, 1973.
10. Smith GL, Temin HM: J Cell Physiol 84:181, 1974.
11. Moses AC, Nissley SP, Short PH, Rechler MM, Poskalny JM: Eur J Biochem 103:387, 1980.
12. Zapf J, Schoenle E, Froesch ER: Eur J Biochem 87:285, 1978.
13. King GL, Kahn RC, Rechler MM, Nissley SP: J Clin Invest 66:130, 1980.
14. Zapf J, Mader M, Waldvogel M, Schalch DS, Froesch ER: Arch Biochem Biophys 168:630, 1975.
15. Marshall RN, Underwood LE, Voina SJ, Foushee DB, Van Wyk JJ: J Clin Endocrinol Metab 39:283, 1974.
16. Rosenfeld RG, Hintz, RL: Endocrinology 107:1841, 1980.
17. Rinderknecht E, Humbel RE: J Biol Chem 253:2769, 1978.
18. Bhaumick B, Goren HJ, Bala RM: Horm Metab Res 13:515, 1981.
19. Bhaumick B, Armstrong GD, Hollenberg MD, Bala RM: Can J Biochem 60:923, 1982.
20. Hunter WM, Greenwood FC: Nature 194:495, 1962.
21. Cuatrecasas P, Hollenberg MD: Adv Protein Chem 30:251, 1976.
22. Bala RM, Bhaumick B: J Clin Endocrinol Metab 49:770, 1979.
23. Cuatrecasas P: Proc Natl Acad Sci USA 69:318, 1972.
24. Hock RA, Hollenberg MD: J Biol Chem 255:10731, 1980.
25. Pilch PF, Czech MP: J Biol Chem 254:3375, 1979.
26. Laemmli UK: Nature (Lond) 227:680, 1970.
27. Cleveland DW, Fischer SG, Kirschner MW, Laemmli UK: J Biol Chem 252:1102, 1977.
28. Fairbanks G, Steck TL, Wallach DFH: Biochemistry 10:2606, 1971.
29. Bhaumick B, Bala RM, Hollenberg MD: Proc Natl Acad Sci USA 78:4279, 1981.
30. Chernausek SD, Jacobs S, Van Wyk JJ: Biochemistry 20:7345, 1982.
31. Massague J, Pilch PF, Czech MP: Proc Natl Acad Sci USA 77:7137, 1980.
32. Massague J, Czech MP: J Biol Chem 257:5038, 1982.
33. Massague J, Guillette BJ, Czech MP: J Biol Chem 256:2122, 1981.
34. Armstrong GD, Bhaumick B, Bala RM, Hollenberg MD: Clin Res 30:522A, 1982.