Immunoprecipitation of insulin receptors by antibodies against Class 1 antigens of the murine H-2 major histocompatibility complex

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Insulin receptors from C57BL/6J mouse (H-2^b) liver membranes were specifically labeled with ^{125}I-photoreactive insulin by UV irradiation. Membranes were solubilized and the capacity of various antibodies reacting with the major histocompatibility complex to immunoprecipitate insulin receptors was tested. About 5% of the labeled receptors were immunoprecipitated by a conventional mouse antiserum against H-2^b histocompatibility antigens and by a monoclonal antibody against Class 1 antigens of the H-2^b haplotype (K^b and D^b). No immunoprecipitation was obtained with a monoclonal antibody against Class 2 antigens of I-A^b or against Class 1 antigens of the H-2^k haplotype. Insulin receptors can thus be specifically immunoprecipitated by antibodies against class I histocompatibility antigens.

Insulin receptor *Histocompatibility antigen* *Monoclonal antibody* *Immunoprecipitation* *Photoaffinity labeling*

1. INTRODUCTION

The insulin receptor is a multimeric complex composed of two major subunits: the α-subunit (M_r 130000) containing the insulin binding site [1,2], and the β-subunit (M_r 95000) containing a protein kinase activity leading to a self-phosphorylation of the receptor in the presence of insulin [3,4]. In addition to these 2 major components, a M_r 45000 polypeptide has also been observed [5,6] and is thought to represent a proteolytic fragment of the β-subunit [6]. The two following observations have led us to reinvestigate the nature of this M_r 45000 component of the insulin receptor. Exposure of intact hepatoma cells to trypsin leads to a complete degradation of the M_r 130000 and the M_r 45000 components, whereas the M_r 90000 subunit is not affected (unpublished). This suggests that the M_r 45000 polypeptide is a naturally occurring component of the receptor in intact cells, rather than a proteolytic fragment of the M_r 90000 β-subunit. Further, the human Burkitt lymphoma cell line Daudi, that fails to express the M_r 45000 heavy chain of Class 1 histocompatibility antigens [7], also fails to bind insulin (unpublished). Taken together, an important question arose concerning the possible structural relationship between the M_r 45000 putative receptor subunit and the histocompatibility antigens. To investigate this, we specifically labeled insulin receptors in mouse liver membranes using a photoreactive insulin analogue and tested the ability of various antibodies reacting with the major histocompatibility complex (MHC) to immunoprecipitate the insulin receptor.

2. MATERIALS AND METHODS

2.1. *Materials*

The photoreactive insulin analogue B2 (2-nitro-4-azidophenylacetyl) des Phe ^{B}1 insulin was prepared by D. Saunders and D. Brandenburg at the Wollforschungsinstitut (Aachen). The analogue retains about 70% of the receptor bind-

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ing affinity and biological potency of native insulin [2]. The photoreactive insulin was iodinated to a specific activity of 200–250 μCi/μg by using the same method as that described for native insulin [8]. All reagents for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) were purchased from Bio-Rad. Serum from patient B9 with autoantibodies to the insulin receptor was a kind gift from C.R. Kahn (Joslin Research Laboratory, Boston MA) [9]. The mouse antiserum reacting with H-2^b histocompatibility antigens (B10A α B10, no.762) was from the CSEAL-CNRS (Orleans). Monoclonal antibodies reacting with K^b and D^b (20-8-4S) [10], with I-A^b (25-9-17S) and with K^k and D^k (3-83P) [11] were generously supplied by D.H. Sachs (NCI, Bethesda MD).

2.2. *Photolabeling of insulin receptors*

Liver membranes were purified from C57BL/6J (H-2^b) or AKR/J (H-2^k) mice as in [12]. Insulin receptors were labeled using the photoreactive insulin as in [2]. Briefly, liver membranes (4 mg protein) were incubated in 2 ml of Krebs–Ringer bicarbonate buffer containing 1% bovine serum albumin, 0.8 mg/ml bacitracin, 2 mM phenyl methyl sulfonyl fluoride, aprotinin (10000 trypsin inhibitors unit/ml) and 300 × 10^6 cpm of ^{125}I-photoreactive insulin for 3 h at 15°C in the dark. Photoactivation was induced by a 5-min exposure of the liver membrane suspension to UV light given by a water-cooled high pressure mercury lamp (Philips HPK 125 W) and filtered through a 'black glass' filter (UVW 55, Hanau).

2.3. *Solubilization of liver membranes and immunoprecipitation*

Following UV-irradiation membranes were collected by centrifugation (18000 × *g*, 5 min) and resuspended in buffer containing Hepes (50 mM; pH 7.4), NaCl (150 mM), bacitracin, aprotinin, phenylmethyl sulfonyl fluoride at the concentrations indicated above, and Triton X-100 (1%). Membranes were solubilized for 90 min at 4°C by continuous stirring. This preparation was centrifuged at 180000 × *g* for 15 min at 4°C (Airfuge, Beckman) and the supernatant was sampled (140 μl/tube) for immunoprecipitation. In brief, solubilized membranes were incubated 16 h at 4°C with different antibodies. Immunoprecipitation with protein A was achieved by addition of *S. aureus* cells (Pansorbin). After 4 h at 4°C the immunoprecipitates were collected by centrifugation (18000 × *g*, 1 min at 4°C) and washed twice with 50 mM Hepes/150 mM NaCl.

2.4. *Gel electrophoresis and autoradiography*

The immunoprecipitates were boiled for 5 min in a solution containing 3% (w/v) SDS, 10% (v/v) glycerol, 10 mM sodium phosphate, 2% (v/v) 2-mercaptoethanol, and 0.01% bromophenol blue. Aliquots of the precipitates were analyzed by one-dimensional SDS–PAGE as in [13], with a 7.5% acrylamide gel as the resolving gel. The M_r's of the standards used were: myosin, 200000; β-galactosidase, 116000; phosphorylase *b*, 94000; bovine serum albumin, 67000; ovalbumin, 43000;

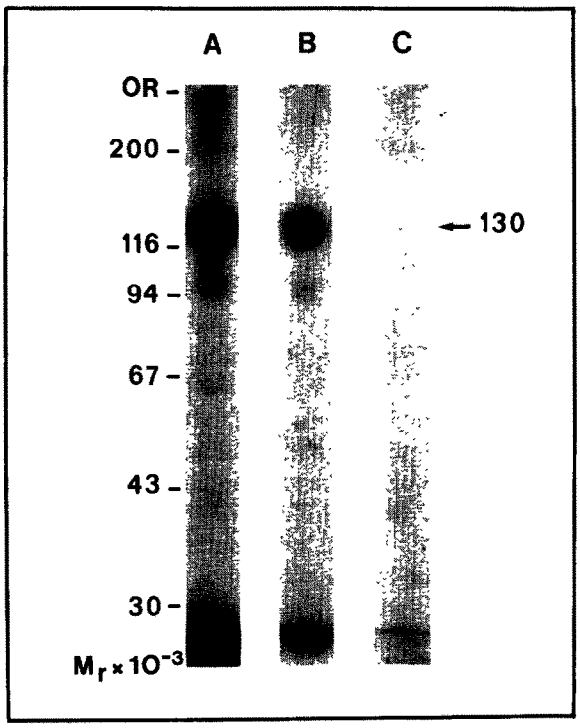

Fig.1. Immunoprecipitation of photolabeled insulin receptors. Liver plasma membranes from C57BL6/J (H-2^b) mice were labeled with photoreactive insulin and either directly solubilized and analyzed by SDS–PAGE under reducing conditions (A), or first immunoprecipitated with anti-insulin receptor antibodies (dilution 1:300) (B) and normal serum (dilution 1:150) (C) prior to gel analysis. An autoradiogram of a gel is shown.

carbonic anhydrase, 30000; soybean trypsin inhibitor, 20000; and lysozyme, 14400. The gels were stained, dried, and autoradiographed by exposing the gels to Kodak-X-Omat film as described [4]. The autoradiograms were scanned in a microdensitometer (Gelman) for quantitative analysis.

3. RESULTS

When photoreactive [^{125}I]insulin was covalently bound to its receptor in mouse liver membranes and membrane proteins directly analyzed without immunoprecipitation by SDS–PAGE under reducing conditions, only one radioactive band was detected on the autoradiogram, with an apparent M_r of 130000 (fig.1A). Using the same technique, a predominant labeling of a M_r 130000 component has also been reported in isolated rat hepatocytes [2] and purified rat liver membranes [14]. This component has been identified as the α-subunit of the insulin receptor in a variety of tissues and cell types [1,2,14]. We next investigated whether antireceptor antibodies precipitate the [^{125}I]insulin–receptor complex. As shown in fig.1B, anti-receptor antibodies were found to precipitate 60–70% of all labeled receptors present in the same amount of plasma membrane, whereas no detectable amount of labeled material could be precipitated by a non-immune serum from normal individuals (fig.1C).

The ability of antibodies reacting with histocompatibility antigens to precipitate photolabeled insulin receptors was then tested using a mouse serum raised against the major histocompatibility complex of H-2^b mice and liver membranes prepared from H-2^b mice (C57BL6/J). Increasing amounts of labeled insulin receptors were precipitated by increasing concentrations of this antiserum (fig.2). At a 1/16 dilution anti H-2^b antibodies precipitated about 5% of the insulin

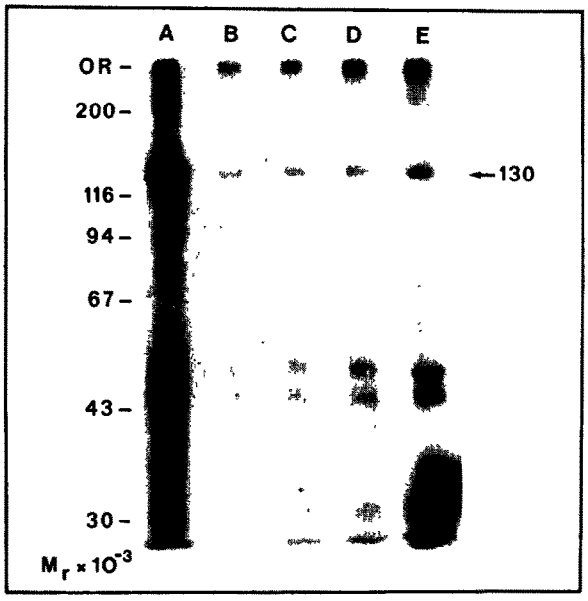

Fig.2. Immunoprecipitation of the M_r 130000 insulin receptor subunit by antiserum against histocompatibility antigens. Insulin receptors from C57BL6/J mouse (H-2^b) liver plasma membranes were labeled with photoreactive insulin and immunoprecipitated with serum containing autoantibodies to receptor (A) at a dilution of 1:300, or serum containing antibodies against H-2^b histocompatibility antigens (B to E) at dilutions of 1:128 (B), 1:64 (C), 1:32 (D), 1:16 (E). The precipitates were analyzed by SDS–PAGE under reducing conditions. An autoradiogram of a gel is shown.

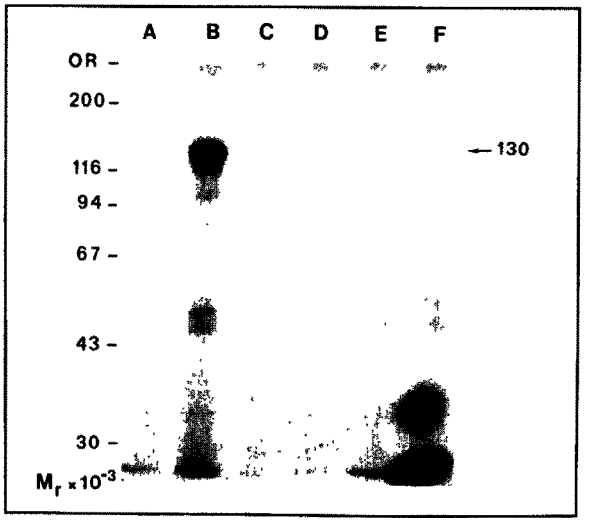

Fig.3. Immunoprecipitation of the M_r 130000 insulin receptor subunit by monoclonal antibodies against histocompatibility antigens. Conditions are similar to those presented in fig.2. The different lanes correspond to the following: (A) monoclonal antibody against I-A^b (dilution 1:32); (B) serum against insulin receptor (dilution 1:300); (C–F) monoclonal antibodies against KD^b at the following dilutions: (C) 1:128; (D) 1:64; (E) 1:32; (F) 1:16.

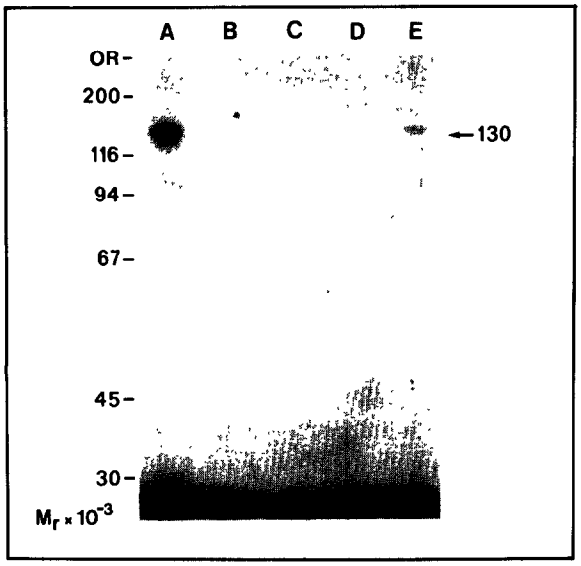

Fig.4. Immunoprecipitation of the M_r 130000 insulin receptors subunit by antibodies against histocompatibility antigens. Insulin receptors from AKR mouse (H-2^k) liver membranes were labeled with photoreactive insulin and immunoprecipitated with (A) serum-containing autoantibodies to the insulin receptor at a dilution of 1:300; (B) a serum from AKR mice at a dilution of 1:64; (C) serum-containing antibodies against H-2^b histocompatibility antigens at a dilution of 1:64; (D) a monoclonal antibody against DK^b at a dilution of 1:64; and (E) a monoclonal antibody against DK^k at a dilution of 1:64.

receptors precipitated by specific anti-insulin receptor antibodies (fig.2).

To further characterize the nature of the anti-MHC antibodies precipitating the insulin receptor we used monoclonal antibodies that recognize specifically the K and D regions of Class 1 histocompatibility antigens of the H-2^b haplotype. Again a dose-dependent immunoprecipitation of the insulin receptor was obtained with anti KD^b monoclonal antibodies. In contrast, a monoclonal antibody against the I-A^b region of the MHC (Class 2 antigens) did not precipitate the receptor (fig.3).

Finally, when insulin receptors from H-2^k mouse liver membranes were photolabeled, only a monoclonal antibody against the KD^k region could precipitate the receptor (fig.4E). No precipitation was obtained with antibodies against Class 1 histocompatibility antigen of the H-2^b haplotype (fig.4B,D) nor with normal mouse serum from H-2^k mice (fig.4C).

4. DISCUSSION

Murine class 1 MHC antigens are composed of a polymorphic trans-membrane polypeptide of M_r 44000 which is associated non-covalently with β_2 microglobulin (M_r 12000). These antigens are expressed on essentially all cell types and their known primary biological function is to guide T-lymphocytes in distinguishing self from non-self.

A physical interaction between class 1 antigens and foreign antigens, is the basis for effective cytotoxic T-lymphocyte activity. Indeed, examples of such interaction at the membrane of target cells have been shown for Semliki–Forest virus proteins [15] or for adenovirus glycoprotein [16]. Here we report that class 1 MHC antigens from liver membranes interact structurally with another protein multimer expressed at the cell surface, namely the insulin receptor. This is in agreement with recent reports on a functional interaction between human major histocompatibility antigens and epidermal growth binding [17], and insulin binding [18] to human cells. Whether a component of the insulin receptor is identical to or part of the MHC remains to be elucidated. In any case the present demonstration of a physical interaction between insulin receptors and the MHC may constitute the basis for a reevaluation of both the physiological function of the MHC and the mechanism of action of insulin.

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