Volume 283, number 1, 15–18 FEBS 09725 May 1991

ADONIS 001457939100433M

Dissociation of the hepatic insulin receptor favours its phosphorylation by casein kinase 2

Ramon Trujillo, Marta Jose, Maria Plana, Elena Molina and Emilio Itarte

Departament de Bioquímica i Biologia Molecular, Facultat de Ciències, Universitat Autònoma de Barcelona, E-08193 Bellaterra (Barcelona), Spain

Received 7 March 1991

The $\alpha\beta$ heterodimeric form of untreated hepatic insulin receptor was a substrate for casein kinase 2, whereas the $\alpha_1\beta_1$ heterotetramer was not. On the contrary, autophosphorylation was detected only in the heterotetramer. Dissociation of the receptor by treatment with dithiothreitol decreased its autophosphorylation but favoured phosphorylation of its β-subunit by casein kinase 2.

Insulin receptor; Casein kinase 2; Protein phosphorylation; Rat liver

1. INTRODUCTION

Insulin receptor is a glycoprotein composed of extracellular α-subunits, which contains the insulin binding site, and transmembrane β-subunits, which possess the insulin stimulated tyrosine kinase activity [1]. In addition to its autophosphorylation on tyrosine, the β-subunit of the insulin receptor has been reported to be a substrate in vitro for serine/threonine kinases such as the cyclic AMP-dependent protein kinase [2], protein kinase C [3], casein kinase 2 [4], and a protein kinase associated to the receptor [5]. Phosphorylation of insulin receptor on serine/threonine residues is well documented to occur in vivo [6–8], and causes a reduction in the tyrosine kinase activity of the receptor.

The receptor extracted from human placenta and rat hepatocytes or hepatoma cells presents several oligomeric forms which correspond to different aggregation states of the α- and β-subunits [9–11]. Dissociation of the $\alpha_2\beta_2$ heterotetramer into $\alpha\beta$ heterodimer causes a loss of the high affinity insulin binding component inherent to the heterotetramer [10]. Furthermore, the $\alpha_2\beta_2$ heterotetramer is the form autophosphorylated in vivo [11], and interaction between two $\alpha\beta$ heterodimers is necessary to show insulin-dependent autophosphorylation in vitro [12].

The aim of the present work was to investigate if the oligomeric state of the insulin receptor also influenced its phosphorylation on serine/threonine residues by casein kinase 2.

Correspondence address: E. Itarte, Departament de Bioquímica i Biologia Molecular, Facultat de Ciències, Universitat Autònoma de Barcelona, Campus de Bellaterra, E-08193 Bellaterra (Barcelona), Spain

Abbreviations: DTT, dithiothreitol; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate

2. EXPERIMENTAL

Insulin was from Eli Lilly. ^{125}I-insulin B-26 was from Amersham. Casein kinase 2 was purified from rat liver cytosol [4]. Liver membranes were prepared from 5-week-old female rats by differential centrifugation. Membrane proteins were extracted by solubilization with 2% Triton X-100 and subjected to chromatography on wheat germ lectin-agarose. The retained proteins were eluted by washing the column with 0.3 M *N*-acetyl-D-glucosamine as in [4].

Autophosphorylation of insulin receptor and its phosphorylation by casein kinase 2 was carried out as described previously [4]. In all cases, samples were analyzed by 0.1% SDS/5% PAGE under non-reducing conditions or by 0.1% SDS/7.5% PAGE under reducing conditions. In some experiments, the samples were also analyzed by non-denaturing polyacrylamide gel electrophoresis using a linear gradient of 2–15% acrylamide, according to [13]. The gels were dried, autoradiographed and the intensity of the bands was quantitated by densitometry.

Cross-linking experiments with 1.25 nM ^{125}I-insulin, in the absence or in the presence of 1 μM unlabeled insulin, were carried out using 1 mM disuccinimidyl suberate as in [14].

3. RESULTS

3.1. *Analysis of the phosphorylated insulin receptor: oligomeric structure of insulin receptor species*

Cross-linking experiments with ^{125}I-insulin of rat liver insulin receptor preparations purified through lectin-agarose showed the existence of two receptor species with apparent M_r of 300 and 200 kDa (Fig. 1A). This pattern is similar to that observed by other groups in rat liver and human placenta [9,10] where they have been shown to correspond to the $\alpha_2\beta_2$ and $\alpha\beta$ species. As expected from previous data [15], the amount of

Published by Elsevier Science Publishers B.V.

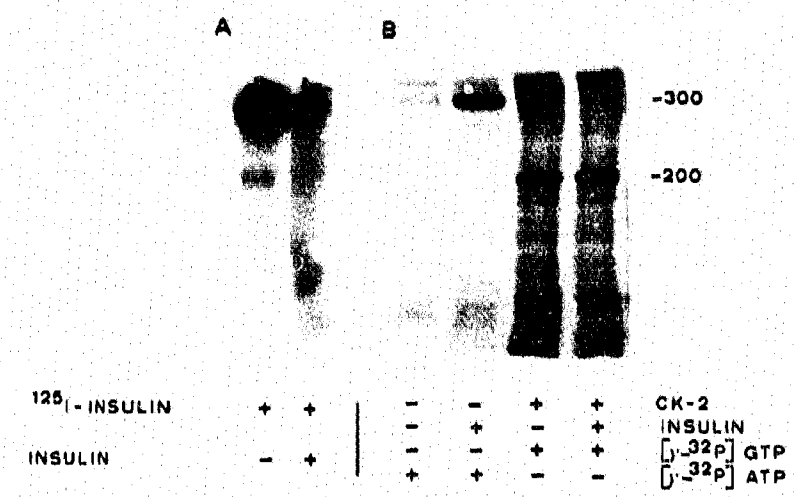

Fig. 1. Oligomeric structure of insulin receptor species. (A) Cross-linking with ^{125}I-insulin (1.2 nM), either in the absence or in the presence of 1 μM unlabeled insulin, was carried out using 1 mM disuccinimidyl suberate under standard conditions. Samples were electrophoresed, dried and autoradiographed. (B) Insulin receptor autophosphorylation (lanes 1 and 2) and phosphorylation by casein kinase 2 (CK-2) (lanes 3 and 4). The partially purified insulin receptor was incubated either in the absence or in the presence of 0.1 μM insulin prior to phosphorylation. Samples were analyzed by SDS-PAGE under non-reducing conditions. The autoradiogram of the gel is shown. The apparent M_r of the bands are indicated in kDa on the side of the autoradiogram.

^{125}I-insulin bound to the $\alpha\beta$ species was very small as compared with that observed with the $\alpha_2\beta_2$ heterotetramer.

The two receptor species showed different abilities to autophosphorylate and to serve as substrates for casein kinase 2 (Fig. 1B). Only the larger M_r species showed autophosphorylation whereas phosphorylation by casein kinase 2 seemed to be restricted to the lower M_r species. The different oligomeric structure of the receptor subjected to autophosphorylation or to phosphorylation by casein kinase 2 was also observed when analyzed by electrophoresis in non-denaturing gels (Fig. 2A). Phosphate incorporation due to autophosphorylation was clearly stimulated by insulin whereas phosphorylation by casein kinase 2 was not. Both the high and low M_r species gave rise to bands that migrate with M_r in the range of 95–103 kDa when analyzed in SDS-PAGE under reducing conditions (Fig. 2B). The presence of the 95–103 kDa doublet may arise from different levels of phosphorylation of the β-subunit, as has been reported to occur with insulin receptor from H-35 hepatoma cells [16].

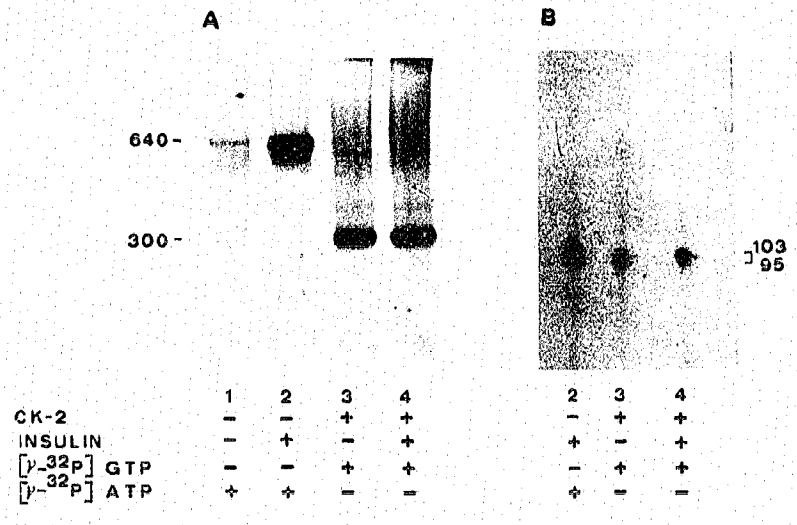

Fig. 2. Analysis of phosphorylated insulin receptor under non-denaturing conditions. (A) Insulin receptor was autophosphorylated either in the absence or in the presence of 75 nM insulin, or phosphorylated by casein kinase 2 (CK-2) using either 0.025 mM [γ-^{32}P]ATP or 0.025 mM [γ-^{32}P]GTP and analyzed in non-denaturing gels. (B) Electrophoretic mobility in SDS-PAGE under reducing conditions of the bands extracted from the non-denaturing gel shown in (A). The apparent M_r of the bands are indicated in kDa on the side of the autoradiogram.

3.2. *Influence of DTT on insulin receptor phosphorylation*

Reduction with DTT causes the cleavage of the heterotetrameric form of insulin receptor into $\alpha\beta$ dimers [10]. Since the form of the receptor which is preferentially phosphorylated by casein kinase 2 corresponded to the $\alpha\beta$ dimer, we assayed the influence of prior treatment of insulin receptor preparations with DTT on its phosphorylation by this kinase.

As shown in Fig. 3A, DTT-treatment led to the appearance of autophosphorylated $\alpha\beta$ heterodimers and free β-subunits. However, the level of total ^{32}P-incorporation decreased as the concentration of DTT in the phosphorylation assay increased. In contrast, the presence of DTT increased considerably the phosphorylation of the receptor by casein kinase 2, but this increase was more prominent at DTT concentrations that give rise to the appearance of free β-subunits.

In addition to the 95–103 kDa band, treatment with DTT prior to incubation with casein kinase 2 led to a marked increase in the phosphorylation of a 275–300 kDa band. This band was not observed in samples that were first phosphorylated with casein kinase 2 and then treated with similar amounts of DTT

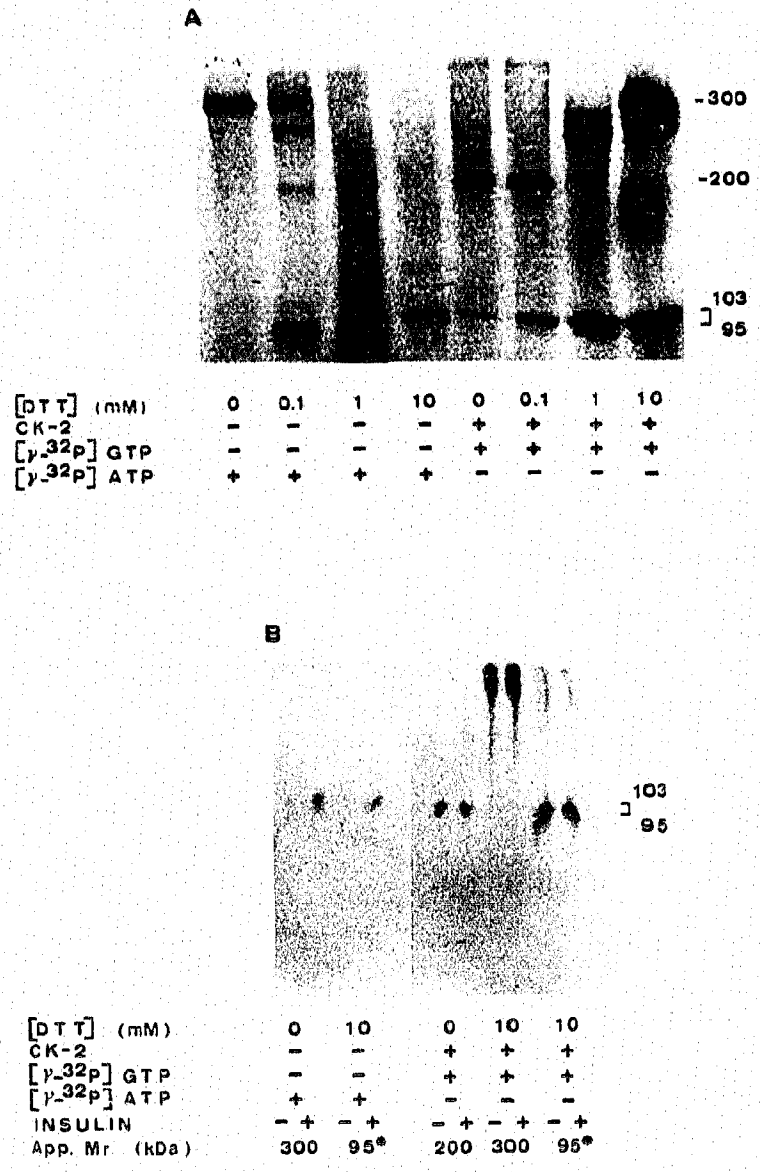

Fig. 3. Effect of DTT on insulin receptor phosphorylation. (A) Insulin receptor was treated with different concentrations of DTT in the presence of 75 nM insulin prior to phosphorylation by casein kinase 2 (CK-2) and analysis by SDS-PAGE under non-reducing conditions. (B) The bands from a gel similar to that shown in (A) were extracted, treated with β-mercaptoethanol and analyzed in SDS-PAGE under reducing conditions. The app. M_r indicated at the bottom of the figure refers to that determined for each band under the conditions shown in (A). The 95–103 kDa doublet was referred as 95*. The apparent M_r of the bands are indicated in kDa on the side of the autoradiogram.

(data not shown). This rules out that its presence is due to any effect of DTT facilitating its entrance in the SDS-PAGE gels. The apparent M_r of the high M_r band phosphorylated by casein kinase 2 did not vary when analyzed in SDS-PAGE under reducing conditions (Fig. 3B).

4. DISCUSSION

The data obtained on the phosphorylation of insulin receptor by casein kinase 2 suggest that the phosphorylation sites for this kinase are hindered in the $\alpha_2\beta_2$ heterotetramer and become accessible upon its dissociation into $\alpha\beta$ heterodimer, and especially in the free β-subunits. This is in contrast to the autophosphorylation of the receptor which took place mainly in the $\alpha_2\beta_2$ heterotetramer, as already reported by other groups [11,12,15]. This fact is interesting since the $\alpha\beta$ heterodimer has been shown to represent an inactive state of the receptor, whereas the heterotetramer would correspond to the active receptor [15].

The possible physiological meaning of this different susceptibility to autophosphorylation and to phosphorylation by casein kinase 2 remains to be elucidated. Nevertheless, our data indicate that the inactivation of insulin receptor by dissociation into $\alpha\beta$ heterodimers is accompanied by strong conformational changes in the β-subunit and reinforce the idea that changes in the oligomeric state of the insulin receptor have a strong influence on its properties.

Acknowledgements: This work was supported in part by Grants SC1.0223.C from the EEC and PB 86-0374 from DGICYT (Spain).

REFERENCES

[1] Rosen, O.M. (1987) Science 237, 1452–1458.
[2] Roth, R.R. and Beaudoin, J. (1987) Diabetes 36, 123–126.
[3] Bollag, G.E., Roth, R.A., Beaudoin, J., Mochly-Rosen, D. and Koshland, D.E. (1986) Proc. Natl. Acad. Sci. USA 83, 5822–5824.
[4] Grande, J., Pérez, M. and Itarte, E. (1988) FEBS Lett. 232, 130–134.
[5] Lewis, R.E., Perry Wu, G., MacDonald, R.G. and Czech, P.M. (1990) J. Biol. Chem. 265, 947–954.
[6] Kasuga, M., Zick, Y., Blith, D.L., Karlsson, F.A., Häring, H.U. and Kahn, C.R. (1982) J. Biol. Chem. 257, 9891–9894.
[7] Pang, D.T., Sharma, B.R., Shafer, J.A., White, M.F. and Kahn, R. (1985) J. Biol. Chem. 260, 7131–7136.
[8] Takayama, S., White, M.F. and Kahn, C.R. (1988) J. Biol. Chem. 263, 3440–3447.
[9] Massagué, J. and Czech, M.P. (1980) Diabetes 29, 945–947.
[10] Böni-Schnetzler, M., Scott, W., Waugh, S.M., DiBella, E. and Pilch, P.F. (1987) J. Biol. Chem. 262, 8395–8401.
[11] Le Marchand-Brustel, Y., Ballotti, R., Grémeaux, T., Tanti, J.F., Brandenburg, D. and Van Obberghen, E. (1989) J. Biol. Chem. 264, 21316–21321.
[12] Boni-Schnetzler, M., Kaligian, A., DelVecchio, R. and Pilch, P. (1988) J. Biol. Chem. 263, 6822–6828.
[13] Kubar, J. and Van Obberghen, E. (1989) Biochemistry 28, 1086–1093.
[14] Pilch, P.F. and Czech, M.P. (1980) J. Biol. Chem. 255, 1722–1731.
[15] Flörke, R.R., Klein, H.W. and Reinauer, H. (1990) Eur. J. Biochem. 191, 473–482.
[16] Tashiro-Hashimoto, Y., Tobe, K., Koshio, O., Izumi, T., Takaku, F., Akanuma, Y. and Kasuga, M. (1989) J. Biol. Chem. 264, 6879–6885.