INTERACTION OF NEUROCUPREIN WITH ADRENALINE

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1. Introduction

Neurocuprein is an acid copper-containing protein of unknown physiological function isolated recently from grey and white matter of bovine brain as well as from brain areas of *Substantia nigra* and *locus caeruleus* [1–3]. The protein consists of one polypeptide chain with mol. wt 10 000 and contains 1 copper atom/molecule. Neurocuprein has no amine oxidase, dopamine-β-hydroxylase and superoxide dismutase activities. The protein does not exhibit activating or inhibitory effects on cytochrome oxidase and superoxide dismutase [4]

It was noted during the purification procedure that the protein remains reduced in the course of many stages. The preliminary study has shown that the reducing agent is of catecholamine nature. In connection with this observation and in order to elucidate the possible role of neurocuprein it would be of interest to study the interaction of the protein with catecholamines and other neurotransmitters

2. Materials and methods

Neurocuprein was isolated by a novel procedure from the fresh white or grey matters, instead of acetone powders used in the previous works [2,3]. In the novel procedure organic solvent and ammonium sulphate fractionations were omitted at all steps of the purification The procedure consists schematically of the following stages.

Tissues were homogenized in a Waring blender with 0.01 M acetate buffer (pH 6.0). The homogenate was centrifuged at 10 000 × *g* for 1 h and to the supernatant obtained DE-32 cellulose was added to adsorb acidic proteins by a batchwise procedure. Then, cellulose was transferred to a column and washed in sequence by 0.05 M, 0.1 M and 0.5 M buffers (pH 6.0). The yellowish copper-containing fraction eluted with the last buffer was dialyzed against 0.01 M buffer, and the dialyzate was applied onto a column of DE-32 cellulose (1.5 × 4). After washing the column with 100–150 ml 0.2 M buffer the protein was eluted with the same buffer containing 0.4 M NaCl. This fraction was dialyzed and finally purified on a DEAE–Sephadex, A-50, column from which the greenish fraction was eluted with 0.2 M buffer containing 0.4 M NaCl. This preparation was electrophoretically homogeneous and had physicochemical properties similar to neurocuprein isolated from acetone powders of bovine brain. The reduction of neurocuprein was carried out by adding dithionite with subsequent gel-filtration through Sephadex G-25 (superfine) to remove the excess of the reductant.

Adrenochrome was prepared by adding 2- or 3-fold excess of ferricyanide to a solution of adrenaline in acetate buffer (pH 6.0). After 5 min incubation the mixture was passed through a column with Biogel P-2. The red band of adrenochrome was collected and used immediately after its elution. Concentrations of adrenochrome were evaluated from molar extinction of the absorbtion band at 500 nm of 750 $M^{-1} cm^{-1}$, and concentrations of ferrocyanide were determined using a molar extinction of $10^3 M^{-1} cm^{-1}$ for the band at 420 nm.

Adrenaline and noradrenaline used here were supplied by 'Whatman', Sephadexes were obtained from 'Pharmacia' and Biogel from 'Serva'. Other chemicals were manufactured in USSR.

Optical spectra were recorded in 10 mm cells at 20°C. Thunberg cells were used for experiments in

anaerobic conditions EPR spectra were obtained at −180°C on a Varian E-4 instrument Standard conditions of EPR measurements were microwave frequency, 9.14 GHz; amplitude modulation, 6.3 G, microwave power, 10 mW.

3. Results and discussions

The addition of adrenaline or noradrenaline to solutions of oxidized neurocuprein resulted in the rapid decrease of EPR signal intensity of neurocuprein (fig.1). Simultaneously the formation of red colored compound was also observed. The optical spectrum of this compound is shown in fig.2. Titration experiments carried out under anaerobic conditions and pH 6.0 with different initial concentrations of adrenaline/neurocuprein led to the conclusion that maximum intensity of the band at 500 nm is observed at the molar ratio adrenaline/neurocuprein near to 1 2 (fig.3). Thus, the colored compound having the broad band at 500 nm seems to be a product of two-electron oxidation of adrenaline or noradrenaline by neurocuprein. This suggestion was further supported by the titration of adrenaline by the one-electron oxidant, ferricyanide. The spectrum of the product formed was found to be identical to one obtained in the neurocuprein–adrenaline system with the stoichiometry adrenaline/ferrocyanide of 1:2 This

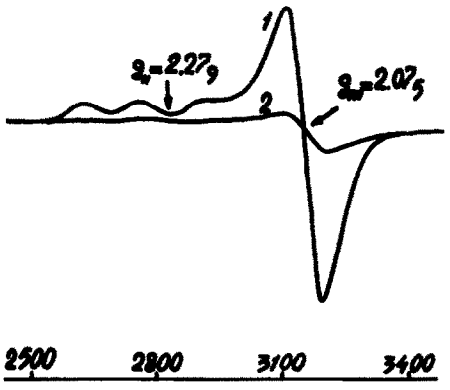

Fig 1 The change of the EPR signal intensity of neurocuprein under the effect of adrenaline. To 10^{-4} M of oxidized neurocuprein (1) 3-fold excess of adrenaline was added at anaerobic conditions and pH 6 0 and the spectrum 2 was recorded after incubation for 5 min

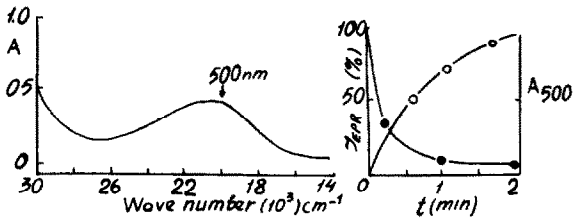

Fig.2. The optical spectrum of the product formed in the course of the reaction neurocuprein with adrenaline (left) and kinetic curves (right) for the drop of the EPR signal intensity (•) and the formation of the product (○)

spectrum is characteristic for adrenochrome. Hence, it may be concluded that 1 mol adrenaline can reduce 2 moles of neurocuprein, as the protein contains only 1 cupric atom.

At aerobic or anaerobic conditions (pH 6.0), adrenochrome was stable at least for 1 h, and neurocuprein obtained by the adrenaline reduction was practically not autoxidized for 1–2 h. It was found however that the addition of ferricyanide to neurocuprein reduced by adrenaline led to the formation of an EPR spectrum the shape and parameters of which are sharply different from those observed in native oxidized neurocuprein before its treatmen by adrenaline (fig.4A). It is important to note that when neurocuprein preliminary reduced by dithionite was oxidized by ferricyanide, the EPR signal arising was the same as in fig.1. On the other hand, the addition of a large excess of ferricyanide to oxidized neurocuprein brings about the disappearence of the signal [2] as the result of the line broadening.

Thus, the signal shown in fig.4A, possibly reflects

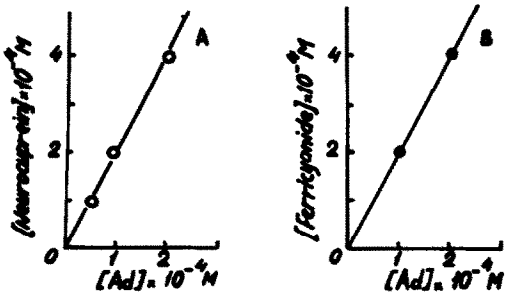

Fig 3. Titrations of adrenaline by oxidized neurocuprein (A) and ferricyanide (B) at pH 6 0 and anaerobic conditions.

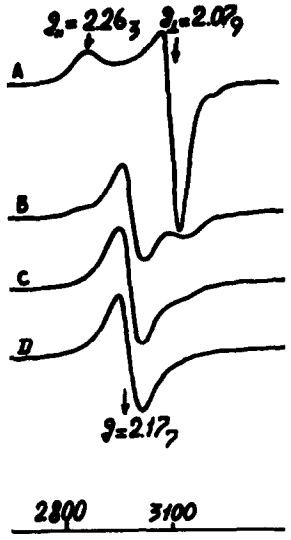

Fig.4 EPR spectra of neurocuprein after the addition of ferricyanide to the protein preliminarily reduced by adrenaline (A) and in the course of the titration of the reduced protein by adrenochrome. 0.5 (B), 1 (C) and 2 (D) equivalents of adrenochrome were added to the reduced protein.

the presence of adrenochrome in neurocuprein solution. Therefore, the effect of adrenochrome on oxidized and reduced neurocuprein was also considered. The titration of the oxidized protein by adrenochrome results in EPR spectra shown in fig.4(B–D). As it follows from these data, adrenochrome modifies the surrounding of copper in neurocuprein. The spectrum observed in the presence of adrenochrome has no hyperfine structural components, resembling the EPR spectrum of hydrated Cu^{2+}. Similar spectrum was observed at the addition of reduced neurocuprein to adrenochrome, indicating the oxidation of the protein.

However, when reduced neurocuprein was titrated by a fresh mixture of adrenaline and ferricyanide rather then adrenochrome, obtained from this mixture by gel-filtration, then the signal arising has the shape similar to fig.4A. Although at present it is difficult to make certain conclusions on the way of the formation of the EPR signal of this type it may be suggested that the signal is formed in the course of the interaction of the reduced protein with the semiquinone radical (half-reduced form) of adrenaline.

Experiments on the interaction of neurocuprein with other neurotransmitters were also carried out. No changes in either intensity or shape of the EPR signal were observed when the oxidized protein was incubated with 2–10-fold excess of acetylcholine, serotonine, tryptamine or tyramine for 2–3 h at 23°C and pH 6.0. However the decrease of the EPR signal intensity of neurocuprein was noted at the titration of the protein by dopamine and L-DOPA. These redox reactions proceed more slowly than in the case of adrenaline and noradrenaline. Besides, it was necessary to add much more (non-stoichiometric) quantities of dopamine or L-DOPA for the effective reduction of the protein.

The reaction of adrenaline was compared with the interaction of hydrated Cu^{2+} and adrenaline. It was shown that adrenaline does not reduce hydrated copper. Instead of this the formation of a complex between adrenaline and Cu^{2+} was observed. This result is in accordance with the early observation [5] of complex formation between catecholamines and hydrated copper.

We suppose that the non-enzymic redoc reaction of neurocuprein with adrenaline and noradrenaline described here, reflects the important role of the protein in neuronal processes, particularly in the detoxification of adrenochrome-like compounds and in the neutralization of excess catecholamines.

References

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