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The Parkinsonism-associated protein DJ-1/Park7 prevents glycation damage in human keratinocyte

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ABSTRACT

Reducing sugars and dicarbonyls form covalent adducts with proteins through a nonenzymatic process known as glycation, which inactivates proteins, is increased in diabetic patients and is associated with diabetic complications, including retinopathy, cataracts, nephropathy, neuropathy, cardiomyopathy and skin defects. We recently characterized DJ-1/Park7 as a protein deglycase that repairs proteins from glycation by glyoxal and methylglyoxal, two major glycating agents which are responsible for up to 65% of glycation events.

In this study, we investigated the ability of DJ-1 to prevent protein glycation in keratinocytes. Glycation of collagen and keratinocyte proteins was tested by measuring ultraviolet absorption and fluorescence emission. Protein glycation in HaCaT keratinocytes was investigated by immunodetection with anti-advanced glycation endproduct antibodies, after DJ-1 depletion or overexpression. *In vitro*, DJ-1 prevented glycation of collagen and keratinocyte protein extracts. In cell culture, DJ-1 depletion by small interfering RNAs resulted in a 3-fold increase in protein glycation levels. Moreover, protein glycation levels were decreased several-fold in cells overexpressing DJ-1 after addition of the Nrf2 inducer sulforaphane or after transfection with a DJ-1 plasmid. Thus, the DJ-1 deglycase plays a major role in preventing protein glycation in eukaryotic cells and might be important for preventing skin glycation.

1. Introduction

Glycation is a non-enzymatic reaction discovered by Louis Camille Maillard [1] that occurs between reducing sugars or dicarbonyls (glyoxal and methylglyoxal) and thiol or amino groups of proteins, nucleic acids and aminolipids [2]. Protein glycation begins with a condensation reaction between carbonyl groups and amino acids, afterwhich a series of dehydrations, oxidations and rearrangements leads to a myriad of products, including Schiff bases, Amadori products, advanced glycation end-products (AGEs), and protein cross-links [2]. Glycation results in protein inactivation and aggregation, mutations and tumorigenesis, and is involved in aging, atherosclerosis, hypertension, and neurovegetative, renal, autoimmune, and post-diabetic diseases [3]. Glycation occurs in healthy cells because reducing sugars and dicarbonyls are continuously produced by metabolic reactions: Methylglyoxal is formed spontaneously from triosephosphates and other minor pathways, and glyoxal is formed by lipid peroxidation and the degradation of monosaccharides and glycated proteins [2]. Glycation by methylglyoxal and glyoxal may represent up to 65% of glycation events [3]. Defense against glycation involves aldoketoreductases, glyoxalases and efflux pumps, which scavenge reactive carbonyls [2], and fructosamine-3-kinases [4] and DJ-1 [5], which repair proteins that have been glycated by glucose and glyoxals, respectively. Whereas the ubiquitously expressed protein DJ-1 has frequently been involved in oxidative stress protection [6], its powerful deglycase activity suggests that it may efficiently protect cells by repairing methylglyoxal- and glyoxal-glycated cysteine, arginine and lysine residues of proteins [5]. Diabetic organisms have increased levels of glucose and dicarbonyl compounds (the plasma methylglyoxal (MGO) level is increased by up to 5–6-fold in diabetic patients [7]),

Abbreviations: MGO, methylglyoxal; AGE, advanced glycation endproduct.
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and, consequently, they suffer from increased glycation damage [3]. Serum albumin, fibrinogen, immunoglobulins, collagen, cristallin, lipoproteins, integrins, keratins and many other proteins display increased glycation levels in diabetic patients and undergo a functional decline which is responsible for vascular stiffness, cardiomyopathy, nephropathy, neuropathy, cataract, autoimmune diseases, and skin alterations [7].

Glycation is responsible for skin dysfunction and aging. Accumulation of AGEs has been detected in fibroblasts and keratinocytes, and is increased in aged skin, UV-irradiated skin and diabetes [8]. AGEs cause damage that may be the origin of skin defects such as loss of elasticity, drying, thinning, stiffening, defects in wound repair, and the appearance of wrinkles. Glycation of collagen impairs its degradation by matrix metalloproteases and its interaction with integrins, and triggers the formation of aged skin *in vitro* [9]. The AGE carboxymethyllysine has been detected in the epidermis of healthy persons [10], and UV-B irradiation enhances glycation of skin keratin [11]. AGEs induce premature senescence in fibroblasts and keratinocytes, and decrease cell viability and migration of keratinocytes [12]. Several reports suggest that DJ-1 plays a role in skin homeostasis. DJ-1 is overexpressed in keratinocytes after oxidative stress [13] and during the re-epithelialization of wounded human skin equivalents [14]. Moreover, decreased DJ-1 levels were observed in patients with atopic dermatitis [15]. In this report we show that the DJ-1 deglycase prevents protein glycation in keratinocytes. The ability of DJ-1 to prevent protein glycation in eukaryotic cells suggests that it might be useful for alleviating skin glycation and post-diabetic diseases.

2. Materials and methods

2.1. Purification of human DJ-1

DJ-1 was prepared as described previously (5).

2.2. Preparation of keratinocyte extracts

The immortal human keratinocyte line HaCaT was cultured in Dulbecco's Modified Eagle Media (Life Technologies) containing antibiotics and supplemented with 10% fetal calf serum and 2 mM L-glutamine. The cells were maintained at 37 °C in a 5% CO_2 incubator. Whole-cell protein extracts were prepared from 10 cm dishes of confluent cells using cell lysis buffer containing 25 mM Tris–HCl pH 7.5, 100 mM $CaCl_2$, 1 mM EDTA, 1 mM EGTA, 0.5% NP40, 1% Triton X-100, and a protease inhibitor cocktail (11836145001, Roche) and incubated for 30 min on ice. Then, cells were centrifuged at 8000 rpm at 4 °C for 10 min, and the supernatant was used.

2.3. Deglycation of collagen and keratinocyte protein extracts

Protein extracts from HaCaT cells were incubated for 48 h at 37 °C (at a concentration of 6 mg/ml) with 50 mM MGO, in the absence or presence of 20 μM DJ-1 (an extremely high MGO concentration was used to overwhelm the endogenous glyoxalase and deglycase activities of keratinocyte extracts). Calf skin collagen (Sigma) was solubilized in 100 mM acetic acid, neutralized with NaOH and incubated at a concentration of 1 mg/ml for 36 h at 37 °C in 50 mM sodium phosphate pH 7.8 with 10 mM MGO, either alone or in the presence of 2 μM DJ-1. Protein glycation was analyzed by measuring UV absorption between 300 and 360 nmn and fluorescence emission between 400 and 500 nm ($\lambda_{ex} = 370$ nm), which are both characteristic of glycated proteins (5).

2.4. Glycation levels in keratinocytes

Exponentially growing HaCaT cells at 40% confluency were treated with sulforaphane, small interfering RNAs (siRNAs) or a plasmid containing DJ-1 DNA. Thereafter, these cells were compared with control cells in terms of DJ-1 expression and glycation of protein extracts. Protein extracts were subjected to SDS-PAGE on 10% polyacrylamide gels, transferred onto nitrocellulose membranes and probed with anti-DJ-1 antibodies (Abcam) and anti-AGE antibodies (Cell Biolabs Inc.).

2.5. Transfection of keratinocytes with siRNAs and plasmids

Exponentially growing HaCaT cells at 30% confluency were transfected with three distinct siRNAs specific for DJ-1 and a control scrambled siRNA (50 nM each, SR307752, Origene) using the transfection reagent Lipofectamine RNAiMAX (13778-150, Life Technologies), according to the manufacturer's instruction. Transfected cells were cultured for 24 and 48 h and cell lysates were probed for DJ-1 expression and protein glycation.

Exponentially growing HaCaT cells at 40% confluency were transfected with the DJ-1/Park7 plasmid (green fluorescent protein (GFP)-tagged human transcript variant 1, pCMV6-AC-GFP Park7, RG201645, Origene) or with the empty vector using the Effectene® transfection reagent (301427, Qiagen) according to the manufacturer's instructions. Cell lysates were probed for DJ-1 expression and protein glycation.

3. Results

3.1. DJ-1 prevents glycation of collagen and keratinocyte extracts

The glycation status of keratinocyte extracts was quantified by measuring UV absorption and fluorescence emission. The extract incubated with MGO alone displayed strong absorption between 300 and 360 nm, whereas extracts incubated without MGO, or with MGO and DJ-1, displayed negligible absorption at these wavelengths (Fig. 1A). Moreover, a keratinocyte extract incubated with MGO and DJ-1 displayed a 2-fold lower fluorescence intensity (at 400–500 nm) than that incubated with MGO alone (Fig. 1B).

We investigated whether DJ-1 could prevent collagen glycation *in vitro*. Collagen incubated with MGO and DJ-1 displayed a 5-fold lower UV absorption at 330 nm than collagen incubated with MGO alone (Fig. 1C). The fluorescence emission at 430 nm ($\lambda_{ex} = 370$ nm) of collagen incubated with MGO and DJ-1 was 10-fold lower than that of collagen incubated with MGO alone (Fig. 1D). These results show that DJ-1 protects both collagen and keratinocyte extracts from glycation *in vitro*.

3.2. Decrease in keratinocyte glycation by sulforaphane

Sulforaphane is an isothiocyanate obtained from cruciferous vegetables, which induces the expression of cytoprotective enzyme via activation of the Nrf2 stress regulator [16,17]. Exponentially growing HaCaT cells were treated with 3 μM sulforaphane and compared with control cells in terms of protein glycation and DJ-1 expression. Sulforaphane treatment lead to an increase in DJ-1 expression (Fig. 2A) and a decrease in protein glycation (Fig. 2B). DJ-1 expression was increased by 1.8-fold at 10 h after sulforaphane addition, whereas glycation measured 48 h after sulforaphane addition was impressively decreased. Several proteins displayed a 2–5-fold decrease in their glycation level.

These results show that protein glycation in keratinocytes specifically affects many proteins (the pattern of glycated protein is different from that of proteins revealed by Ponceau staining), and