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Glycated albumin (Amadori product) induces activation of MAP kinases in monocyte-like MonoMac 6 cells

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Abstract

Increased levels of glycated, Amadori-modified albumin are a risk factor for diabetic vascular disorders. Glycated albumin binds to specific receptors and induces cellular signaling pathways, the complexity of which is largely unknown. Binding of glycated albumin to MonoMac 6 cells leads to an activation of MAPK p44/42 (ERK1/2) and p38 with subsequent translocation of NF-κB into the nucleus. The activation of MAPK is in part mediated by protein kinase C activation, but a PKC-independent pathway via MEK-1 is also involved. Protein tyrosine kinases do not play a role in the activation of NF-κB. The results may have pathophysiological significance, because the MonoMac 6 cell line is not greatly different from blood monocytes.

Keywords: Glycated albumin; Fructoselysine; Receptor; Signal transduction; MonoMac 6

1. Introduction

Chronic hyperglycemia in diabetes leads to an increased formation of Amadori products through the nonenzymatic glycation reaction between glucose and free amino groups in proteins. Initially, a Schiff base is formed which rearranges into an aminoketose (Amadori product). The main Amadori product in plasma proteins is fructoselysine. These Amadori adducts undergo further irreversible reactions to form advanced glycation end products (AGEs). It has been established that these AGEs play an important role in aging of tissues and in the pathogenesis of late diabetic complications, in chronic renal failure, in disturbances of peritoneal dialysis and in some degenerative brain diseases, for instance Alzheimer's disease [1,2]. In addition, several studies have demonstrated that Amadori products mainly in the form of glycated albumin are also associated with diabetic macro- and microangiopathies [3–7]. Glycation induces alterations in the properties of proteins. Thus for example, glycated, Amadori-modified albumin exerts its effects by binding to specific receptors on several cell types. Such sites were found on monocytes, peritoneal and alveolar macrophages, the monocytic cell lines MonoMac6 (MM6), U937, THP-1, and HL-60, as well as on endothelial cells and fibroblasts [8–10]. Membrane-bound nucleolin, nucleophosmin and a myosin heavy chain derivative with molecular masses of 110, 150 and 200 kDa respectively have recently been shown to interact with glycated albumin through the fructoselysine moieties. Binding of glycated albumin to MM6 cells induced secretion of the proinflammatory cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). These cells binding of glycated albumin led to activation of protein kinase C-ε (PKC) and this was linked to translocation of activator protein-1 (AP-1) and nuclear factor-κB (NF-κB) into the nucleus. Glycated albumin also stimulated activation of protein tyrosine kinases (PTK) and the PKC inhibitor GÖ6976 prevented all of these effects. Genistein, an inhibitor of tyrosine kinases prevented the activation of AP-1, but not the activation of NF-κB, which was only dependent on

Abbreviations: AGEs, advanced glycation end products; AP-1, activator protein-1; ERK, extracellular signal-regulated kinase; GA, glycated albumin; IL-1β, interleukin 1β; MAPK, mitogen-activated protein kinases; NA, native, nonglycated albumin; NF-κB, nuclear factor-κB; PKC, protein kinase C; PTK, protein tyrosine kinase; ROS, reactive oxygen species; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; TNF, tumor necrosis factor

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PKC activity [8–14]. Recently, Naitoh et al. [15] also showed the release of TNF-α through glycated albumin in human monocyte-like THP-1 cells.

Cohen and Ziyadeh and Chen et al. [16,17] demonstrated binding sites for an Amadori-modified amino acid sequence in glycated albumin on endothelial and mesangial cells. Glycated albumin stimulated PKC-β activation, TGF-β1 production and increased collagen synthesis in mesangial and glomerular endothelial cells, which may be important for the development of diabetic nephropathy. Activation of the mitogen-activated protein kinases (MAPK) extracellular signal-regulated kinase (ERK) 1/2 by glycated albumin is required for the inhibition of cell growth and enhanced matrix protein synthesis in mesangial cells [18]. Glycated albumin also activated ERK and NF-κB in macrophages and induced oxidative stress, which is partially responsible for NF-κB translocation [19]. The glycated protein is involved in the development of insulin resistance in skeletal muscle cells due to inhibition of the PI3K/PKB insulin signal transduction pathway through activation of PKC-α, while leaving ERK pathway unchanged [20].

The existence of fructoselysine-specific receptors, which bind glycated albumin on monocytes/macrophages supports the hypothesis that glycated albumin may stimulate monocytes and activate various signal transduction pathways and transcription factors. The activation of PKC and PTK in MM6 results in a translocation of AP-1 and NF-κB into the nucleus and an increased synthesis of proinflammatory cytokines [9,13]. It is not known, whether activated PKC directly stimulates NF-κB activation and which role MAPK and oxidative stress may have in eliciting this pathophysiologically important reaction. We demonstrate here that glycated albumin promotes activation of the MAPK ERK1/2 (p44/42) and p38 in the presence of the antioxidant *N*-acetyl-L-cysteine in the monocyte-like cell line MonoMac 6.

2. Materials and methods

2.1. Albumins

Glycated and nonglycated human serum albumins were purchased from Sigma (Steinheim, Germany). Glycated albumin contained 2.7–3.5 mol fructoselysine per mol albumin. In glycated and nonglycated albumin fluorescent AGEs and bacterial lipopolysaccharide were not detectable, carboxymethyllysine was present in only minute amounts. The protein is not borohydride reduced [20].

2.2. Cell culture

MM6 cells (DSMZ, Braunschweig, Germany) were seeded into 6-well plates at density of 1×10^6/well and grown overnight in RPMI 1640 (PAA, Pasching, Austria) supplemented with 10% fetal calf sera, 100 μg/ml kanamycin, 1 mM sodium pyruvate, 2 mM glutamine, 1× non-essential amino acids (Biochrom Seromed, Berlin, Germany) and 500 μM *N*-acetyl-L-cysteine for prevention of oxidative stress. The cells were cultivated for a further 24 h before the indicated amounts of native or glycated albumin were added for 30 min.

In selected experiments, cells were preincubated for 30 min with the MEK-1 inhibitor PD 98059, the specific inhibitor of p38 MAPK SB 203580, the specific inhibitor of PKC-ε Ro-32-0432, or genistein, a protein tyrosine kinase (PTK) inhibitor (Calbiochem, Darmstadt, Germany).

2.3. Activation of p38 MAPK and ERK

MM6 cells were washed with ice-cold PBS pH 7.4 and solubilized in lysis buffer (25 mM Tris–HCl (pH 7.4), 50 mM NaF, 100 mM NaCl, 5 mM EGTA, 1 mM EDTA, 1% (v/v) triton, 92 mg/ml saccharose, 1 mM sodium vanadate, 1 mM benzamidine, 0.1 mM PMSF, 2 μM microcystin, 0.1% (v/v) mercaptoethanol). Cellular lysates (50 μg protein) were fractionated on SDS-PAGE (10%) and analyzed by immunoblotting.

Immunoblot analysis was performed using a phospho-p38 MAPK antibody, which recognizes phosphorylated threonine 180 and tyrosine 182 residues of p38 MAPK, or using a phospho-p44/42 MAPK antibody, which recognizes endogeneous levels of p42/p44 MAPK (ERK1/2) only when phosphorylated at threonine 202 and tyrosine 204 of human ERK (Cell Signaling, www.cellsignal.com). The primary antibody against total p38 MAPK was from Calbiochem, San Diego, CA, U.S.A. The primary antibody against ERK1/2 was from Sigma, Steinheim, Germany. Blots were washed and then incubated with the corresponding anti-rabbit or anti-mouse–horseradish-peroxidase conjugate (1:3000 dilution) and detected by enhanced chemiluminescence reagent (ECL, Amersham, Buckinghamshire, UK). Appropriate exposures were quantitated by densitometry.

2.4. Electrophoretic mobility shift assay

1×10^7 MM6 cells were incubated in the presence of the indicated amounts of ligands and 500 μM *N*-acetyl-cysteine for 2 h at 37 °C. Cells were collected and washed twice with ice-cold PBS. The cell pellet was resuspended in 1 ml 10 mM HEPES (pH 7.6), 15 mM KCl, 2 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM dithiothreitol, 0.1% NP-40, 1 mM PMSF, 10 μg/ml aprotinin, 1 μg/ml pepstatin, 2 μg/ml leupeptin and incubated on ice for 10 min. The samples were then centrifuged at 1000×*g* for 5 min at 4 °C. The nuclear pellet was resuspended in 50 μl HEPES (pH 7.9), 0.42 M NaCl, 25% glycerol, 0.2 mM EDTA, 0.5 mM dithiothreitol and protease inhibitors as above and mixed on ice for 15 min. The nuclear extract was centrifuged at 15,000×*g* for 10 min at 4 °C. The supernatant (nuclear proteins) was collected and stored at −70 °C. NF-κB consensus oligonucleotide (5′-AGT TGA GGG TTT CCC AGG C-3′) was 5′-end labeled with ^{32}P γ-ATP (Amersham) using T4 poly-nucleotide kinase (Promega) to specific activity of 5000 to 20,000 cpm/100 fmol. Nonincorporated radioactivity was removed using Microspin G25 columns (Amersham). 6 μg of nuclear protein was incubated with 0.5 ng of labeled oligonucleotide in binding buffer (10 mM Tris–HCl (pH 7.5), 50 mM NaCl, 1 mM $MgCl_2$ 0.5 mM EDTA, 0.5 mM dithiothreitol, 4% glycerol, 0.05 mg/ml poly(dI–dC)) for 20 min at room temperature in a final volume of 10 μl. Free oligonucleotide and oligonucleotide protein complexes were separated by electrophoresis on a native 6% polyacrylamide gel. The gel was dried and exposed to a X-ray film with intensifying screen overnight at −70 °C. Specificity of binding was ascertained by competition with a 100-fold excess of unlabeled consensus oligonucleotide.

2.5. Detection of activated transcription factor NF-κB p65

1×10^7 MM6 cells were incubated in the presence of the indicated ligands and 500 μM *N*-acetyl-L-cysteine for 2 h at 37 °C. For inhibition experiments cells were pretreated for 30 min with either the PTK inhibitor genistein (100 μM), the PKC inhibitor Ro-32-0432 (200 nM), the specific MAPK p38 inhibitor SB 203580 (10 μM) or the specific MEK-1 inhibitor PD 98059 (50 μM), respectively.

Cells were collected and washed twice with ice-cold PBS. The cell pellet was resuspended in 1 ml 10 mM HEPES (pH 7.6), 15 mM KCl, 2 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM dithiothreitol, 0.1% NP-40, 1 mM PMSF, 10 μg/ml aprotinin, 5 μg/ml antipain, 2 μg/ml leupeptin and incubated on ice for 10 min. The samples were then centrifuged at 1000×*g* for 10 min at 4 °C. The nuclear pellet was resuspended in 50 μl PBS (pH 7.4), 1% NP-40, 0.5% sodiumdesoxycholate, 0.1% SDS, 1 mM dithiothreitol and the protease inhibitors as above and mixed on ice for 15 min. The nuclear extract was centrifuged at 15,000×*g* for 10 min. The supernatant (nuclear proteins) was collected and stored at −70 °C. Protein content was quantified using a BioRad protein assay.

NF-κB quantification was performed with an NF-κB p65 ELISA Kit from Stressgen Bioreagents Corporation (Victoria, Canada). Equal amounts of