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Review

Glycation of the high affinity NGF-receptor and RAGE leads to reduced ligand affinity

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ABSTRACT

AGEs are posttranslational modifications generated by irreversible non-enzymatic crosslinking reactions between sugars and proteins – a reaction referred to as glycation. Glycation, a feature of ageing, can lead to non-degradable and less functional proteins and enzymes and can additionally induce inflammation and further pathophysiological processes such as neurodegeneration.

In this study we investigated the influence of glycation on the high affinity NGF-receptor TrkA and the AGE-receptor RAGE. We quantified the binding affinity of the TrkA-receptor and RAGE to their ligands by surface plasmon resonance (SPR) and compared these to the binding affinity after glycation. At the same time, we established a glycation procedure using SPR. We found that glycation of TrkA reduced the affinity to NGF by a factor of three, which could be shown to lead to a reduction of NGF-dependent neurite outgrowth in PC12 cells. Glycation of RAGE reduced binding affinity of AGEs by 10-fold.

1. Introduction

Advanced glycation endproducts (AGEs) have a growing role in different disciplines of sciences. First, in the research of frequent age-dependent human diseases (including neurodegeneration, arteriosclerosis or diabetes), second, in the food industry (browning), and, third, in biotechnology (storage and composition of recombinant proteins and enzymes). Glycation of proteins causes dysfunctions in ageing cells and tissues (Monnier, 1989). Glycation is an endogenous process during the metabolism of sugars resulting in protein crosslinks, protein denaturation, and finally in increased protein accumulation during ageing. The irreversible and protease-resistant glycated protein-crosslinks are called advanced glycation endproducts (AGEs) (Stirban et al., 2014). The initial reaction for formation of AGEs is a non-enzymatic glycosylation (i.e. glycation) between reactive carbonyl groups of monosaccharides and the ϵ-amino group of lysine or its free amino group. In addition, amino groups of several other amino acids such as arginine, tryptophan or cysteine are potential sites for glycation. The resulting Schiff base adducts rearrange to so-called Amadori products. After further oxidation and dehydration reactions, which include formation of radical oxygen species (ROS) as side products, yellow-brown fluorescent and thermo-stable AGEs are generated. The accumulation of reactive carbonyl carbolites or glycooxidation products is referred to as carbonyl stress. Two of these physiologically occurring carbolites of monosaccharides are glyoxal and methylglyoxal (Li et al., 2014). AGEs are a heterogeneous group of in part unknown compounds. Well known and characterized are carbomethyllysine (CML) and carboxylethyllysine (CEL), pentosidine, or non-oxidative AGEs such pyrroline, glyoxal lysine dimer (GOLD) or methylglyoxal lysine dimer (MOLD). The reactive dicarbonyl precursors glyoxal and methylglyoxal form CML and CEL, which serve as biomarkers and can be detected by specific antibodies (Krautwald and Münch, 2010; Requena et al., 1996).

ROS, which are generated during AGE formation, are also involved in cell damage, apoptotic processes (Loske et al., 1998) and early ageing processes (Uribarri et al., 2007). Taken together, glycation promotes ageing in multiple ways and is known to have an immense influence on a variety of age-dependent diseases, such as atherosclerosis, cataract, (diabetic) nephropathy or neurodegenerative disorders such as Alzheimer's disease (Tan et al., 2007).

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Alzheimer's disease is the most common neurodegenerative disease that affects 1–2% of the population older than 65 years and increases to 50% for 85 year old people (Imtiaz et al., 2014). Involvement of accumulated AGEs in different regions of the brain in Alzheimer's disease was first proposed by Smith et al. and Colaco et al. nearly twenty years ago (Colaco et al., 1996; Smith et al., 1995). Münch et al. showed in 2002 an accumulation of intracellular AGEís in up to 95% of pyramidal neurons in patients with Alzheimer's disease (Münch et al., 2002). These findings were confirmed by the detection of intracellular AGEs in neurons in the context of formation of neurofibrillary tangles and neuronal cell damage or death (Levi-Montalcini et al., 1954; Lüth et al., 2005).

Nerve growth factor (NGF) was described as a neuronal survival factor for sympathetic and sensory neurons (Levi-Montalcini et al., 1954). After binding to the high affinity thyrosine kinase receptor (TrkA), NGF promotes survival, differentiation and neurite (axonal) outgrowth (Lärkfors et al., 1987; Sofroniew et al., 2001). Furthermore, the NGF/TrkA pathway plays an important role during development of the nervous system at several ontogenetic stages (Sofroniew et al., 2001). Binding of NGF to TrkA activates the MAP kinase, RAS, PI3K and phospholipase Cγ1 pathways. Although Lärkfors et al. have already demonstrated in 1987 a decreased level of NGF in aged rats (Lärkfors et al., 1987; Terry et al., 2011), little is known about age-related changes in the NGF/TrkA pathway.

Recently, we demonstrated that AGE modification reduces cell adhesion, NGF-mediated neurite outgrowth and activation of ERK in PC12 cells (Bennmann et al., 2014). Espinet et al. proposed in 2015 that AGE accumulation leads to an imbalance of the proNGF/NGF ratio and an inhibition of the NGF-mediated neuroprotection (Espinet et al., 2015).

AGEs bind to the multi-ligand receptor of advanced glycation endproducts (RAGE). RAGE was identified in 1992 by Neeper et al. as a receptor for AGEs with a molecular weight of 42.8 kDa. RAGE belongs to the immunoglobulin superfamily and shares homology to other immunoglobulin cell surface proteins, such as NCAM or MUC18 (Neeper et al., 1992). Since these proteins are cell adhesion molecules, Sessa et al. proposed an involvement of RAGE in cell adhesion (Sessa et al., 2014). RAGE is expressed in endothelial cells (Pollreisz et al., 2010), lung epithelial cells (Demling et al., 2006), in the immune system on monocytes and macrophages (Ohashi et al., 2010; Wang et al., 2010), in neuronal cells (Nah et al., 2007), and in smooth muscle cells (Zhu et al., 2012). Functionally, RAGE is involved in lung homeostasis, bone metabolism, in the adaptive and innate immune response, and in several neuronal functions such as nerve regeneration, migration and neuronal outgrowth (Ott et al., 2014). RAGE binds a wide variety of AGEs. One frequently studied ligand of RAGE is glycated bovine serum albumin (AGE–BSA). It is believed that binding of AGE–BSA to RAGE activates several signaling pathways (Ko et al., 2013; Lu et al., 2004; Nah et al., 2008). Mice lacking RAGE are viable and healthy but appear to be resistant to some diseases such as arteriosclerosis and neurodegeneration (Liliensiek et al., 2004).

We were interested in investigating the role of receptor glycation in the NGF/TrkA- and the RAGE/AGE-BSA-mediated signal transduction pathway. Therefore we investigated, first, the binding affinity of isolated RAGE to AGE-BSA via surface plasmon resonance (SPR), second, the binding affinity of NGF to glycated TrkA receptor, and, third, the influence of receptor glycation on neurite outgrowth.

We determined K_D values of 13 μM for glucose-BSA (glycation of BSA with glucose) and 19 μM for fructose-BSA (glycation of BSA with fructose) as ligands of RAGE. However, after glycation of RAGE we measured a reduced binding affinity for glucose-BSA and no detectable binding for fructose-BSA. We observed a reduced binding of NGF to glycated TrkA receptor by a factor of three. Finally, we confirmed the physiological relevance of this effect by measuring a reduction of NGF-mediated neurite outgrowth by reducing the NGF concentration by 3-fold in PC12 cells.

2. Material and methods

2.1. NGF /TrkA kinetics by surface plasmon resonance

Receptor binding interactions were performed using a surface plasmon resonance (SPR) Biacore 2000 system (GE Healthcare, Freiburg, Germany). NTA sensor chips (GE Healthcare, Freiburg, Germany) were used for coupling of His-tagged TrkA receptor (Sino biological Inc., Beijing, China). Each flow cell was activated individually by injecting 500 μM $NiCl_2$ for 1 min at 20 μl/min followed by injecting 0.2 M EDC/0.05 M NHS for 7 min at 5 μl/min. 50 μg/ml TrkA receptor was injected for 1–5 min at a flow rate 2 μl/min until a desired level of immobilization was reached. About 400 response units (RU) ± 50 RU were coupled, which is equivalent to an amount of 400 pg receptor/mm^2 ± 150 pg/mm^2. This amount corresponds to the calculated analyte binding capacity (theoretical RU) of the chip. The real-time measurements of binding kinetics were performed with several different immobilized TrkA flow cells for independent measurements. Excess reactive esters were blocked using 1 M ethanolamine for 7 min at 5 μl/min. A non-receptor coupled flow cell served as appropriate reference surface. A running buffer composed of 10 mM HEPES, 150 mM NaCl, 50 μM EDTA, 0.005% Tween 20, pH 7.4 (HBS buffer) at a flow rate of 5 up to 10 μl/min was used. Binding isotherms were determined at 23 °C.

To measure the kinetics of NGF (ImmunoTools, Friesoythe, Germany) over TrkA on the chip, NGF concentrations in threefold serial dilutions (0, 0.06, 0.2, 0.6, 1.85, 5.5, 16, 50 nM) on three independent TrkA immobilized surfaces were injected for 3 min at 30 μl/min. A 30 s dissociation phase was used. The measurement conditions were chosen to minimize potential mass transport effects. Finally, the dissociation constant was calculated by three separate experiments on three flow cells. Surface was regenerated in two steps, first, with a 1:1 mix of running buffer, pH 9.3, and 10 mM NaOH for 1 min at 10 μl/min, and, second, with 10 mM NaOH for 10 s at 30 μl/min. The sensogram data from real-time bimolecular interaction analysis were evaluated with BIAevaluation software.

2.2. Preparation of glycated BSA

1 mM endotoxin free BSA (Thermo Fisher Scientific, Waltham, USA) was dissolved in 50 mM potassium phosphate buffer, pH 7.3. The buffer was supplemented with 1 mM EDTA (Carl Roth, Karlsruhe, Germany). A potassium phosphate stock solution with a ratio of K_2HPO_4 (77%) to KH_2PO_4 (23%) was required. 0.5 M D(−) glucose, D(−) fructose and D(−) ribose (Sigma–Aldrich, St. Louis, Germany) were separately added to each BSA solution. As control, BSA dissolved in potassium phosphate buffer, pH 7.3, with 1 mM EDTA was used. The four preparations were incubated at 50 °C for 40 days. After incubation, the glycated BSA and BSA control solutions were dialyzed against double distillated water for 72 h. Each solution was lyophilized, resolved by sterilized PBS, pH 7.4 (GE Healthcare, Freiburg, Germany), and adjusted to a concentration of 10 mg/ml (150 μM). We refer to the indefinite glycated BSA solutions by their glycation argents, glucose-BSA, fructose-BSA, or ribose-BSA.

2.3. Detection of BSA glycation by Coomassie staining

20 μg of each BSA solution was separated on 8% SDS-polyacrylamide gel (C.B.S., Scientific Company, Del Mar, USA). The gel was stained with 1% Coomassie brilliant blue (Serva Electrophoresis GmbH, Heidelberg, Germany) in 10% acetic acid (Carl Roth, Karlsruhe, Germany), 40% ethanol (Sigma–Aldrich, St. Louis,