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ORIGINAL PAPER

# Human astrocytes and aortic endothelial cells actively convert the oxidized form of albumin to the reduced form: reduced albumin might participate in redox regulation of nerve and blood vessel systems

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**Abstract** Human serum albumin (HSA) is a mixture of mercaptalbumin (HMA, reduced form) and nonmercaptalbumin (HNA, oxidized form), i.e., a protein-thiol redox couple in the extracellular fluid (ECF), and it might have antioxidant properties. Forty-two patients with orthopedic disorders participated in this study and were divided into two groups according to their age (young and older groups). By using HPLC to separate HSA into HMA and HNA, we analyzed the percentages of HMA and HNA in serum and lumbar cerebrospinal fluid (CSF). We also examined the redox activity of cultured normal human astrocytes, aortic endothelial cells, and dermal fibroblasts for HSA-thiol. The mean HMA value from the serum of the older group was significantly lower than that of the young group, whereas that from CSF was not significantly different between the two groups; CSF albumin is almost completely in the reduced form, and no age-related differences were observed. Cultured astrocytes and aortic endothelial cells showed conversion of HNA to HMA, whereas dermal fibroblasts showed no such redox activity. From the results obtained from in-vivo and in-vitro studies, HMA is considered to participate in redox regulation in the ECF, for example in the CSF that surrounds the central nervous system (CNS), and in blood serum.

**Keywords** Albumin · Astrocyte · Cerebrospinal fluid (CSF) · Protein thiol · Redox state

**Abbreviations**

| | |
|---|---|
| HSA | Human serum albumin |
| HMA | Human mercaptalbumin |
| HNA | Human nonmercaptalbumin |
| ROS | Reactive oxygen species |

## Introduction

Serum albumin is synthesized in the liver and is exported as a simple, non-glycosylated protein containing about 580 amino acids. This protein is the most abundant protein in the circulatory system and is also present in the interstitial fluids of body tissues [1]. One of the most important features of its structure is the presence of a highly reactive Cys-34 thiol group, called mercaptalbumin (the reduced form; in humans: human mercaptalbumin, HMA) in which the thiol group is in the free state [2]. In contrast, albumin in which the thiol group is bound to thiol-containing compounds is called nonmercaptalbumin (the oxidized form; in humans: human nonmercaptalbumin, HNA) [2]. The major HNA compound is a mixed disulfide with cystine or oxidized glutathione (the reversible form of HNA; tentatively called HNA-1) whereas others include oxidation products higher than the mixed disulfide, for example

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the sulfenic (–SOH), sulfinic ($–SO_2H$), and sulfonic ($–SO_3H$) states (the irreversible forms of HNA; called HNA-2) [3]. In living systems, human serum albumin (HSA) is known to be a mixture of HMA and HNA, i.e., a major part of the protein-thiol redox potential in the extracellular milieu, and it might have antioxidant properties because of the relatively high concentrations of protein-thiol in the body tissues [4].

We have developed a convenient HPLC method for separation of HSA into HMA and HNA [5, 6] and have extensively studied the dynamic changes of the HSA-thiol redox state under various physiologic [7–10] and pathophysiologic states, for example hepatic [11, 12], renal [13–18], diabetic [19, 20], and other diseases [21–23].

Cerebrospinal fluid (CSF) is a modified extracellular fluid (ECF) present in the central nervous system (CNS). The protein content and concentration of the CSF is very different from that of the serum, because transport of substances from the blood into the CSF is highly restricted by the blood–CSF barrier [24]. The major CSF protein is albumin, which has a relatively low molecular mass compared with other serum proteins, for example immunoglobulins [24]. Although quantitative determination of CSF albumin in normal and diseased brain tissues has been described in several papers [25–27], there are no reports concerning the redox state of CSF albumin-thiol. Our HPLC method has been proved to be a useful technique for analysis of the reduced and oxidized albumin (HMA and HNA) fractions in ECF, especially when the sample size is small and the concentration is low, as is the case for CSF albumin in the cerebrospinal space [24].

In this study we have successfully obtained important evidence concerning the maintenance of the redox pool in the CSF, even in the elderly, as monitored via the CSF albumin-thiol redox state. Moreover, in an in-vitro study it was found that both normal human astrocytes and aortic endothelial cells actively convert HNA to HMA, whereas dermal fibroblasts had no such redox activity.

## Materials and methods

### Subjects

Forty-two patients with orthopedic disorders, for example lumbar disc herniation, lumbar spondylosis, and lumbar spinal cord stenosis participated in this study (26 men and 16 women with ages ranging from 16 to 80 years (mean ± SD: 56.7 ± 17.5 years)), as selected by the Department of Orthopedics, Gifu University Graduate School of Medicine, Gifu, Japan. They showed no signs of systemic diseases, for example diabetes mellitus or hepatic or renal dysfunction, other than their orthopedic disorders. Patients who showed signs of inflammation or damage related to blood–brain barrier function were excluded from the study. The local ethics committee approved this study protocol, and informed consent was obtained from all patients. All procedures complied with the Declaration of Helsinki. When the patients underwent a myelography for diagnostic purposes, approximately 1 ml of lumbar CSF was tapped at the beginning of the operation through a lumbar puncture, with special care taken to avoid blood contamination. Approximately 5 ml blood was drawn just before or after the myelographic examination, and serum was collected by centrifugation at 3,000 rpm for 15 min at 4°C. The specimens were immediately stored at −80°C until analysis.

### HPLC system for measurement of the albumin redox state

Measurement of the albumin-thiol redox state was performed by use of a previously reported HPLC method [18]. Briefly, the HPLC system consisted of an AS-8010 autosampler (injection volume: 2 µl for serum and 100 µl for CSF) and a CCPM double-plunger pump in conjunction with an SC-8020 system controller (all from Tosoh, Tokyo, Japan). The chromatograph was obtained with a Finnigan UV6000LP photodiode-array detector (detection range: 200–600 nm in 1-nm steps; Thermo Electron, Waltham, MA, USA). A Shodex-Asahipak ES-502 N 7C column (10 × 0.76 cm I.D., DEAE-form for ion-exchange HPLC; Showa Denko, Tokyo, Japan; column temperature: 35 ± 0.5°C) was used in this study. Elution was performed with a linear gradient with ethanol (at the concentrations: 0 to 1 min: 0%, 1 to 50 min: 0 → 10%, 50 to 55 min: 10 → 0%, 55 to 60 min: 0%) for specimens in a 0.05 M sodium acetate and 0.40 M sodium sulfate mixture (pH 4.85) at a flow rate of 1.0 ml/min. The buffer solution was deaerated by bubbling helium through it.

The HPLC profiles obtained from these procedures were subjected to numerical curve fitting with simulation software (PeakFit, version 4.05, SPSS Science, Chicago, IL, USA); each peak shape was approximated by a Gaussian function. The values for the fractions of HMA [f(HMA)], HNA-1 [f(HNA-1)], and HNA-2 [f(HNA-2)] (%) were obtained by use of the following equations:

$$\mathrm{f(HMA)}(\%) = [\mathrm{HMA}/(\mathrm{HMA} + \mathrm{HNA\text{-}1} + \mathrm{HNA\text{-}2})] \times 100$$

$$\mathrm{f(HNA\text{-}1)}(\%) = [\mathrm{HNA\text{-}1}/(\mathrm{HMA} + \mathrm{HNA\text{-}1} + \mathrm{HNA\text{-}2})] \times 100$$

$$f(\text{HNA-2})(\%) = [\text{HNA-2}/(\text{HMA} + \text{HNA-1} + \text{HNA-2})] \times 100.$$

### Cell culture

Normal human astrocytes (donor: unknown, CC-2565, lot 4F0755), normal human aortic endothelial cells (54-year-old male, CC-2535, lot 7F3087), and normal human dermal fibroblasts (33-year-old female, CC-2511, lot 6F3305) were obtained from Lonza Walkersville (Walkersville, MD, USA). HSA (A1653, lot 36K7545) was obtained from Sigma–Aldrich (St Louis, MO, USA) and used without further purification.

The cells were maintained in the growth media supplied by the manufacturer (astrocytes: AGM BulletKit, aortic endothelial cells: EGM-2 BulletKit, dermal fibroblasts: FGM-2 BulletKit) under a 5% $CO_2$ atmosphere. Trypsinized cells were subcultured in 24-well plates at densities of 1 or $2 \times 10^5$ cells/well on day 0. HSA was dissolved in each growth medium immediately before being added to the wells at a final concentration of 10 mg/ml and filter-sterilized. On the next day, the medium was aspirated, and fresh medium with or without HSA was added to the wells. HSA-containing medium was also added to the wells without cells, as a control. Aliquots of the medium were subjected to measurement at time 0. At the indicated times (3, 6, 9, and 24 h), 500 µl supernatant was aspirated and frozen at −80°C until the HPLC analysis. Using independent cultures, the data are presented as the means ± SE from a representative experiments performed in triplicate. Experiments were repeated twice, and essentially the same results were obtained.

### Statistical analysis

In the clinical study, values are expressed as means ± SD unless otherwise stated. We used Stat View 5.0 statistical software (SAS Institute, Cary, NC, USA). The Mann–Whitney's *U* test was used for comparisons between the young and older groups, while the Wilcoxon signed-rank test was used for comparisons within groups. *P* values less than 0.05 were considered to be significant. To measure the magnitude of correlation, we used Pearson's correlation coefficient (*R*). The correlation was determined to be significant when the *P* value was less than 0.05 with Fisher's Z transformation.

## Results

### Redox state of lumbar CSF and serum albumin

In the relationship between the age of the 42 subjects and the values (%) for HMA fraction (f(HMA)) of albumin from the serum and lumbar CSF, the f(HMA) values were correlated significantly not with that for the CSF (open triangles) ($R = 0.086$, $P = 0.59$) but with that for the serum ($R = -0.635$, $P < 0.0001$), as detected by linear-regression analysis (Fig. 1). Based on this result, subjects were divided into two groups according to their age (young group: 16–36 years (29.1 ± 6.6 years, $n = 10$), older group: 51–80 years (65.4 ± 8.3 years, $n = 32$)), and we compared the fractions of HMA and HNA of serum albumin, together with those of lumbar CSF albumin. The HPLC results are summarized in Table 1. According to the results given in Table 1, the statistical significance of the values (%) for HMA and HNA (f(HMA) and f(HNA)) are shown in Fig. 2a and b, respectively.

As shown in Fig. 2a, the value (%) for f(HMA) from the serum of the older group (69.5 ± 4.7%, closed column) was significantly lower than that from the serum of the young group (76.4 ± 3.7%, open column) ($P < 0.001$, Mann–Whitney's *U* test). The mean f(HMA) value of 76.4% in young group is in accordance with that previously reported for healthy young subjects (74.6%) [9], and that of 69.5% in the older group is also in accordance with that previously reported for elderly patients with senile cataracts (66.4%) [22]. In the serum, f(HMA) decreases with increasing age, indicating that redox capacity is likely to decrease age-dependently in the ECF. These phenomena agree well with many reports of age-related increases of oxidative states in the elderly. On the other hand, the f(HMA) values from the CSF were 93.0 ± 6.1% for the young group and 93.1 ± 2.8% for the older group, i.e. there is no significant difference between them. Furthermore, it is intriguing that the albumin in the CSF is almost all in the reduced form and that this does not differ with age.

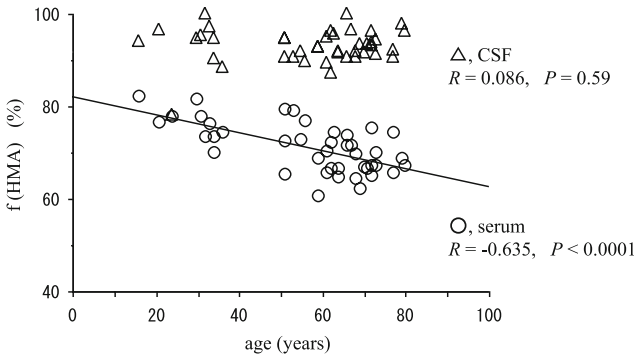


**Fig. 1** Correlation between f(HMA) values of albumin and age of 42 subjects (serum, *open circles*; CSF, *open triangles*). There is a significant negative correlation between age and f(HMA) value from the serum ($R = -0.635$, $P < 0.0001$), however, no correlation is observed between age and f(HMA) value from the CSF ($R = 0.086$, $P = 0.59$)

**Table 1** Values (%) for f(HMA), f(HNA-1), and f(HNA-2) of serum and lumbar CSF albumins from patients with orthopedic disorders

| | Subjects (average age) | *n* | f(HMA) (%) | f(HNA-1) (%) | f(HNA-2) (%) |
|---|---|---|---|---|---|
| Serum | Young group (29.1 ± 6.6) | 10 | 76.4 ± 3.7 | 22.0 ± 3.9 | 1.6 ± 0.4 |
| | Older group (65.4 ± 8.3) | 32 | 69.5 ± 4.7 | 28.6 ± 4.5 | 1.9 ± 0.6 |
| CSF | Young group (29.1 ± 6.6) | 10 | 93.0 ± 6.1 | 6.7 ± 6.1 | 0.3 ± 0.7 |
| | Older group (65.4 ± 8.3) | 32 | 93.1 ± 2.8 | 5.8 ± 2.2 | 1.1 ± 0.9 |

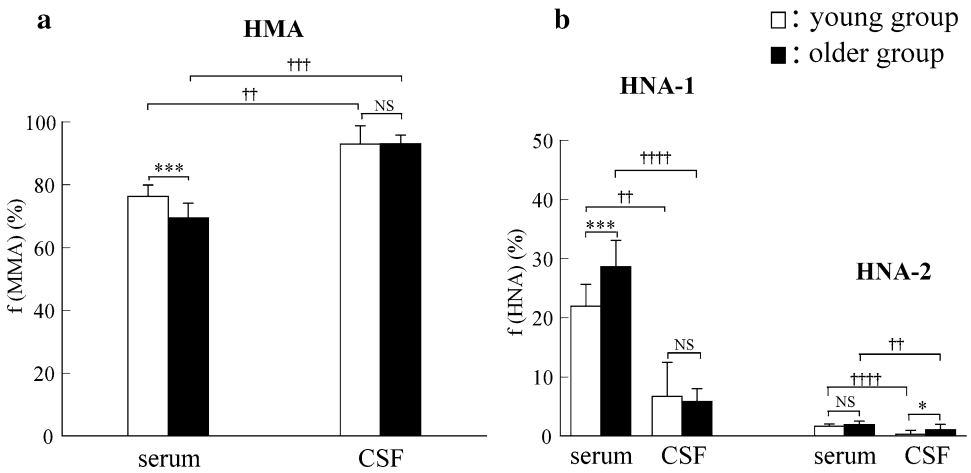


**Fig. 2** Values (%) of **a** f(HMA) and **b** f(HNA) (f(HNA-1) and f(HNA-2)) of albumin in serum and CSF from the young group ($n = 10$, *open columns*) and older group ($n = 32$, *closed columns*). Each *column* and *bar* represents the mean value and SD. Mann–Whitney's *U* test was used for comparisons between groups (***$P < 0.001$, *$P < 0.05$, NS: not significant), and the Wilcoxon signed-rank test was used for comparisons within groups ($^{\dagger\dagger\dagger\dagger}P < 0.0001$, $^{\dagger\dagger\dagger}P < 0.001$, $^{\dagger\dagger}P < 0.01$)

Figure 2b shows the value (%) for oxidized albumin (HNA). With regard to the oxidized albumin, we further analyzed the values (%) for HNA-1 (the "reversibly" oxidized form) and HNA-2 (the "irreversibly" oxidized form) fractions in the serum and CSF. For the values of HNA-1, there was a significant difference in the serum between the two groups ($P < 0.001$, Mann–Whitney's *U* test), whereas there was no significant difference in the CSF between the two groups. For the HNA-2 values, there were no significant differences in the serum, whereas a significant difference was observed in the CSF ($P < 0.05$, Mann–Whitney's *U* test).

## Conversion of HNA to HMA in three kinds of normal human cultured cells

In order to clarify which tissue regulates or maintains the redox state of albumin-thiol in the ECF, a commercial HSA product was incubated with three kinds of normal human cultured cells; astrocytes, aortic endothelial cells, and dermal fibroblasts. To examine whether conversion of HNA to HMA occurs in these cultured cells, a very low HMA content of HSA product (Sigma–Aldrich; A1653, lot 36K7545) was used in this study (HMA: 12.0%, HNA-1: 47.8%, HNA-2: 40.2%): usually one cannot obtain a high HMA content of HSA product because of auto-oxidation during the processes of manufacturing HSA from large-scale pooled blood.

Figure 3 shows the time-dependent changes in the f(HMA) value (%) of HSA incubated at 37°C with (a) astrocytes, (b) aortic endothelial cells, or (c) dermal fibroblasts (closed circles: cell (+), open circles: cell (−)). As shown in the figures, the HMA content of the astrocytes (11.9%) and aortic endothelial cells (11.1%) gradually increased during the first 9 h and then reached a plateau until 24 h (around 20%). However, in the absence of these cells, the HMA content decreased because of auto-oxidation. In the case of dermal fibroblasts, however, the HMA content was almost unchanged with and without the cells.

Figures 4 and 5 show the time-dependent changes in f(HNA-1) and f(HNA-2) values (%) of HSA incubated at 37°C with (a) astrocytes, (b) aortic endothelial cells, or (c) dermal fibroblasts (closed circles: cell (+), open circles: cell (−)). As shown in Fig. 4, the amounts (%) of HNA-1, i.e., the reversibly oxidized albumin in astrocytes and aortic endothelial cells decreased from 47–49% to 40–42% in a time-dependent manner, whereas almost no change was observed in that in fibroblasts. It can be seen from Fig. 5 that there were almost no changes in HNA-2 content (%) among the three types of cells, because HNA-2 includes highly oxidized species, for example the sulfenic (–SOH), sulfinic ($–SO_2H$), or sulfonic ($–SO_3H$) states of oxidized albumin, i.e., the "irreversibly" oxidized form. However, in the case of astrocytes, HNA-2 content decreased slightly from 41 to 38%, indicating that the

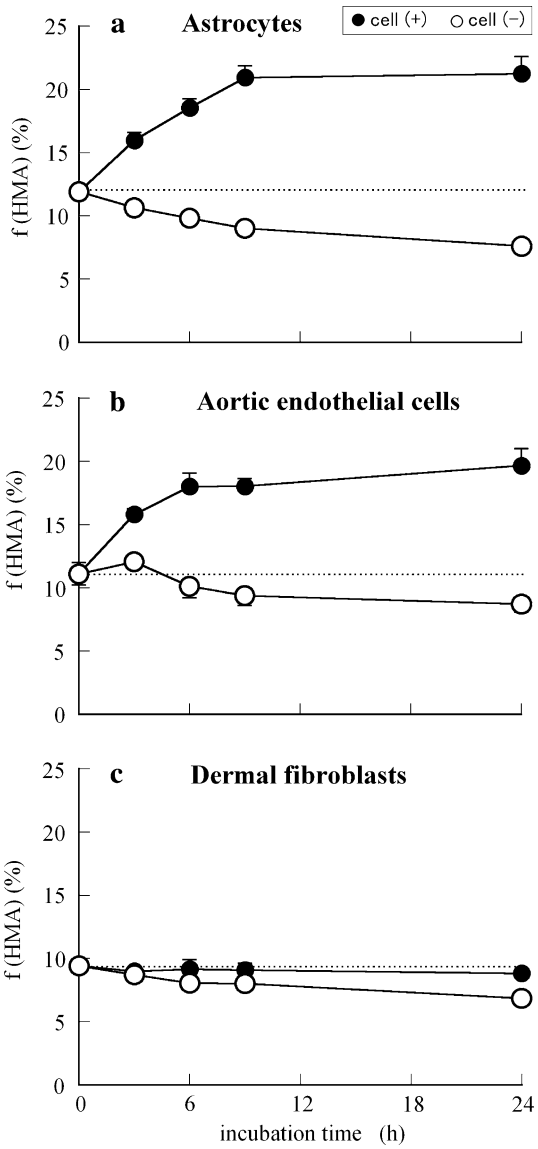


**Fig. 3** Time course of f(HMA) values (%) of HSA incubated at 37°C in each growth medium in the absence (*open circles*) or presence (*closed circles*) of cell culture ($2 \times 10^5$ cells/well): normal human **a** astrocytes, **b** aortic endothelial cells, and **c** dermal fibroblasts. Using independent cell cultures, the data presented are the means ± SE obtained from a representative experiment performed in triplicate

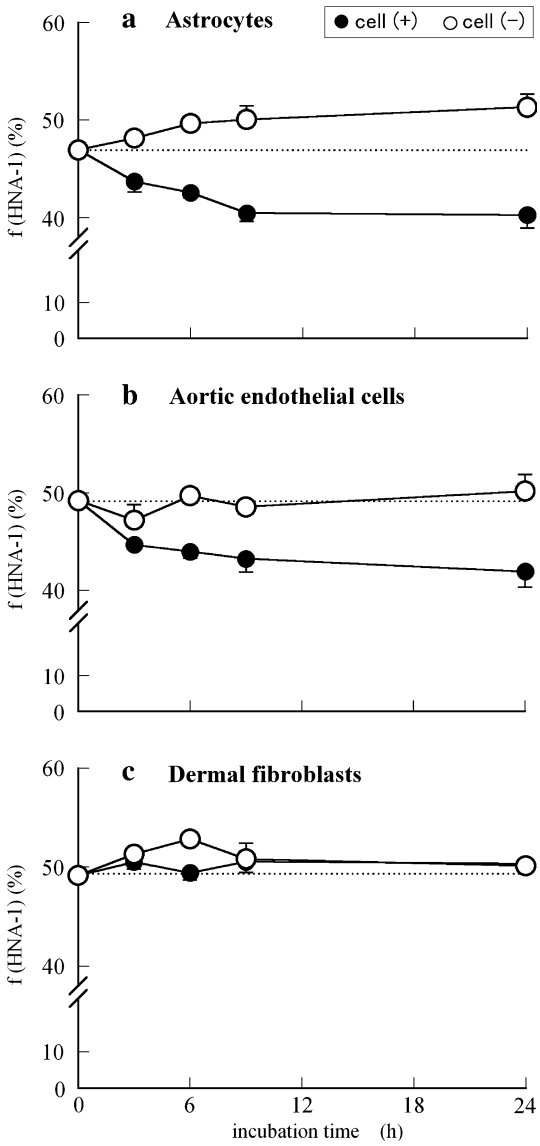


**Fig. 4** Time course of f(HNA-1) values (%) of HSA incubated at 37°C in each growth medium in the absence (*open circles*) or presence (*closed circles*) of cell culture ($2 \times 10^5$ cells/well): normal human **a** astrocytes, **b** aortic endothelial cells, and **c** dermal fibroblasts. Using independent cell cultures, the data presented are the means ± SE obtained from a representative experiment performed in triplicate

ability of astrocytes to convert the HNA-2 to HMA might be stronger than that of other two kinds of cells.

Each growth medium usually contains various kinds of supplemental growth factors and other active substances. However, in the absence of the cells, no increase in f(HMA) (Fig. 3) nor any decrease in f(HNA) (Figs. 4 and 5) was observed, suggesting that there were no active substances in the growth medium that affected the HMA ↔ HNA conversion of HSA.

From the set of in vitro results shown in Figs. 3, 4, and 5, it is assumed that normal human astrocytes and aortic endothelial cells, especially astrocytes, actively convert the oxidized form of albumin to the reduced form, whereas dermal fibroblasts have no such redox activity.

## Discussion

Even in normal circumstances, small amounts of reactive oxygen radicals and other free radicals are produced, resulting in cellular damage associated with peroxidation

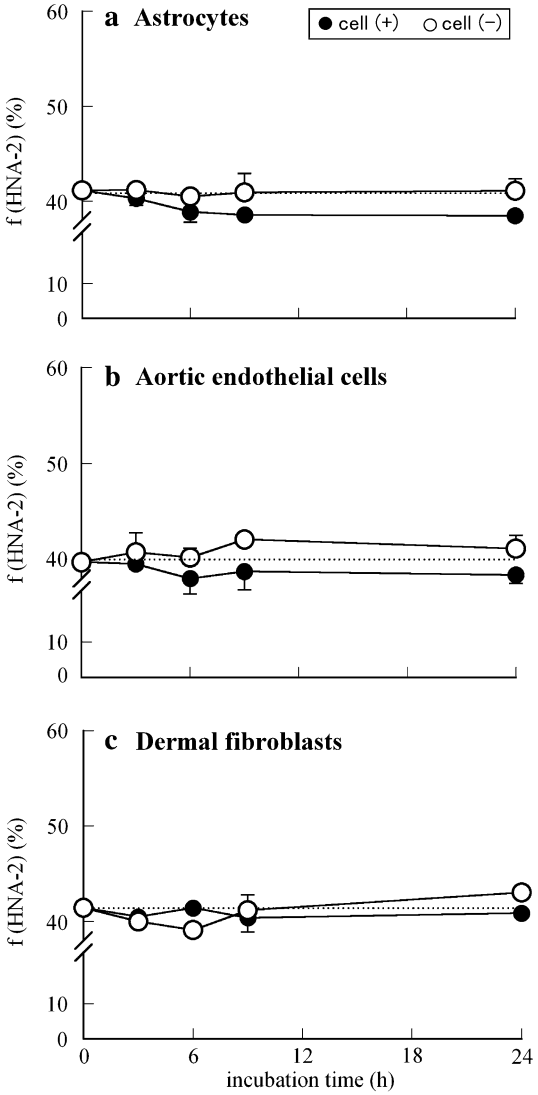


**Fig. 5** Time course of the f(HNA-2) values (%) of HSA incubated at 37°C in each growth medium in the absence (*open circles*) or presence (*closed circles*) of cell culture ($2 \times 10^5$ cells/well): normal human **a** astrocytes, **b** aortic endothelial cells, and **c** dermal fibroblasts. Using independent cell cultures, the data presented are the means ± SE obtained from a representative experiment performed in triplicate

of membrane lipids. In this regard, animals have developed many defenses to protect themselves from free radicals in the intracellular and extracellular environment [28]. Both ICF and ECF contain low-molecular mass antioxidants such as vitamin C, vitamin E, and thiols, which are actively involved in defense against reactive oxygen species (ROS) [28]. Moreover, with regard to thiols, and especially protein cysteinyl thiols, the oxidation of protein thiols to mixed disulfides and their reduction back to thiols might be an effective antioxidant system in both fluids [29]. Redox-dependent signaling events involving the post-translational modification of proteins are now accepted as an important regulatory process under physiological conditions [30, 31]. In cells, Cohen's group demonstrated in their review that the functions or signaling mechanisms of intracellular proteins such as sarco/endoplasmic reticulum $Ca^{2+}$ ATP-ase and p21ras are significantly affected by irreversible oxidation of their key thiols in disease [32]. On the other hand, with regard to extracellular protein thiols, albumin, with its single thiol group (Cys-34), is the most abundant protein in the ECF including the interstitial fluid; it is, hence, responsible for the largest fraction of reactive thiol in the ECF. Therefore, thiol-disulfide exchange reactions of albumin, i.e., dynamic changes in the redox state of albumin, might be considered to participate in the maintenance of a constant redox potential, and the f(HMA) value for HSA might reflect the redox buffer capacity of the human body.

The CSF is a modified ECF in the cerebrospinal space, which makes up the internal environment of the CNS. Because the volume of the CSF is relatively small and the albumin concentration is also very low in the CSF, there have been no studies of the functional role or analysis of the redox state of human albumin in CSF. As can be seen in Table 1 and Figs. 1 and 2, it is interesting to note that the values for f(HMA) from the CSF are significantly different from the corresponding serum values both in the young and older groups: the albumin in the CSF is almost all in the reduced form and this does not change with age. As already mentioned, to protect cell functions against ROS, antioxidant substances such as vitamin C and E, uric acids, glutathione, and thiol-containing amino acids are present in tissues [28]. From the literature, the mean concentrations of vitamin E in the serum and CSF of a normal control group (60.8 years old, $n = 78$) were 33.2 μM and 30.1 nM, indicating that the vitamin E level in CSF is much lower than that in serum [33]. On the other hand, mean concentrations of vitamin C in the serum and CSF were 42.8 and 163.8 μM in the normal control group ($n = 63$), indicating that the vitamin C concentration in the CSF is approximately four times the corresponding serum value, by means of facilitative transport through the choroid plexus [34]. Compared with other organs, because of the presence of an effective antioxidant system, the brain is able to defend itself against ROS that are continuously generated during oxidative metabolism. With regard to the defense system in brain tissue, some researchers emphasize in their reviews that glutathione is one of the most important detoxifying substances for ROS in the brain, i.e., metabolic interaction of glutathione occurs between astrocytes and neurons [35, 36]. Glutathione also regulates many protein functions by glutathiolation and vice versa; some proteins, for example protein disulfide isomerase and thioredoxin, regulate glutathione metabolism by a reversible thiol–disulfide exchange reaction [37]. From this point of view, it is

possible to say that albumin may also function in the regulation of glutathione metabolism, because the Cys-34 of HMA can bind reversibly to the oxidized form of glutathione or cystine in the ECF.

From the existence of strong antioxidant biomolecules in the CNS demonstrated above, together with our result concerning the high level of reduced human albumin in the CSF (Fig. 2a), it is necessary to explore the relationship between neural cell functions and albumin-thiol redox state. Astrocytes, the main glial cells in the CNS, play a major role in supporting the development of neurons because of the production of growth factors and cytokines; hence, we examined how astrocytes correlate with the dynamics of the redox regulation of albumin-thiol by using cultured normal human astrocytes and comparing them with that of aortic endothelial cells and dermal fibroblasts.

As shown in Figs. 3, 4, and 5, the values for f(HMA) of HSA incubated both in astrocytes and aortic endothelial cells increased gradually and maintained high f(HMA) values until 24 h. In contrast, the values for f(HNA-1) decreased both in astrocytes and aortic endothelial cells. These results indicate that both types of cell actively generate the thiol-disulfide exchange reaction, i.e., the HNA-1 → HMA conversion of human albumin. Moreover, this conversion effect was larger in astrocytes than in the aortic endothelial cells. It is interesting to note that astrocytes can also convert HNA-2 to HMA (Fig. 5a). As already mentioned, HNA-2 is composed of highly oxidized species, for example the sulfenic, sulfinic, and sulfonic states of oxidized albumin, i.e., "irreversibly" oxidized albumin. However, it is reported for HNA-2 that HSA sulfenic acid (HSA-SOH) is generated by the reaction of HMA with hydrogen peroxide and peroxynitrite, and it has been proposed that this serves as an intermediate in the formation of low-molecular-mass disulfides [38]. Very recently, it was reported that HSA-SOH can react rapidly with plasma thiols and is a central intermediate in redox modulation; the role of stable HSA-SOH was also discussed [39]. Therefore, it might be reasonable to classify HSA-SOH in HNA-2, because it can be converted to HMA by the strong redox effect present in astrocytes (Fig. 5a). Glial cells, especially astrocytes, constantly provide substantial amounts of cysteine and/or glutathione to maintain their levels in the local environment surrounding neurons [35, 36, 40]. Moreover, astrocytes can internalize albumin by receptor-mediated endocytosis, and albumin in cells regulates the synthesis of the neurotrophic factor oleic acid [41]. From this evidence, together with our current results, it can be speculated that the albumin in the CSF participates in redox regulation between astrocytes and neurons via thiol-disulfide exchange reactions with cysteine and/or glutathione.

Albumin, hemoglobin, and fibrin were the first proteins of the body to be studied. Among them, albumin is probably the most studied because of its being the most abundant soluble protein and the most prominent protein in sera. On the basis of a number of physicochemical studies on the structure and functions of bovine and human albumins from the 1960s, the HMA ↔ HNA conversion of HSA is well known to occur in the non-enzymatic process [42], and, hence, HMA and HNA can be obtained by ion-exchange chromatography on DEAE-Sephadex A-50 [43] and by affinity chromatography on activated thiol Sepharose 4B [44]. However, as has recently been reviewed [30], enzymes such as glutaredoxin, thioredoxin, and peroxiredoxin are potent antioxidants and multifunctional enzymes in protein thiolation/dethiolation processes in cells and plasma; further study oriented toward discovery of enzymes involved in this conversion in cultured cells is needed, using new methods such as RNAi.

In summary:

1. this study presents the first evidence that the redox state of human CSF albumin has significantly high f(HMA) and low f(HNA) values, compared to the corresponding values in serum from patients with orthopedic disorders. Although further studies comparing the results from age-matched healthy subjects are also needed, studies in humans are limited, especially studies of normal human CSF. This is, of course, because of ethical concerns. Our data on cultured cells show that astrocytes have a strong ability to convert oxidized albumin to reduced albumin, which may act as an effective antioxidative system in the brain, together with the high levels of vitamin C [34] and glutathione [35, 36] in the CSF;
2. from the study on cultured aortic endothelial cells it is speculated that during circulation through the body in normal circumstances, a high level of HSA-thiol is constantly maintained by the reduction activity of the endothelial cells of whole blood vessels, but not by dermal fibroblasts in connective tissues.

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