GENETICS

Interaction of Ceruloplasmin with the Plasma Membrane Receptors of CV-1 Cells and Its Feedback Regulation

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It is shown that cultured cells of the CV-1 line possess the capacity for high-affinity binding of ceruloplasmin, show a kinetics of saturation, and internalize ceruloplasmin. Propagation of cells in medium supplemented with fetal calf serum depleted of ceruloplasmin results in a two-fold increase of high-affinity receptor expression on the cell surface. This phenomenon is not accompanied by any change in the receptor-ligand affinity. Ceruloplasmin binding to the cell surface and subsequent internalization do not lead to its marked degradation.

Key Words: *ceruloplasmin; ceruloplasmin receptor; copper transport*

The uptake of copper (Cu), a trace element essential for all cell types, by the organism, its transport along the pathways of intercellular communication, delivery to the cells of various organs, entry into the cells, intracellular transfer to the sites of synthesis and assembly of Cu-containing enzymes, and removal from the cells and organism are implemented by the system of Cu-transferring proteins. This system consists of two types of proteins: membrane-bound and soluble. Their balanced functioning ensures the transport of protein-entrapped Cu ions to all cell compartments. Soluble proteins are represented by ceruloplasmin (CP) and a family of CP-related proteins, including plasma CPs [5], milk CPs [2,7], bile CPs [6,11], CP-like proteins of cerebrospinal, amniotic, and seminal fluids [1,9,10], and intracellular CP-like protein [3]. Membrane-bound components include CP receptors [4] and two Cu-transport-related ATPases of the P type [12,13].

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The mechanism of interprotein Cu transfer is still poorly understood. In view of this, we undertook a study of the molecular mechanism of interaction between serum CP, a universal Cu carrier in the blood flow, and the specific CP membrane receptor of green monkey kidney cells (CV-1 line).

MATERIALS AND METHODS

Experiments were carried out on CV-1 cell confluent monolayers. The cell line was obtained from the Collection of Cell Cultures, Institute of Cytology, Russian Academy of Sciences. Highly purified preparations of human and rat CP (A_{610}/A_{280} equal to 0.045 and 0.044, respectively) were manufactured in our Department by M. M. Shavlovskii and E. T. Zakharova. Radioactive iodine labeling of CP was performed by the chloramine T method. CP was removed from fetal calf serum (FCS; Gibco) by incubation of 10% FCS solution in Dulbecco's minimal essential medium (DMEM; Flow Lab.) with DEAE-sepharose in a v/w ratio of 10:0.5. The serum CP concentration was measured by oxidase

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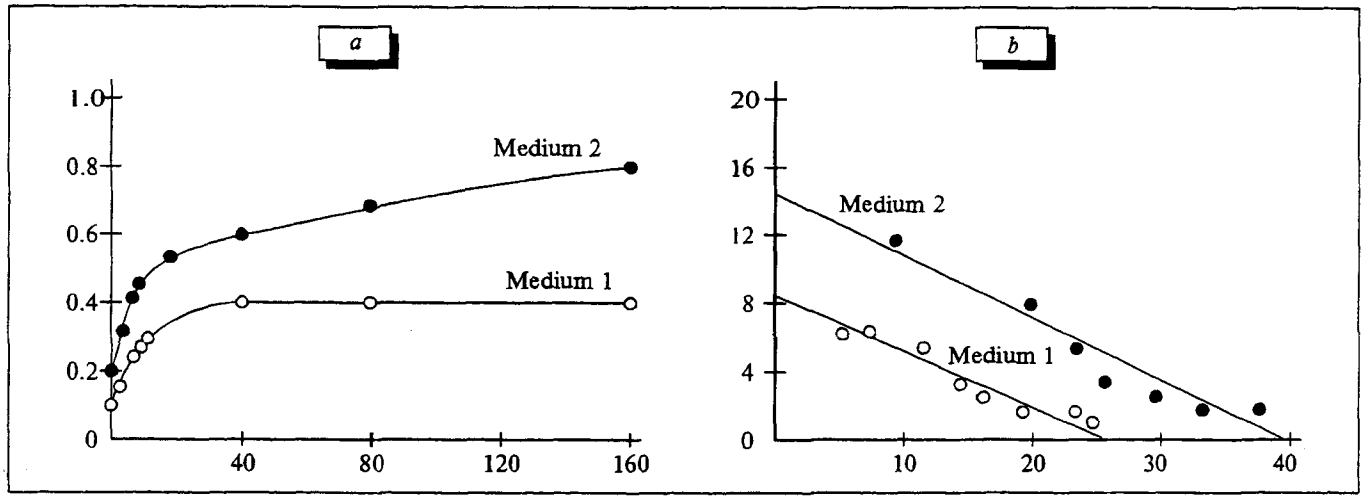

Fig. 1. Kinetics of saturating ^{125}I–CP binding to CV–1 cell monolayer (*a*) and graphic analysis of this process (*b*). Cells were incubated with ^{125}I–CP for 16 hours at 0°C. Ordinate: *a*) amount of bound CP per well, ng; *b*) ratio of bound/unbound CP, %.

activity using p-phenylenediamine as the substrate. CP-binding activity was studied as following. Cells were placed in the wells of microplates (Flow Lab.), 10,000 cells per well, and cultured for 24 hours in complete DMEM supplemented with 10% FCS (medium 1), and then in one-half of the wells the medium was replaced with medium 2 (DMEM plus FCS depleted of CP), and the cells were further cultured for 2 days. The culture medium was then discarded, and the cell monolayers were washed with phosphate-buffered saline (PBS; 0.125 M NaCl solution buffered with 10 mM Na-phosphate, pH 7.4). The wells were refilled with 0.2 ml medium 2, and ^{125}I-labeled CP (^{125}I; Izotop, Russia) with a specific activity of 1.3×10^6 cpm/μg was added in the dose range of 2 to 150 ng per well. After incubation at 0°C for 16 hours the radioactivity of unbound CP was estimated in culture medium aliquots. The remaining medium was aspirated, cell monolayers were washed 7 times with PBS and lysed in a hot solution of 1 M NaOH and 5% Na-dodecylsulfate (SDS; Serva). Radioactivity was recorded in a Nuclear Chicago liquid scintillation counter. Nonspecific binding of radiolabeled CP was estimated by performing the experiment in the presence of unlabeled CP added in a 100-fold excess (0.2 to 15 μg in various wells). For comparison of the CP-binding capacity of the cells grown under the conditions of normal and low CP concentration, and for the study of CP internalization and degradation, cells were suspended in medium 1 and placed in 60 mm Petri dishes, 10^5 cells per dish. Twenty-four hours later in half of the dishes medium 1 was replaced with medium 2 and after further 48-hour culturing 2 ml of medium 2 containing 100 ng of labeled CP were added to each dish. The dishes were incubated for 2.5 hours at 0°C or 37°C. Cell monolayers were washed with cold PBS and treated with 1 ml dextran sulfate (Pharmacia) dissolved in Hanks solution in a concentration of 4 mg/ml at 0-4°C for 60 min. CP-binding activity was judged by the level of radioactivity released from the cell surface by dextran sulfate, CP internalization by the radioactivity of cell lysates, and degradation by the radioactivity of acid-soluble material of the culture fluid [10]. The molecular weight of cell surface-bound and internalized CP polypeptides was assessed by SDS polyacrylamide gel electrophoresis [8], followed by autoradiography of the dried gel.

RESULTS

Comparison of CP-binding activity of CV-1 cells grown in medium 1 and medium 2 showed the capacity of these cells for high-affinity CP binding, which depends on the CP concentration in

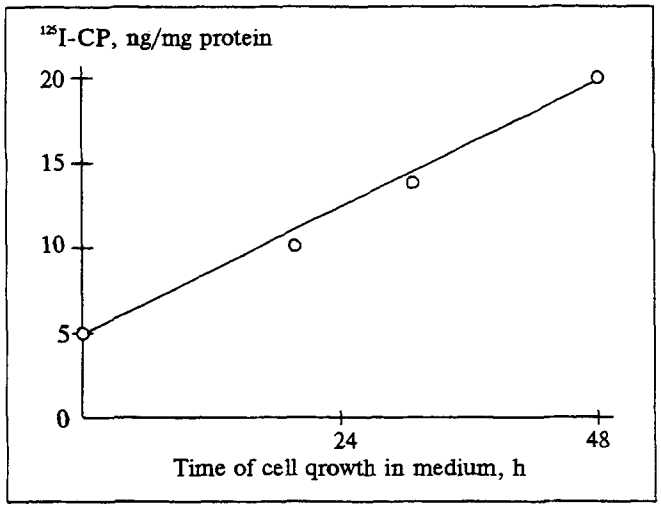

Fig. 2. CP binding by CV–1 cell monolayer as a function of time of culturing in CP–deficient medium.

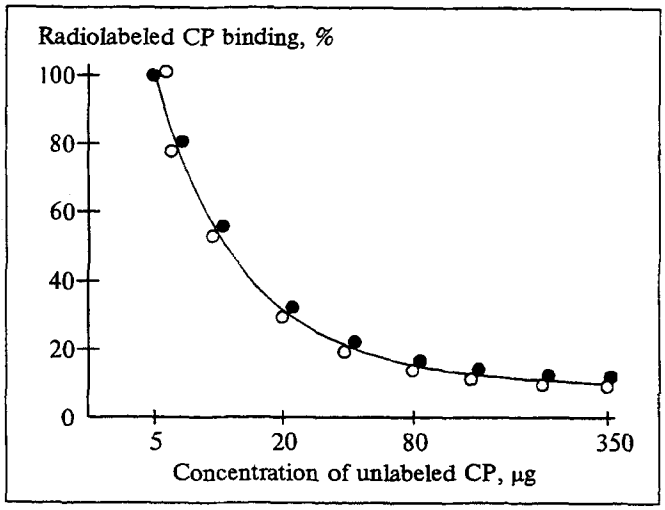

Fig. 3. Competition of human CP (dark circles) and rat CP (white circles) with radiolabeled human CP for binding sites in CV−1 cell monolayer. 40 ng of labeled CP and various amounts of unlabeled human or rat CP were added to each well containing 2×10^5 cells. The level of binding in the absence of unlabeled CP was taken as 100%.

the culture medium and is characterized by a kinetics of saturation (Fig. 1, *a*). Cells grown in CP-depleted medium 2 exhibited a 2-fold increase of CP binding as compared to those grown in medium 1 (Fig. 1, *a*). Graphic analysis of CP binding in Scatchard coordinates (Fig. 1, *b*) revealed that, regardless of the culturing conditions, the affinity of CP receptors was of comparable level (Kd=97 and 65 nM in the case of medium 1 and medium 2, respectively). However, the number of CP-binding sites on the cells exponentially growing in medium 2 exceeded that of the cells grown in medium 1 almost twofold (180×10^3 and 110×10^3 per cell, respectively).

The increase in the capacity of cell to bind ^{125}I-CP was in direct proportion to the time of cell culturing in CP-deficient medium 2 (Fig. 2). As can be seen in Fig. 3, rat CP efficiently competed with human CP for the binding sites on the CV-1 cell surface.

CP binding, internalization, and degradation by CV-1 cells were tested at both 0-4°C and 37°C. The CP-binding activity of cell surface receptors proved to be independent of temperature and its level was about 10^5 CP molecules per cell. A rise of temperature from 0°C to 37°C resulted in an almost 3-fold increase of CP internalization (3×10^4 and 8×10^4 CP molecules per cell, respectively). Acid-soluble products of CP degradation in the culture medium analyzed after incubation of cells with radiolabeled CP at either temperature were practically absent. Analysis of CP released from the cell surface by dextran sulfate by means of gel-electrophoresis under denaturing conditions showed that binding *per se* did not alter the molecular weight or quantitative ratio of the detectable polypeptides (Fig. 4). At the same time, following internalization intracellular CP was represented mainly by peptides of low molecular weight.

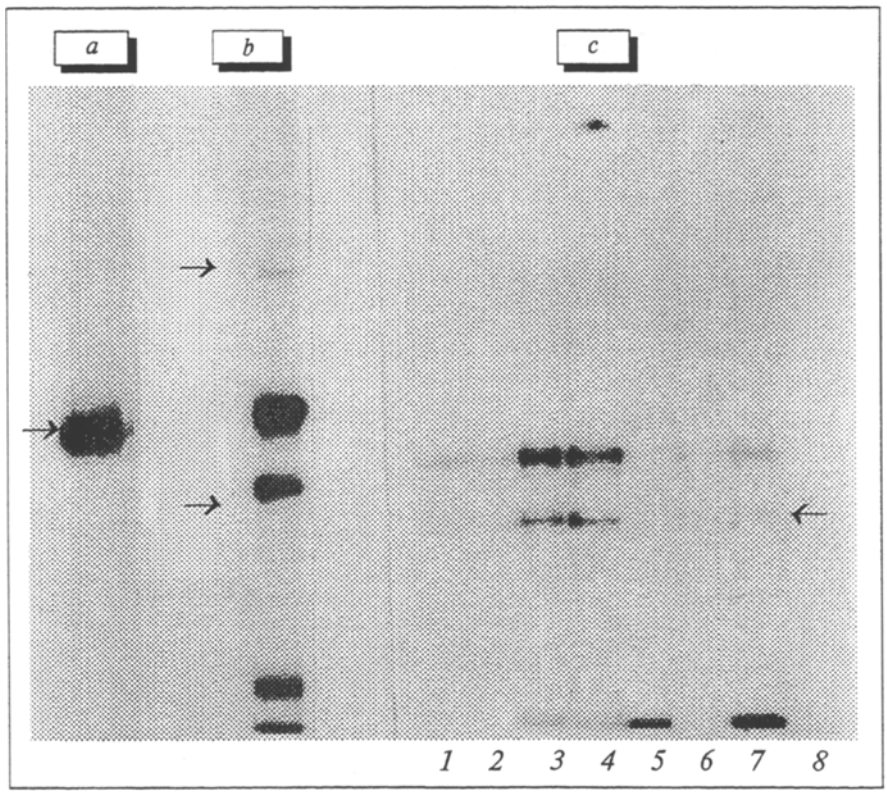

Fig. 4. Electrophoretic analysis of ^{125}I−CP bound and internalized by CV−1 cells incubated under different conditions. *a*) electrophoresis in 7.5% polyacrylamide gel under nondenaturing conditions. Arrow points to position of CP−marker band, detected by oxidase activity. *b*) electrophoresis in 7.5% polyacrylamide gel in the presence of SDS. Arrows point to position of protein markers: CP (130 kD) and bovine serum albumin (65 kD). *c*) electrophoresis under denaturing conditions of ^{125}I−CP released from the cell surface by dextran sulfate (*1*−*4*) and internalized CP (*5*−*8*). Arrow points to position of albumin (65 kD). *1, 2, 5, 6*: cells were grown in medium 1; *3, 4, 7, 8*: cells were grown in medium 2. CP binding was performed at 37°C (*1, 3, 5, 7*) and at 0°C (*2, 4, 6, 8*).

The absence of products of CP degradation in the culture medium may be attributed to intracellular protease-mediated modification of CP, which, however, does not reach the point of its entire degradation to acid-soluble products.

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