Chemistry of Natural Compounds, Vol. 38, No. 4, 2002

CONFORMATIONAL CHANGES OF PROTEIN CONJUGATED TO METAL COMPLEXE

A. A. Abduvaliev,[1] M. S. Gil′dieva,[2] and V. P. Tatarskii[3]

UDC 611-012;615.213;615.277.3.015.42

The binding targets of a copper acetylacetonate complex modified by tegafur are protein NH_2 groups. Chemical binding occurs between the conjugate components. Bovine serum albumin is not denatured by complexation in the studied conjugate.

Key words: protein conjugate, copper acetylacetonate, tegafur, electron paramagnetic resonance, fluorescence, gel electrophoresis, protein conformation.

Certain transition-metal ions and their compounds that act as haptens and biocatalysts can be used as indicators of the effectiveness of intracellular substrates and antitumor preparations on malignancies. Square planar complexes of transition metals with low redox potentials that can expand their coordination sphere with functional groups of proteins are especially interesting. Complexes of copper acetylacetonates with natural amines and their analogs, which exhibit polyfunctionality on the inhibition of tumor growth and antimetastasis [1, 2], are examples of these. After the elucidation of the mechanism of action of these preparations on the molecular level, in particular, on the conformation of the proteins, they can be used as effective labels for evaluating the functional features of polyproteide structures. The paramagnetism of the copper comlexes and the delocalization of spin density from the metal onto the axial ligands enables electron paramagnetic resonance (EPR) and optical electronic spectroscopy to be applied for elucidating the detailed mechanisms of reaction of these molecules with biological substrates.

Herein we present results of investigations on conformational changes in bovine serum albumin (BSA) conjugated to the complex of copper acetylacetonate, $Cu(C_5H_7O_2)_2$ [$Cu(acac)_2$], and the known antitumor preparation tegafur (Tf), N′-(2-furanidyl-5-fluorouracil). The sixth coordination site in the five-coordinate complex $Cu(acac)_2 \cdot Tf$, which has square pyramidal geometry and C_{2v} symmetry, is free for reaction with highly basic nucleophilic groups of the protein [3].

Atomic absorption analysis established that the copper concentration in the conjugate of BSA with $Cu(acac)_2 \cdot Tf$ is 12.8 mg/L. According to the molecular weight of the complex and protein, one molecule of the conjugated protein contains 12-14 $Cu(acac)_2 \cdot Tf$ molecules. The nature of the binding between the protein and active center of the complex was determined using EPR and fluorescence spectroscopy.

The EPR spectrum of the $Cu(acac)_2 \cdot Tf$ complex (Fig. 1) contains a broad singlet with unresolved hyperfine structure (HFS, line width 138 G) and a g-factor of 2.18. The EPR spectrum of the BSA—$Cu(acac)_2 \cdot Tf$ conjugate has a characteristic four-component HFS due to coupling of the unpaired electron density of Cu with projections of its nuclear spin of 3/2 (HFS constant 64 G, g = 2.12).

The EPR spectra showed that the $Cu(acac)_2 \cdot Tf$ targets protein NH_2-groups and forms complexes with them. The shape are of the EPR spectra and the values of the magnetic parameters of the signals indicate that the BSA—$Cu(acac)_2 \cdot Tf$ conjugate is an isolated Cu structure of low symmetry.

1) Pilot Plant "Nikhol," ROSC MH, Republic of Uzbekistan; 2) Republic Oncological Scientific Center, MH, Republic of Uzbekistan; 3) HAEMATO Basics GmbH, Luckenwalde, Germany, e-mail: genetika@pochtamt.ru. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 290-292, July-August, 2002. Original article submitted April 1, 2002.

0009-3130/02/3804-0347$27.00

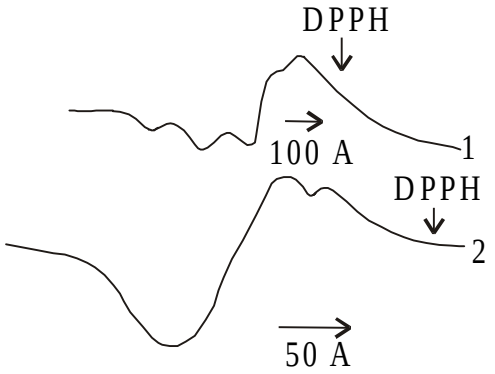

Fig. 1

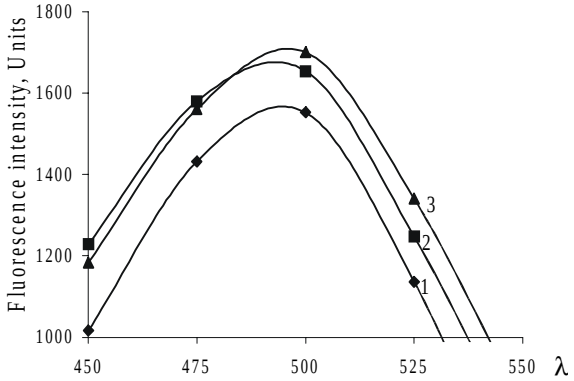

Fig. 2

Fig. 1. The EPR spectrum of the BSA conjugate with $Cu(C_5H_7O_2)_2 \cdot Tf$ (1) and EPR spectrum of $Cu(C_5H_70_2)_2 \cdot Tf$ (2).
Fig. 2. Fluorescence spectra (arb. units) of $Cu(C_5H_7O_2)_2 \cdot Tf$ (1), BSA (2), and the BSA conjugate with $Cu(C_5H_7O_2)_2 \cdot Tf$ (3).

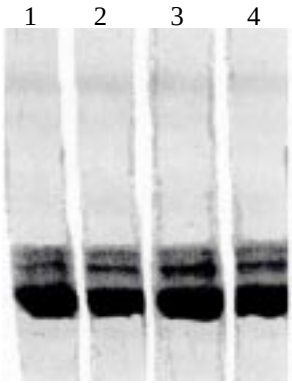

Fig. 3. Electrophoregrams of the BSA conjugate with $Cu(C_5H_7O_2)_2 \cdot Tf$ and conjugates of compounds included in it in 15% polyacrylamide gel. Coumassie G-250 dye. BSA conjugate with $Cu(C_5H_7O_2)_2 \cdot Tf$ in 0.01 M carbonate buffer (CB), pH 9.5 (15 μg/10 μL) (1), BSA in 0.01 M CB, pH 9.5 (15 μg/10 μL) (2), BSA conjugate with Tf in physiological solution (15 μg/10 μL) (3), BSA conjugate with $Cu(C_5H_7O_2)_2$ in physiological solution (15 μg/10 μL) (4).

Conformational changes of the protein after complexation by $Cu(acac)_2 \cdot Tf$ were evaluated by fluorescence methods (Fig. 2). It can be seen that the fluorescence energies of the conjugate and the starting components comprised by it are slightly different. Thus, the fluorescence intensity of the conjugate at 510 nm is greater than for the starting compounds, e.g., native BSA. This is consistent with an increase of the extinction coefficient and, as a result, a conformational change of the protein and suggests that the three-dimensional quaternary structure of BSA changes. Chromophores that were previously shielded manifest themselves as an increase of fluorescence intensity. The small shift of the fluorescence peak of the conjugate compared with native BSA and $Cu(acac)_2 \cdot Tf$ indicates that the corresponding transitions change.

Nevertheless, conjugation of BSA to the Cu complex does not lead to changes that would destroy the structural integrity of the biopolymer. Electrophoregrams of the conjugate and its components did not show significant shifts indicative of fragmentation or denaturation of the protein (Fig. 3).

Considering the lack of visible chromatographic changes (Fig. 3) and the insignificant differences of the fluorescence lines between BSA and its conjugate (Fig. 2), it seems that the Cu complex does not react directly with functional groups of the chromophores and does not contribute to quenching the fluorescence. Although it is known that complexes of acetylacetonates with Tf and melfalan are good traps of induced chlorotetracycline fluorescence [3]. Therefore, the metal complexes are

incorporated into the protein structure. However, in biological systems with highly basic chemical groups, they can participate in ligand exchange with the Cu complex, i.e., replace the protein in the conjugate.

In this instance the protein conjugate can be viewed as a carrier for transportation and prolonged action of antitumor metal complexes. It should be noted that the metal complexes affect the protein by conformationally changing its tertiary structure.

Thus, the results indicate that $Cu(acac)_2 \cdot Tf$ reacts with BSA by distributing diffusely and individually over its structure. The conformation of the biopolymer changes but is not destroyed.

EXPERIMENTAL

We used BSA (Pilot Plant "Nikhol" of ROSC MH RUz, Tashkent), Tf, and $Cu(acac)_2 \cdot Tf$ (HAEMATO Basics GmbH, Luckenwalde, Germany) in experiments on the preparation of the BSA—$Cu(acac)_2 \cdot Tf$ conjugate.

Conjugates of BSA with $Cu(acac)_2 \cdot Tf$, Tf, and $Cu(acac)_2$ were synthesized as follows. BSA (150 mg) in NaCl (5 mL, 0.9%) was incubated with metal complex (20 mg) for 3 h at room temperature. After the incubation was finished, the resulting solution was centrifuged for 20 min at 8000 rpm and purified of low-molecular-weight compounds by repeated dialysis against phosphate buffer (0.2 M, pH 7.3) containing NaCl (0.15 M). The conjugate solution was converted to $NaHCO_3$ (0.1 M, pH 9.5) by dialysis and centrifuged for 30 min at 8000 rpm. The BSA concentration in the solution was found spectrophotometrically by the common method of determining protein using Coomassie G-250 dye at wavelength 495 nm.

The amount of $Cu(acac)_2 \cdot Tf$ in the conjugate (1 g/L in doubly distilled water) was determined by atomic absorbance analysis on a Hitachi Z8000 spectrophotometer (Japan) with a background correction for the Zeeman effect. EPR spectra were recorded on a SEPR-03 instrument in physiological solution (pH 5.0) containing DMSO (10%) at room temperature. The concentrations of the studied compounds was 1 mg/mL. Fluorescence spectra were recorded on a SFR-1 instrument (Institute of Instrument Building, Pushchino, Russia); spectra of $Cu(acac)_2 \cdot Tf$ (1 mg/mL), by excitation at 210 nm in NaCl solution (0.15 M, pH 7.3); of BSA and BSA—$Cu(acac)_2 \cdot Tf$ (1 mg/mL), by excitation at 235 nm in carbonate buffer (0.1 M, pH 9.5). The chromatographic study was performed by the common method of vertical gel-electrophoresis in an AVGE-2 apparatus in polyacrylamide gel with Coomassie G-250 dye.

REFERENCES

1. V. P. Tatarskii, *International Symposium on the Development of Antimetastatic and Antiangeogenic Agents* [in Russian], Taejon, Korea, August 1997, p. 203.
2. A. A. Abduvaliev, A. A. Yusupova, and V. P. Tatarskii, *Problems in Oncology* [in Russian], A Collection of Scientific Articles, Tashkent (2001), No. 1, 29.
3. V. P. Tatarskii, Author's Abstract of a Doctoral Dissertation in Biological Sciences, Tashkent (1994).