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Scavenger receptor of human monocytic leukemia cell line (THP-1) and murine macrophages for nonenzymatically glycosylated proteins

Kyoko Takata [1], Seikoh Horiuchi [1], Norie Araki [1], Masanobu Shiga [2], Mikihiko [1] Saitoh [2] and Yoshimasa Morino [1]

[1] *Department of Biochemistry, Kumamoto University Medical School, and* [2] *Dojindo Laboratories, Kumamoto (Japan)*

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Long-term incubation of proteins with glucose undergo a series of nonezymatic reactions to form advanced glycosylation end product (AGE) with fluorescence and brown color. The receptor for AGE-proteins was demonstrated in murine macrophages (Vlassara et al. (1985) Proc. Natl. Acad. Sci. USA 82. 5588). Our recent study with rat macrophages revealed that the receptor also recognized proteins modified with aliphatic aldehydes such as formaldehyde or glycolaldehyde, indicating its close identity to a scavenger receptor for aldehyde-modified proteins (Takata, K. et al. (1988) J. Biol. Chem. 263. 14819). This notion was tested in the present study with human monocytic leukemia cell line (THP-1 cells), human monocyte macrophages and murine peritoneal macrophages. Endocytic uptake of AGE-proteins and aldehyde-modified proteins was inhibited in a cross-competitive fashion. The receptor activities of THP-1 cells for AGE-albumin and aldehyde-modified proteins were induced synchronously by phorbol 12-myristate 13-acetate. Furthermore, upon reduction by $NaBH_4$ of the Schiff base formed between proteins and glucose or aldehydes, no ligand activity was generated. However, once the ligand activity was generated, $NaBH_4$ was no longer effective for the ligand activity. Thus, a structure in common between AGE-proteins and aldehyde-modified proteins may be crucial for recognition by the human macrophage receptor.

Introduction

Scavenger receptors mediate endocytic uptake of chemically modified proteins [1]. Previous studies have shown the presence of two different receptors, one for chemically modified LDL [2], and the other for aldehyde-modified proteins [3]. The former receptor named 'acetyl-LDL receptor' has been intensively studied as a potential mechanism for foam cell formation in atherosclerotic lesions [2,4,5]. Recent studies focus on the identification of a natural ligand for this receptor, and the oxidative process occurred in vivo to LDL is proposed as a major potential candidate [6–10]. In contrast, less is known about the physiological significance of the receptor for aldehyde-modified proteins. Although this receptor was originally reported as being specific for f-Alb [11–13], subsequent studies on its ligand specificity revealed that proteins modified by formaldehyde as well as by other alipathic aldehydes such as glycolaldehyde, glyceraldehyde and propionaldehyde were recognized as effective ligands [14]. It is therefore proposed that the receptor might act as a general scavenger receptor for aldehyde-modified proteins in vivo. However, its physiological role has remained unknown due largely to lack of the information on its natural ligand.

Nonenzymatic glycosylation of proteins, called the Maillard reaction [15], has recently been highlighted in terms of its possible correlation with diabetic complications and aging processes [16–19]. The nonenzymatic

Abbreviations: AGE, advanced glycosylation end product; BSA, bovine serum albumin; f-Alb, formaldehyde-modified BSA; glycol-Alb, glycolaldehyde-modified BSA; FFI, 2-(2-furoyl)-4(5)-(2-furanyl)-1H-imidazole; FFI-HA, 4-furanyl-2-furoyl-1*H*-imidazole-1-hexanoic acid; FFI-BA, 4-furanyl-2-furoyl-1*H*-imidazole-1-butyric acid; FFI-tyramine, 4-furanyl-2-furoyl-1*H*-imidazole-1-tyramine; PMA, phorbol 12-myristate 13-acetate; TCA, trichloroacetic acid; HPLC, high-performance liquid chromatography, Hepes, 2-(4-hydroxyethyl)-1-piperazineethanesulfonic acid.

Correspondence: S. Horiuchi, Department of Biochemistry, Kumamoto University Medical School, Honjo, 2-2-1, Kumamoto 860, Japan.

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glycosylation is one of the well-known posttranslational modifications that occur readily in vivo. Modification of proteins with glucose occurs via a common route, i.e., Schiff base formation, which may eventually lead to AGE-products [16–18]. Our recent experiments [20] with rat sinusoidal liver cells and peritoneal macrophages showed that AGE-proteins were recognized by the receptor identical or closely similar to that for aldehyde-modified proteins [20], suggesting the biological importance of this scavenger system in diabetic complications and the aging process. It was also shown [20] that FFI, a structure proposed to be characteristic of AGE-products [21,22] did not serve as a signal for the receptor recognition. In the present study, we determined whether the notion obtained by the previous study using rat macrophages [20] could apply to human macrophages. Some experiments were also done in parallel with murine peritoneal macrophages to compare our results with those of original investigators [21,22].

Materials and Methods

Chemicals. BSA (Fraction V), human serum albumin, human hemoglobin and PMA were purchased from Sigma. BSA was chromatographed on a Sephacryl S-200 column and monomeric BSA was used for ligand preparation. Formaldehyde, glycolaldehyde, glyceraldehyde and D-glucose were purchased from Wako Chemical Co. (Osaka, Japan). $Na^{125}I$ (15.8 mCi/μg of iodine) and $NaB\,[^3H]_4$ (40.9 μCi/μmol) were obtained from DuPont-New England Nuclear. Other chemicals were of the best grade available from commercial sources.

Ligand preparation and iodination. AGE-BSA, AGE-human serum albumin and AGE-human hemoglobin were prepared according to the method of Chang et al. [22]. Briefly, BSA (2.0 g), human serum albumin (0.5 g) and human hemoglobin (0.5 g) were dissolved with 3.0 g D-glucose in 10 ml of 0.5 M sodium phosphate buffer (pH 7.4). Each sample was deoxygenated with nitrogen and sterilized by ultrafiltration. After incubation at 37°C for 60 days, the samples were dialyzed against 20 mM sodium phosphate buffer (pH 7.4) containing 0.15 M NaCl. Each protein was also incubated in parallel without glucose. Absorption spectra of AGE-BSA were taken on a Hitachi spectrophotometer model 100-50 at a concentration of 1.0 mg/ml in 0.05 M sodium phosphate buffer (pH 7.4). Fluorescence excitation spectra were taken on a Hitachi 850 spectrofluorometer at 0.1 mg/ml of AGE-BSA in the same buffer at 440 nm emission wavelength. BSA incubated in parallel without glucose was used as a control. Aldehyde-modified proteins such as f-Alb and glycol-Alb were prepared as described [14]. Parallel incubation of BSA with glucose or aldehydes was done in the presence of 50 mM $NaBH_4$ [20]. To examine the effect of $NaBH_4$ on the ligand activity, AGE-BSA, f-Alb and glycol-Alb were incubated at 37°C for 4 h with 50 mM $NaBH_4$. AGE-BSA, f-Alb and FFI-BSA were labeled with ^{125}I with Iodo-Gen (Bio-Rad) to a specific radioactivity of 790, 710, and 810 cpm/ng, respectively. These labeled ligands were > 98% TCA-precipitable. The protein was determined as described [23].

Chemical synthesis of FFI and its derivatives. 2-(2-Furoyl)-4(5)-(2-furanyl)-1H-imidazole (FFI), 4-furanyl-2-furoyl-1*H*-imidazole-1-hexanoic acid (FFI-HA), 4-furanyl-2-furoyl-1*H*-imidazole-1-butyric acid (FFI-BA), and 4-furanyl-2-furoyl-1*H*-imidazole-1-tyramine (FFI-tyramine) were synthesized as reported previously [24] according to the original methods [21,22]. Chemical structures were confirmed by ^{1}H-NMR ($CDCl_3$) spectroscopy.

Preparation of FFI-BSA conjugates. FFI-HA and FFI-BA were coupled to BSA with water-soluble carbodiimide as reported previously [20,24]. Incorporation of FFI in these BSA conjugates was calculated on the basis of the molecular absorption of FFI (ε_{365} 9664); 7.6 mol/mol for FFI-HA-BSA and 5.7 mol/mol for FFI-BA-BSA.

Determination of early-stage products of the Maillard reaction. Amounts of fructosamine were determined as an Amadori product of AGE-proteins by two methods. First, each AGE-protein (3–5 mg) was incubated with 16 mM NaB^3H_4 in 1.0 ml of 0.1 M sodium phosphate buffer (pH 7.0) for 10 min at 25°C and then for 50 min at 4°C. The sample was then dialyzed extensively against 0.01 M sodium phosphate buffer (pH 7.0) and the incorporated radioactivity was determined as described [25]. In the second method, fructosamine was determined by the colorimetric assay based upon the property of fructosamine to act as an reducing agent in alkaline solution [26] by using Fructosamine Test (Hoffman-Roche, Switzerland). Albumin containing 1-deoxy-1-morpholinofructose was used as a standard.

Determination of FFI in AGE-proteins. Determination of FFI in AGE-proteins was performed as described [24]. Briefly, AGE-proteins were hydrolyzed in 6 M HCl at 110°C for 11 h, evaporated to dryness, and incubated with aqueous ammonium hydroxide (25%) for 10 min at room temperature. The reaction mixture was determined for FFI both by the HPLC system and the radioimmunoassay as described previously [24] with the lower limit of detection being < 0.2 μg and < 2 ng of FFI, respectively.

Cellular assay with murine peritoneal macrophages. Elicited peritoneal macrophages were harvested from female Balb/C mice (18–20 g) 6 days after intraperitoneal injection of Brewer's thioglycolate broth (Difco) and suspended in RPMI 1640 medium supplemented with 20% fetal calf serum, 100 units/ml of penicillin and 100 μg/ml of streptomycin (medium A) as reported previously [27,28]. The cells ($1.0 \cdot 10^6$) were seeded in each plastic dish (16 mm diameter, Corning) and in-

cubated for 2 h at 37°C in a 5% humidified CO_2-incubator. The monolayers were washed three times with 1.0 ml of Eagle's minimum essential medium containing 3% BSA buffered with 20 mM Hepes to pH 7.4 (medium B) and used for the uptake experiments. Each well was incubated at 37°C with varying concentrations of ^{125}I-labeled ligand in 0.5 ml of medium B. After a 4-h incubation, 0.3 ml of the culture medium was withdrawn from each well and its TCA-soluble radioactivity was measured to determine the intracellular degradation [20]. The remaining cells were washed three times with 1.0 ml of ice-cold medium B and five times with phosphate-buffered saline. Cells were dissolved for 3 h at 37°C in 1.0 ml of 0.1 M NaOH to determine the cell-associated radioactivity and cellular protein. Each well contained 25–30 μg of cell protein. Total cellular association was defined as amounts of ^{125}I-labeled ligand obtained under these conditions. Nonspecific cellular association was determined by parallel incubations with 1.0 mg/ml of unlabeled same ligand. Specific cellular association was calculated by subtracting nonspecific cell association from total cell association. For competition experiments, cells were incubated with ^{125}I-labeled ligand in the presence of unlabeled ligand to be tested.

Cellular assays with THP-1 and human monocyte macrophages. THP-1 cells, established cell line from human monocytic leukemia [29], were purchased from American Type Culture Collection. Each well (16 mm in diameter) received $5.0 \cdot 10^5$ cells in 1.0 ml of medium A plus 0.2 mg/ml of PMA (medium C) and cultured for 3 days unless otherwise specified. Monolayers were supplemented with 1.0 ml of medium C at 3 days' intervals. After washed three times with 1.0 ml of medium B, each well was incubated for 4 h except for the time course study (Fig. 2 and 6B). Each well contained 148 ± 12 μg of cellular protein. Human blood monocyte macrophages from healthy volunteers were prepared by Ficoll-isopaque gravity sedimentation [30]. Each well was seeded with 1.0×10^7 cells in 1.0 ml of medium A. After a 1 h incubation, adherent cells were cultured for 3 days in medium A and used for experiments. The cellular protein per each well was 57 ± 10 μg. Throughout cellular assays described above, in order to correct for possible ligand absorption to a culture well or spontaneous release of free iodine from ^{125}I-ligand, incubations were done in parallel without cells. The blank values thus obtained (less than 5% of total cell-associated ^{125}I-ligand) were subtracted from corresponding experimental values. Each value in all figures and tables represents the mean of triplicate assays.

Results

Physicochemical and biological properties of AGE-BSA

AGE-BSA used in the present study had a UV absorption maximum at 278 nm with a 330–335 nm shoulder peak which was not observed with BSA incubated without glucose. The excitation fluorescence spectrum of AGE-BSA measured at the emission maximum of 440 nm shows a major excitation maximum at 370 nm and a minor maximum at 290 nm. BSA incubated in parallel without glucose did not give any fluorescence peaks. Amounts of the stable Amadori products of our AGE-proteins were determined as fructosamine; AGE-BSA contained 214.0 ± 34.0 cpm/μg protein which is > 9.5-fold higher than that of BSA incubated without glucose (Table I). FFI was proposed as one of the major fluorescence compounds generated in the advanced stage of the Maillard reaction [21,22]. Amounts of FFI generated upon hydrolysis of AGE-proteins and subsequent treatment with ammonia was 2.5–2.8 μg/mg protein, while FFI was virtually undetectable in control BSA (Table I).

TABLE I

Determination of fructosamine and FFI in AGE-proteins

	Fructosamine		FFI [d]	
	NaB^3H_4 [a] (cpm/μg protein)	MDF [b] (nmol/μg protein)	radioimmunoassay (μg/mg protein)	HPLC (μg/mg protein)
AGE-BSA	214.0 ± 34.0	2.25 ± 0.19	2.8 ± 0.4	2.5 ± 0.2
BSA [e]	22.5 ± 3.3	n.d. [c]	< 0.002	< 0.2
AGE-human serum albumin	172.4 ± 23.8	2.79 ± 0.41	2.3 ± 0.3	2.4 ± 0.2
Human serum albumin [e]	30.8 ± 3.1	n.d.	< 0.002	< 0.2
AGE-hemoglobin	123.0 ± 29.8	0.81 ± 0.10	1.6 ± 0.3	1.4 ± 0.3
Hemoglobin [e]	47.0 ± 19.6	n.d.	< 0.002	< 0.2

[a] Determined by reduction with NaB^3H_4 and expressed as cpm/μg protein.

[b] Determined for the reducing activity with 1-deoxy-1-morpholinofructose (DMF) as a standard.

[c] n.d.; not detectable. The lower limit of detection was less than 0.02 nmol of MDF/μg protein.

[d] FFI in AGE-proteins was determined both by the radioimmunoassay and the HPLC analyses after acid hydrolysis and subsequent reaction with ammonia. These data were cited from our previous work [20] to compare our AGE-proteins with those of original investigators [28].

[e] BSA, human serum albumin, and hemoglobin incubated in parallel without glucose.

The biological property of our AGE-BSA was determined with murine peritoneal macrophages, cells used by the original investigators [27,28]. The cellular binding of ^{125}I-labeled AGE-BSA was competed for by AGE-BSA and its specific binding exhibited a typical saturation curve, and AGE-BSA bound to the cells was endocytosed and degraded intracellularly (data not shown), suggesting the presence of the cell-surface receptor for AGE-BSA. From these data it is evident that our AGE-BSA was both physicochemically and biologically indistinguishable from that of Vlassara et al. [27,28].

Endocytic uptake of AGE-BSA by THP-1 cells

THP-1 cells are induced to differentiate into macrophages by PMA [29,31]. We first determined whether or not the receptor for AGE-proteins was expressed by human macrophages. When the cells were well-differentiated into macrophages after 3 days' incubation with PMA, cell-associated ^{125}I-AGE-BSA exhibited a saturation pattern against the ligand concentrations (Fig. 1). Furthermore, amounts of the cell-associated ^{125}I-AGE-BSA were inhibited by > 80% by an excess unlabeled AGE-BSA. Upon the Scatchard analyses [32], the specific cell-associated ^{125}I-AGE-BSA gave an apparent K_d for uptake of $4.6 \cdot 10^{-7}$ M (Fig. 1), indicating the presence of a membrane receptor for AGE-BSA. We next examined the post-binding events occurring after the binding of AGE-BSA to the surface membranes. Shortly after the initial increase in the cell-associated ligand, the TCA-soluble radioactivity in the medium increased sharply with time up to 3 h, reaching a plateau thereafter (Fig. 2). Thus, it is evident that cell surface-bound AGE-BSA is subjected to endocytic degradation by THP-1 cells as is the case with murine [28,33] and rat macrophages [20].

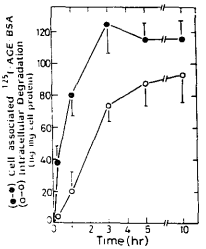

Fig. 2. Endocytic uptake and degradation of ^{125}I-AGE-BSA by THP-1 cells. Cells were incubated with 9 μg/ml ^{125}I-AGE-BSA for the indicated periods and the specific endocytic uptake (●) and the specific degradation (○) were determined. Each bar shows the range.

Does 'FFI' serve as a signal for receptor recognition?

Previous studies using murine peritoneal macrophages demonstrated that FFI acted as a signal for the recognition of AGE-BSA by the receptor [27,28,34,35]. However, this was not the case with rat peritoneal macrophages and sinusoidal liver cells [20]. In an attempt to solve the discrepancy, we first determined effects of FFI-BSA on the interaction of ^{125}I-AGE-BSA with murine peritoneal macrophages. The cell-association of ^{125}I-AGE-BSA was effectively inhibited by unlabeled AGE-BSA in a dose-dependent fashion. However, FFI-BSA had no effect on the process (Fig. 3).

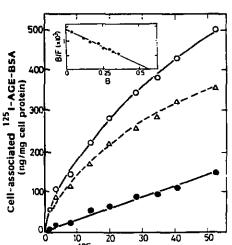

Fig. 1. Cell-associated ^{125}I-AGE-BSA as a function of ligand concentrations in THP-1 cells. Cells were incubated for 4 h with ^{125}I-AGE-BSA in the presence (nonspecific cell-association; ●) or absence (total cell-association; ○) of 1.0 mg/ml unlabeled AGE-BSA. △ denotes the specific endocytic uptake and its Scatchard analysis is shown in the inset.

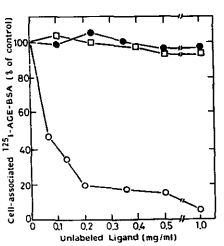

Fig. 3. Effect of FFI-BSA on uptake of ^{125}I-AGE-BSA by murine macrophages. Each dish was incubated with 7.4 μg/ml ^{125}I-AGE-BSA in the presence of unlabeled FFI-HA-BSA (●), FFI-BA-BSA (□) or AGE-BSA (○). After incubation for 4 h, the cell-associated radioactivity was determined. The 100% value determined in the absence of any competing compounds was 3.1 μg/mg of cell protein. FFI per se had no effect on this process up to 10 μM (data not shown).

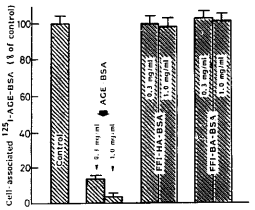

Fig. 4. Effect of FFI-BSA on uptake of ^{125}I-AGE-BSA by THP-1 cells. Cells were incubated for 4 h with 10 μg/ml ^{125}I-AGE-BSA in the presence of 0.3 or 1.0 mg/ml FFI-HA-BSA, FFI-BA-BSA and AGE-BSA. The 100% value was 115 ng/mg protein/4 h.

Moreover, ^{125}I-FFI-BSA per se did not exhibit any specific interaction with the cells under these conditions (data not shown). When the same experiment was performed with THP-1 cells, FFI-BSA also had no effect on endocytic uptake of ^{125}I-AGE-BSA (Fig. 4). Thus, the involvement of FFI in the receptor recognition is unlikely.

Effect of AGE-BSA and aldehyde-modified proteins on their endocytic uptake by murine macrophages

A scavenger receptor for aldehyde-modified proteins is known to occur [11,36] and some of its ligand specificity have been characterized [12–14]. Our recent experiments with rat macrophages revealed that this receptor was identical to that for AGE-proteins [20]. This notion was tested with murine macrophages. As Fig. 5 shows, both AGE-BSA and aldehyde-modified proteins competed with ^{125}I-AGE-BSA for its cell-association, although f-Alb was less effective when compared with glycol-Alb and other glycolaldehyde-modified proteins (Fig. 5A). Conversely, the cell-association of ^{125}I-labeled f-Alb was similarly inhibited by AGE-BSA and aldehyde-modified proteins (Fig. 5B). It is likely therefore that AGE-BSA and aldehyde-modified proteins are endocytosed by murine macrophages via a route identical to each other.

Scavenger receptor activity versus differentiation of THP-1 into macrophages

To know the interaction of f-Alb with THP-1 cells, cells were incubated with ^{125}I-f-Alb under the conditions identical to those for Fig. 2. Amounts of the cell-associated ^{125}I-f-Alb versus ligand concentration obeyed a saturation kinetics with an apparent K_d for uptake of $2.2 \cdot 10^{-7}$ M (Fig. 6A), and the cell-associated f-Alb underwent subsequent intracellular degradation (Fig. 6B). The same phenomenon was observed with glycol-Alb (data not shown). Thus, it is evident that THP-1 cells also possess the receptor for aldehyde-modified proteins as rat macrophages do [20].

Receptor-mediated scavenger function is characteristically expressed in macrophages or macrophage-derived cells [1–3]. The time-dependent increase in the receptor activity for AGE-BSA and f-Alb was compared. The cell-association of ^{125}I-AGE-BSA and its subsequent degradation sharply increased after addition of PMA and reached a maximum level within 3 days, followed by a gradual decline after 10 days (Fig. 7A). A similar time-dependent pattern was also obtained when

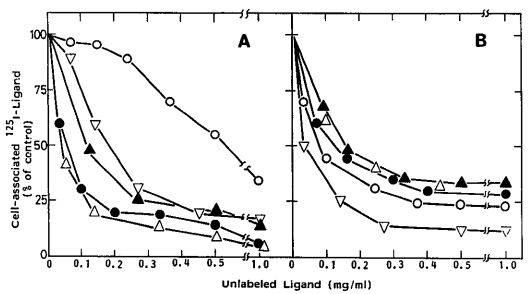

Fig. 5. Cross competitive effect of AGE-BSA and aldehyde-modified proteins on their uptake by murine macrophages. Cells were incubated for 4 h with 7.2 μg/ml ^{125}I-AGE-BSA (A) or 5.1 μg/ml ^{125}I-f-Alb (B) in the presence of a ligand to be tested, and determined for the cell-associated radioactivity. Unlabeled ligands used were f-Alb (○), glycol-Alb (△), glycolaldehyde-modified human serum albumin (▲), glycolaldehyde-modified hemoglobin (▽), or AGE-BSA (●). The 100% values for ^{125}I-AGE-BSA and ^{125}I-f-Alb were 3.5 and 0.49 μg/mg cell protein, respectively.

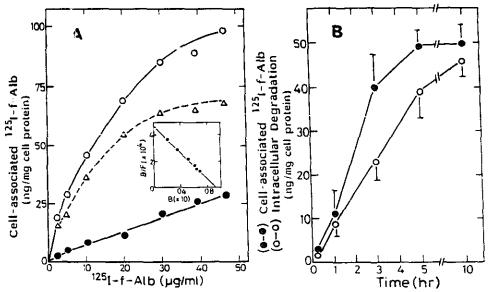

Fig. 6. Uptake and degradation of ^{125}I-f-Alb by THP-1 cells. (A) Cells were incubated for 4 h with ^{125}I-f-Alb in the presence (●) or absence (○) of 1.0 mg/ml of unlabeled f-Alb. The Scatchard plot of the specific uptake (Δ) is shown in the inset. (B) The cells were incubated with 10 μg/ml of ^{125}I-f-Alb for indicated periods and the specific endocytic uptake (●) and the specific degradation (○) were determined. Each bar shows the range.

^{125}I-f-Alb was used as a ligand (Fig. 7B). Upon cross-competition experiments, the cell-association of ^{125}I-AGE-BSA was inhibited by f-Alb and glycol-Alb. In the same fashion the cell-association of ^{125}I-f-Alb was competed for by AGE-BSA (Table II). These results suggest that AGE-BSA, f-Alb and glycol-Alb are recognized by the same receptor.

Ligand specificity of receptor for AGE-BSA and for aldehyde-modified proteins

The ligand specificity of the AGE-BSA receptor was compared with that for the receptor for aldehyde-modified proteins in two ways. We first examined the effect on the ligand activity of $NaBH_4$, a reagent known to reduce Schiff base formed between aldehydes and protein lysyl residues. As Table II shows, BSA modified with glycolaldehyde or formaldehyde in the presence of $NaBH_4$ competed neither with ^{125}I-f-Alb nor ^{125}I-AGE-BSA for their cell-association to THP-1 cells. Similarly, no ligand activity was generated after BSA had been incubated with glucose in the presence of $NaBH_4$. In sharp contrast, however, once the ligand activity was generated, treatment of AGE-BSA, f-Alb and glycol-Alb with $NaBH_4$ had no effect on the ligand activity.

In the second experiments, AGE-proteins other than AGE-BSA such as AGE-human serum albumin and hemoglobin were tested for their ligand activity. These AGE-proteins were found to act as effective ligands when determined by their inhibitory effect on the endocytic uptake of ^{125}I-AGE-BSA, whereas proteins incubated in parallel without glucose displayed no ligand activity (Table III, left-hand column). When these

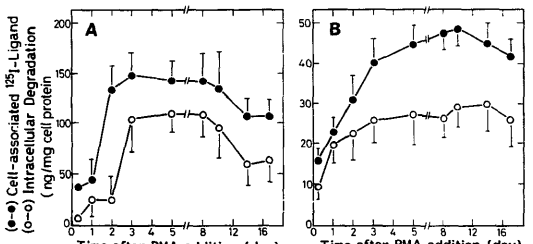

Fig. 7. Time-dependent uptake of ^{125}I-AGE-BSA and ^{125}I-f-Alb by THP-1 cells after PMA addition. After preincubation with PMA for indicated intervals. THP-1 cells were incubated with 10 μg/ml of ^{125}I-AGE-BSA (A) or ^{125}I-f-Alb (B). After a 4 h-incubation, the cell-associated radioactivity (●) and TCA-soluble radioactivity in the culture medium (○) were determined. Each bar denotes the range.

TABLE II

$NaBH_4$ treatment of AGE-proteins and aldehyde-modified proteins on their uptake by THP-1 cells

Cells were incubated for 4 h with 13.8 μg/ml of ^{125}I-AGE-BSA or 10.8 μg/ml of ^{125}I-f-Alb in the presence of 0.4 mg/ml of each ligand tested, and the cell-associated radioactivity was determined.

Effector	Cell-associated ^{125}I-AGE-BSA		Cell-associated ^{125}I-f-Alb	
	(ng/mg protein per 4 h)	(%)	(ng/mg protein per 4 h)	(%)
None	143.6	100	23.1	100
BSA [a]	146.5	102	24.3	105
BSA + glucose + $NaBH_4$ [b]	158.0	110	24.0	104
AGE-BSA	15.8	11	1.6	7
AGE-BSA + $NaBH_4$ [c]	31.6	22	0.9	4
BSA + formaldehyde + $NaBH_4$ [b]	138.8	98	22.4	97
f-Alb	51.7	36	6.2	27
f-Alb + $NaBH_4$ [c]	54.5	38	5.8	25
BSA + glycolaldehyde + $NaBH_4$ [b]	135.0	94	20.3	88
glycol-Alb	12.9	9	2.8	12
glycol-Alb + $NaBH_4$ [c]	24.4	17	2.8	12

[a] BSA incubated in parallel without glucose was used as a control.

[b] BSA was incubated with glucose, formaldehyde or glycolaldehyde in the presence of $NaBH_4$.

[c] AGE-BSA, f-Alb or glycol-Alb with the full ligand activity was further incubated with $NaBH_4$.

AGE-proteins were tested for their effect on the cell-association of ^{125}I-f-Alb, the identical inhibitory pattern was obtained (Table III, right-hand column). These observations suggest that the ligand activity was generated through Schiff base formation, but Schiff bases or Amadori products themselves did not serve as active ligands. Furthermore, a ligand structure critical for the receptor recognition might be common among AGE-proteins and aldehyde-modified proteins.

Receptor of human monocyte macrophages for AGE-BSA

Finally, the receptor for AGE-BSA was examined with human monocyte macrophages (Fig. 8). The association of ^{125}I-AGE-BSA to these cells was competed for by AGE-BSA and glycol-Alb. Similarly, these ligands inhibited the cell-association of ^{125}I-f-Alb. Moreover, the effect of $NaBH_4$ on the ligand activity of AGE-BSA and glycol-Alb was similar to that observed in the experiment using THP-1 cells (see Table II). These findings indicate that AGE-BSA was endocytosed by human blood monocytic macrophages via a route identical to f-Alb and other aldehyde-modified proteins.

Discussion

The receptor-mediated recognition of AGE-BSA was first demonstrated with murine peritoneal macrophages [28]. In the first part of the present study we compared our AGE-BSA preparation with that used by the original investigators [28]. The results indicate that our preparation is physicochemically and biologically identical to theirs. Thus, data obtained by experiments using our AGE-BSA should be comparable to theirs.

TABLE III

Effect of AGE-proteins on uptake of ^{125}I-AGE-BSA and ^{125}I-f-Alb by THP-1

Experiments were performed under the conditions identical to those of Table II.

Effector	Cell-associated ^{125} I-AGE-BSA		Cell-associated ^{125}I-f-Alb	
	(ng/mg protein per 4 h)	(%)	(ng/mg protein per 4 h)	(%)
None	143.6	100	23.1	100
AGE-BSA	15.8	11	1.6	7
BSA [a]	146.5	102	24.3	105
AGE-human serum albumin	33.0	23	1.4	6
Human serum albumin [a]	140.7	98	21.7	94
AGE-human hemoglobin	23.0	16	1.2	5
Human hemoglobin [a]	147.9	103	22.9	99

[a] BSA, human serum albumin and hemoglobin incubated without glucose were used as a control.

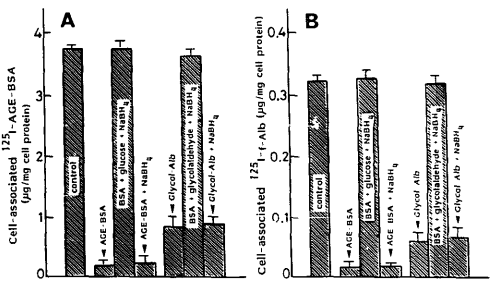

Fig. 8. Ligand specificity of the scavenger receptor of human monocyte macrophages Macrophages were incubated with 2 μg/ml of ^{125}I-AGE-BSA (A) or ^{125}I-f-Alb (B) in the presence of 1.0 mg/ml of unlabeled ligands. Ligands tested were the same as those ... 1 for Table I. The 100% values for ^{125}I-AGE-BSA and ^{125}I-f-Alb were 3.7 and 0.32 μg/mg cell protein per 4 h, respectively. Bars show the range.

Using these ligands we demonstrated that the receptor for AGE-proteins of human macrophages mediates the endocytic uptake of aldehyde-modified proteins. This notion was supported by three lines of evidence. First, cross-competition did occur between AGE-proteins and aldehyde-modified proteins for their cell-association to murine macrophages (Fig. 5) and human macrophages (Fig. 8, Tables II and III), results consistent with our previous study with rat macrophages [20]. Secondly, the receptor activities of THP-1 cells for AGE-BSA and f-Alb were expressed synchronously along with their differentiation into macrophages (Fig. 7). Finally, effects of $NaBH_4$ on the ligand activity was similar between AGE-BSA and aldehyde-modified proteins; reduction by $NaBH_4$ of the Schiff base formed between protein amino groups and glucose or aldehydes failed to generate an active ligand, whereas once generated, the ligand activity was not affected by reduction by $NaBH_4$ (Table II). These results agree well with our previous data with aldehyde-modified proteins [14].

A view that is not consistent with the above notion is also available. Reaction of protein amino groups with aldehydes lead to formation of Schiff base adducts and Amadori rearrangement products, followed by inter- or intramolecular cross-linking reaction. Cross-linking reactions are reported to occur to proteins modified by glycolaldehyde [37] or by glucose [16]. In contrast, due to a lack of any hydroxyl group next to a keto moiety, formaldehyde does form a Schiff base adduct but does not undergo the Amadori rearrangement reaction. However, cross-linking reaction could occur to proteins modified with formaldehyde via a carbinol amine. In fact, treatment of albumin with formaldehyde resulted in polymer formation in addition to monomers as was the case with albumin modified by glycolaldehyde, and these monomeric and polymeric forms exhibited the same ligand activity [14]. It is possible therefore that a structure generated by cross-linking reaction might serve as a common signal for receptor-mediated recognition of AGE-proteins and aldehyde-modified proteins (Table III).

Another difference is the post-binding phenomenon of AGE-proteins. Maximal amounts of cell-associated AGE-BSA was 5–7-fold as high as that of f-Alb (Figs. 1 and 6A, Tables II and III). The inhibitory effect of f-Alb on the cell-association of ^{125}I-AGE-BSA to murine macrophages and human macrophages was somewhat weaker than other aldehyde-modified proteins such as glycol-Alb, glycolaldehyde-modified human serum albumin and hemoglobin (Fig. 5 and Table II). Moreover, amounts of AGE-BSA degraded intracellularly which were expected from those of cell-associated ligand were less than f-Alb or glycol-Alb with murine and human macrophages (Figs. 2 and 6B) and also with rat macrophages [20]. One possible explanation would be that AGE-proteins are resistant to proteolytic digestion. This is the case with AGE-collagen [18]. Our preliminary experiment in vitro also revealed a highly resistant nature of AGE-BSA to tryptic digestion. The internalization step of cell surface-bound AGE-proteins could also be involved. Further studies are needed to clarify this issue.

FFI was shown to act as a critical signal for the biological recognition of AGE-BSA and AGE-myeline by murine macrophages [27,28]. However, FFI-BSA did not compete with ^{125}I-AGE-BSA for its endocytic uptake by THP-1 cells (Fig. 4) nor by murine peritoneal macrophages (see Fig. 3). This was also the case with experiments with rat macrophages [20]. Moreover, the specific cellular interaction of ^{125}I-FFI-BSA was ob-

served neither with murine macrophages (data not shown) nor with rat macrophages [20]. Thus, a structure other than FFI is important for the receptor recognition. In support for this notion, recent reports from two laboratories indicate the absence of FFI in AGE-proteins [24,38].

Although the present study raises a possibility that AGE-proteins act as natural ligands for the receptor for aldehyde-modified proteins, the functional aspect of the receptor remains unknown. In this connection, it was recently reported that AGE-BSA binding to murine macrophages markedly induced the synthesis and secretion of cytokines such as tumor necrosis factor and interleukin-1 [39]. Furthermore, upon treatment with tumor necrosis factor, macrophages increased their binding capacity to ^{125}I-AGE-BSA, indicating that the AGE-protein receptor activity could be regulated by cytokines in an autocrine fashion [40]. Thus, it is likely that the specific recognition of AGE-proteins by the scavenger receptor might be coupled to the immunological modulation in vivo.

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