Determination and Characterization of Vanadium Ion in Serum Albumins

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Abstract

Vanadium was found to be contaminated in commercially available Sigma Grade bovine serum albumin (BSA), fraction V. Vanadium in BSA was estimated as about 17 μg V/g protein based on the result of neutron activation analysis, and approximately 85% of the metal was easily removed by dialysis against *o*-phenanthroline or EDTA The levels of vanadium in Sigma Grade serum albumins from various sources were in the following order, bovine (16.7 μg V/g protein) > ovine (10.0) > rabbit (9.1) > porcine (3.9) > human (0.2). The BSA sample in 0.1 M tris–HCl buffer, pH 7.5 showed the absence of vanadyl (+4 oxidation state) ion, but when a reducing agent such as ascorbic acid or cysteine was added, an intense signal due to the presence of vanadyl ion appeared, confirming that vanadium in BSA is in the vanadate (+5 oxidation state). Furthermore, the ^{51}V NMR spectrum of BSA at pH(D) 7.5 showed the resonance at −569.9 ppm against $VOCl_3$ (0 ppm) as external standard in the presence of EDTA, showing that the metal ion is also present in the vanadate form. The present results point out that commercially available serum albumins should only be used after at least simple purification such as dialysis.

Introduction

Since the vanadium ion is proven to be an essential trace element in many animals [1–3] and a potent inhibitor of Na^+, K^+–ATPase at physiological concentrations [4], much interest has been focused on the physiology, biochemistry and bioinorganic chemistry of this element. Because of difficulty in making accurate measurements of the vanadium ion in biological materials, reliable data on the concentrations of the vanadium ion in animals are limited.

Vanadium concentrations have been reported in human sera [5–7] and marine biological samples [8] by neutron activation analysis (NAA), which is the most reliable method currently used for the determination of vanadium.

During investigations on the chemistry and biochemistry of vanadium ion [9–14], we found that vanadium ion is a contaminant in commercially available bovine serum albumins. In this paper, we present evidence that vanadium in serum albumins is estimated based on the results of NAA and is demonstrated to be present in the vanadate form (+5 oxidation state) by ESR and ^{51}V NMR spectroscopies.

Experimental

Materials and Methods

Fraction V powders of serum albumins used were as follows: Sigma A 4503 and Boehringer 735078; human A 1653, porcine A 2764, ovine A 3264, rabbit A 0639. Bovine trypsins, type I (T 8003) and III-s (T 2395) were also purchased from Sigma Chemical Co. Other chemicals used were of special reagent grades.

To remove paramagnetic species, a 1 mM solution of BSA was dialysed at 4 °C against 0.01 M *o*-phenanthroline (*o*-phen) or EDTA, pH 6.0 for 20 h, followed by several changes of distilled deionized water for 3 days. It was then lyophilized as samples for NAA. A standard vanadium solution was prepared from NH_4VO_3 in 0.45 M H_2SO_4, and was standardized complexometrically with EDTA solution.

Protein samples, and standards for vanadium and sodium analysis, on filter papers, were sealed in double-layered chlorine-free, polyethylene bags, and subjected to NAA. The sealed samples were packed

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in a pneumatic tube and activated by irradiation at 5000 kW for 30 s in the Research Reactor (Kyoto University). The thermal, epithermal and fast neutron fluxes were 2.34×10^{13}, 8.40×10^{11} and 4.80×10^{12} neutrons cm^{-2} s^{-1}, respectively. Measurements of gamma-rays were performed with PGE Ge(Li) detector (active volume, 90 ml and relative efficiency for 1332 KeV gamma-ray of ^{60}Co to 3 X 3 inch NaI was 16.12%), equipped with Northern (4096 channels) pulse height analyser. Subsequent data reduction was achieved with a series of Fortran computer program written by the staff of the Research Reactor Institute of Kyoto University.

ESR spectra were recorded with a JES-FE1XG (X-band) spectrometer with 100 KHz field modulation at 77 K, which was calibrated with a Takeda Riken frequency counter TR 5212, and 1,1-diphenyl-2-picrylhydrazyl (free radical) (DPPH) ($g = 2.0036$) and Mn(II) in MgO ($\Delta H_{3-4} = 86.9$ G) as standards.

^{51}V NMR spectra were measured with a JEOL JNM-FX200, Fourier transform NMR spectrometer operated at 53.40 MHz. D_2O was used as a field frequency lock, using 5 mm ϕ sample tubes. ^{51}V chemical shifts were calibrated with respect to an external $VOCl_3$ standard.

Results

Determination of Vanadium in Albumins by Neutron Activation Analysis

Among the elements in biological samples, chloride and sodium ions occur in concentrations above trace element levels. Immediately after irradiation by neutrons, ^{38}Cl and ^{24}Na have the dominant activities. However, as shown in Fig. 1, which shows the gamma ray spectrogram of Sigma Grade BSA after irradiation by neutron, ^{52}V (half-life, 3.75 min) gave rise to an easily-detectable photon peak at 1434.0 KeV, being well separated from the peaks of ^{24}Na and ^{38}Cl. The

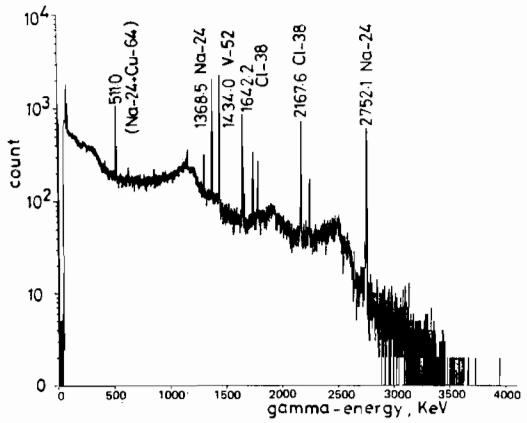

Fig. 1. Gamma-ray spectrogram of fraction V powders of bovine serum albumin (Sigma).

1434.0 KeV peak was used to calculate the vanadium concentration in albumins.

The concentrations of vanadium in commercially available serum albumins, purified albumins after dialysis against *o*-phen or EDTA and bovine trypsins are summarized in Table I. In Sigma Grade BSA (fraction V), 16.7 μg vanadium/g protein was detected, whereas in the BSA after dialysis, the amounts were decreased to 11–14%. The concentration of vanadium in Boehringer Grade BSA was estimated as 0.19 μg/g protein. We also detected cupic ion in the same specimen (a full examination of this ion is being prepared).

TABLE I. Vanadium Contents in Serum Albumins and Trypsins

Protein	Grade	Vanadium content, μg/g protein	
BSA, fraction V	Sigma A 4503	16.69 ± 0.88	($n = 7$)
BSA, dialysed against *o*-phen	Sigma, A 4503	1.98	($n = 2$)
EDTA	Sigma, A 4503	2.35	($n = 2$)
BSA, fraction V	Boehringer, 735078	0.19	($n = 2$)
BSA, dialysed against *o*-phen	Boehringer, 735078	0.15	($n = 2$)
EDTA	Boehringer, 735078	0.10	($n = 2$)
Serum Albumin human	Sigma, A 1653	0.19	($n = 2$)
porcine	Sigma, A 2764	3.87	($n = 2$)
rabbit	Sigma, A 0639	9.10	($n = 2$)
ovine	Sigma, A 3264	10.00	($n = 2$)
Bovine trypsin type III-s	Sigma, T 2395	5.23	($n = 2$)
type I	Sigma, T 8003	0.96	($n = 2$)

The levels of vanadium in Sigma Grade serum albumins from various sources were in the following order, bovine > ovine > rabbit > porcine > human. Vanadium was also detected in Sigma Grade trypsins, particularly, in type III-s sample (5.2 μg/g protein).

Characterization of Vanadium Ion in Bovine Serum Albumin

ESR spectrometry has been proved to be useful for characterization of vanadyl ion (+4 oxidation state as VO^{2+}) in biological samples [15, 16]. The

TABLE II. ESR Parameters of Vanadyl Ion in Albumin and other Vanadyl Complexes

System	A_0[a,b]	$A_\parallel$[a]	$A_\perp$[a]	g_0[c]	$g_\parallel$	$g_\perp$
Vanadyl ion detected in BSA, fraction V (Sigma) (pH 7.5)	102.1	179.6	63.4	1.985	1.942	2.006
Vanadyl–albumin complex[d] (pH 5.0)	100.3	172.8	64.0	1.966	1.939	1.979
	102.8	177.1	65.6	1.965	1.938	1.979
Vanadyl–ascorbate complex[e] (pH 7.0)	92.6	158.2	59.1	1.983	1.954	1.998

[a]Units of 10^{-4} cm^{-1}. [b]Calculated from the relationship, $A_0 = (A_\parallel + 2A_\perp)/3$. [c]$g_0 = (g_\parallel + 2g_\perp)/3$. [d]Ref. 18. [e]H. Sakurai *et al.*, unpublished data. The set of g_0 and A_0 values was obtained from the spectrum at room temperature, and $g_\parallel$ and $A_\parallel$ values were from that at 77 K.

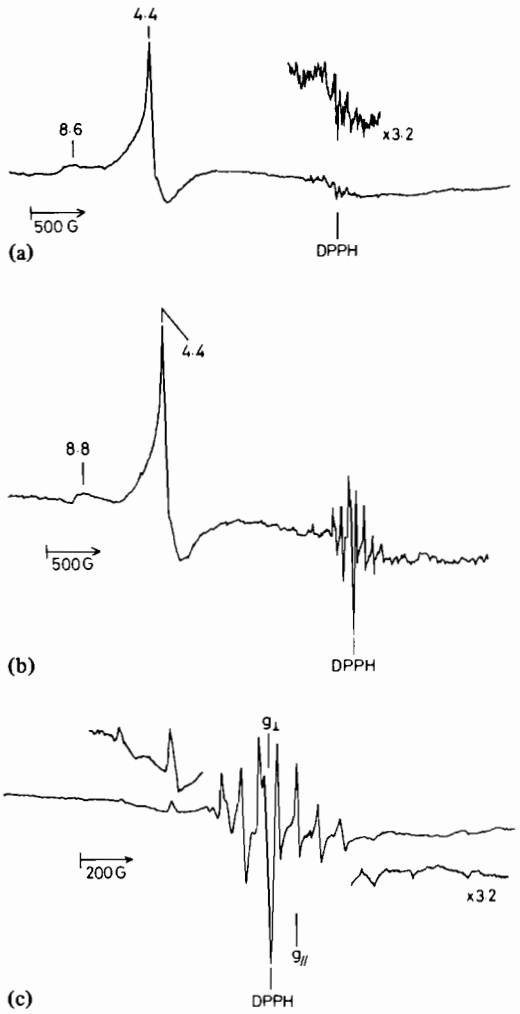

Fig. 2. ESR spectra of bovine serum albumin, fraction V (Sigma) at pH 7.5 and 77 K in the absence of ascorbic acid (a) and in the presence of ascorbic acid (b,c). Concentrations of bovine serum albumin and ascorbic acid were 2.4 mM and 10 mM, respectively.

ESR spectrum at 77 K of Sigma Grade BSA dissolved in 0.1 M tris–HCl buffer (pH 7.5) showed the absence of vanadyl ion, but the presence of ferric ion in the non-heme form (Fig. 2A). However, when a reducing agent such as ascorbic acid or cysteine was added to the BSA solution, a relatively intense signal around $g = 2.0$ appeared (Fig. 2B). This signal was composed of 8-lines (Fig. 2C), which could be assigned to vanadyl ion when compared with the data described previously [17, 18]. Thus, it was confirmed that the vanadium in BSA is in the vanadate form (+5 oxidation state). The ESR parameters for the vanadyl ion detected in BSA after reduction by ascorbic acid were compared with those of vanadyl–BSA complex [18] and vanadyl–ascorbate complexes [19] (Table II). Judging from the A- and g-values, vanadyl ion in BSA has a similar coordination structure to that of the vanadyl–BSA complex [18] and the ion was not ligand-substituted by ascorbate.

ESR spectrum of Boehringer Grade BSA without reduction demonstrated the presence of both non-heme type ferric ion and relatively high amounts of cupric ion (data not shown). These results were consistent with that of NAA in which vanadium was not detected in high amounts, compared with that of Sigma Grade BSA. Precise analysis of ESR spectra of BSA from various sources will be reported elsewhere.

The ^{51}V NMR spectrum of Sigma Grade BSA at pH(D) 7.5 showed a resonance at −569.5 ppm against $VOCl_3$ (0 ppm) as external standard in the presence of EDTA (Fig. 3). Without EDTA, the signal was, however, not detected. Under the same conditions, an EDTA–vanadate complex prepared by EDTA and $NaVO_3$ in D_2O at pH(D) 7.5 gave rise to a main resonance signal at −539.7 ppm (data not shown). Based on the results of ^{51}V NMR spectrometry, the presence of vanadate ion in BSA was proven after ligand substitution by EDTA.

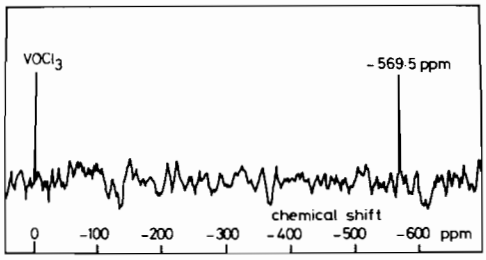

Fig. 3. ^{51}V NMR spectrum of bovine serum albumin, fraction V (Sigma) at pH 7.5 in D_2O in the presence of EDTA. Concentrations of albumin and EDTA were 0.65 mM and 2 mM, respectively.

Discussion

When we used Sigma Grade BSA (fraction V) as standard materials to study the binding properties of paramagnetic transition metal ions, we noticed that ESR spectrum of the BSA samples showed signals due to vanadyl ion in the presence of reducing agent such as ascorbic acid (Fig. 2) or cysteine. Thus the ESR spectra as well as ^{51}V NMR spectra confirmed the presence of vanadium in the +5 oxidation state in the BSA and the concentrations of vanadium in the samples were estimated by NAA (Table I).

The manner of binding of the vanadium ion to BSA is not certain, but approximately 85% of the metal ion in the protein is easily removed by dialysis against *o*-phen or EDTA (Table I). A 15% of total vanadium may, therefore, be assumed to bind more strongly to BSA. Among the serum albumins tested, the sample from the bovine sera contained the highest amount of vanadium. At present, we do not know why the commercial albumin preparation contains high amounts of vanadium.

The number of binding sites of BSA and bovine trypsin to vanadium ion have been reported to be 40 and 14, respectively, at pH 7.5 and 25 °C [20]. On the basis of this result, BSA (M_r = 69 000) and trypsin (M_r = 24 000) binds vanadium ion maximally at 28.7 and 28.8 μg/g protein, respectively. The vanadium concentrations in Sigma Grade BSA and trypsin estimated in the present study showed much lower levels than the calculated maximum concentrations, indicating that the binding sites are not fully saturated by the vanadium ion.

We detected a relatively high amount of cupric ion in BSA samples of Boehringer Grade. A full examination of this ion is being prepared.

The human albumin contained the lowest amount (0.19 μg/g protein) of vanadium of specimens tested, but still contained 7–34 folds higher vanadium levels than the natural human albumins; reliable amounts of vanadium in human serum are reported to be in the range of 0.024–0.939 ng/ml serum being a mean of 0.031 ng/ml serum [5] and 0.26–1.30 ng/ml serum [7]. Since albumin concentration in serum is estimated to be approximately 46.2 g/l serum [21], vanadium in serum is evaluated as 5.6–28.1 ng/g albumin.

Serum albumins have been used in a variety of way in many fields; e.g. as standard material for protein determination, fatty acid carrier for cell cultures [22] and protecting material for macrophage separation [23]. Vanadium ion above trace element levels in serum albumins can not be neglected in use, because it may influence any biochemical reactions. The present results pointed out that commercially available serum albumins should be at least dialysed before use.

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