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Influence of magnetic field on aqueous two-phase extraction of horse ferritin in the polyethylene glycol/hydroxyethyl starch system

Magdalena Zielińska-Dawidziak*, Roman Błaszak, Dorota Piasecka-Kwiatkowska

Department of Biochemistry and Food Analysis, Poznan´ University of Life Sciences, Poland

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ABSTRACT

The presented experiments show the model of expectation of equine spleen ferritin extraction in a new aqueous two-phase system which was formed by mixing polyethylene glycol (PEG) and hydroxyethyl starch (HES). The tendency of the protein to migrate in the analyzed systems was dependent on the concentrations of HES and PEG as well as PEG molecular weight. The highest concentration of ferritin in the top phase (rich in PEG) was recorded in the system composed of 6% PEG 3000 and 3% HES. The obtained concentration was 0.88 mg mL^{-1}. The lowest concentration was 0.42 mg mL^{-1} in the system composed of 5% PEG 6000 and 1% HES.

Next the influence of the magnetic field on ferritin accumulation was analyzed. Selected samples were placed between homogeneous (S/S) or heterogeneous magnetic poles (N/S and S/N). It was observed that after the application of the magnetic field the extraction of ferritin into the PEG rich phase increased in every examined system. That increase was as high as 1.67-fold ferritin concentration in the PEG phase as compared with the total concentration of ferritin in the system before separation. Introduction of the magnetic field to two-phase extraction systems is shown as an effective method of changing the partition coefficient of ferritin.

1. Introduction

Liquid–liquid extraction in aqueous two-phase systems (ATPS) is a separation method of protein, which offers mild conditions of extraction and integrates concentration, purification and clarification processes. This method was applied to purification of natural compounds also because of its low cost. ATPS may be formed for example when water solutions of two incompatible polymers, such as polyethylene glycol (PEG) and dextran, are mixed together [1,2]. In the presented experiments ATPS were formed by mixing polyethylene glycol and the polymer to date infrequently used in ATPS, i.e. hydroxyethyl starch (HES).

The most important inconvenience of ATPS lies in searching for operating conditions, under which the separated molecule is recovered with a sufficient efficiency and purity. The partition coefficient of separated substances depends on various factors, such as properties of the system (hydrophobicity, composition, temperature, pH, charge and the presence of additional substances) and properties of separated molecules (surface properties, size, charge and chirality). Mathematical models for the prediction of partition coefficients have not been developed yet [3–5] and experiments on the separation often start from that point. In the presented experiment analyses concerned the effect of concentrations of used polymers and the molecular weight of PEG as well as the effect of the magnetic field and its direction on ferritin extraction into the PEG-rich phase.

As it was mentioned above, one of the facts which influence extraction in ATPS is charge – the charge of the system and the charge of the protein. The hypothesis which we wanted to confirm is that the placement of ATPS in a magnetic field will change Nernst's partition coefficient of substances, because of the fact that the magnetic field will influence the distribution of the summary charge in the system.

The separated molecules in the analyzed system was ferritin. The protein is very conservative, both in terms of its structure and function, both in animals and in plants. The apoferritin shell is full with an iron core. Plant ferritin is considered as a food supplement in iron deficiency prevention. However, problems with easy, continuous, large-scale separation of the protein have been reported. Due to the conservative properties of this protein we proposed to construct a mathematical model of ferritin separation on the basis of the extraction of equine spleen ferritin, which is commercially available in a defined form (concentration, purity, iron content, etc.).

* Corresponding author: Magdalena Zielińska-Dawidziak, Department of Biochemistry and Food Analysis, Faculty of Food Science and Nutrition, Poznan´ University of Life Sciences, 48 Mazowiecka Street, 60-623 Poznan´, Poland.
Tel.: +48 618487344/+48603138084; fax: +48 618487352.
E-mail addresses: mzd@owl.up.poznan.pl, mzd@up.poznan.pl (M. Zielińska-Dawidziak).

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Another aspect of importance for us was connected with the fact that ferritin is also sensitive to the magnetic field. First of all it is a protein, thus it demonstrates magnetic sensitivity [6]; what is more, the polypeptide shell is filled with an iron core, which should enhance the influence of the magnetic field on the extraction of this protein. The protein is considered as magnetic nanoparticles [7].

Magnetic separation of protein has been developing for over 30 years; however, the separation is based on an interaction of magnetic particles coated by ligands [8–10]. Extraction in an aqueous two-phase system is often improved by additional effects, such as traditional and microwave heating [11], nitrogen flotation [12], introduction of magnetic particles in ATPS [13,14] or Au nanoparticles [15], surfactants and micelles [16–18]. In our experiments we wanted to examine the direct influence of the magnetic field on the extraction of ferritin.

2. Materials and methods

2.1. Aqueous two-phase system

Aqueous two-phase systems were formed by mixing solutions of two polymers – polyethylene glycol (FLUKA, MW: 600; 1000; 3000 and 6000) and hydroxyethyl starch (SIGMA, MW: 2000). The final concentration of polymers in ATPS solutions ranged from 0.5 to 10% (m/v). The amount of 1 cm^3 of each polymer was used.

The protein – equine spleen ferritin (SIGMA, concentration 53.5 mg mL^{-1}) was introduced into the system to obtain a final concentration of 0.6625 mg mL^{-1}. Phases were separated and the concentration of ferritin in the top phase was analyzed.

2.2. Determining protein concentration

The concentration of protein was determined spectrophotometrically, using the absorbance measurement at wavelength 280 nm. Since it was a solution of pure protein, no reference wavelength was used. The calibration curve was prepared within the range of 0–250 μg mL^{-1}.

2.3. The model for prediction of ferritin in ATPS formed from PEG and HES

Experiments analyzing the effect of PEG and HES concentrations and PEG molecular weight were designed using the computer program Design-Experiment 7.0 (Stat-Ease, Inc., GB). Box–Behnken statistical screening design was applied to predict ferritin migration in ATPS formed from HES and PEG. The number of measurement points was 56.

2.4. The effect of magnetic field on ferritin migration in PEG–HES systems

The effect of the magnetic field on ferritin extraction was analyzed for 5 probes, which were subjected to the influence of magnetic field. These 5 systems were formed from PEG with MW = 3000 Da, and the concentration of PEG and HES was 1%, 3% or 5% (m/v). After introducing ferritin and mixing, the systems were placed for 7 h between 2 neodymium magnets N38 with a magnetic flux density of 1.21 T. Three directions of the magnetic field were applied: N/S, S/N and S/S. The scheme of the experiment is presented in Fig. 1. After 7 h of magnetic field action, the phases were separated and the concentration of the protein was analyzed in the PEG-rich phase.

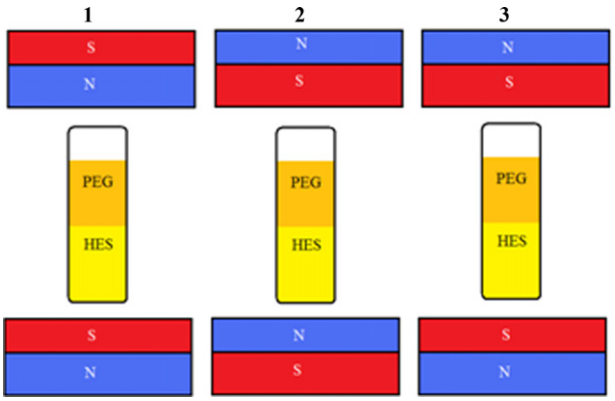

Fig. 1. A diagram of the experiment analyzing the effect of magnetic field on migration of ferritin in the PEG 3000 HES system.

2.5. Statistical analysis

Statistical analyses were performed using Design-Experiment 7.0 (Stat-Ease, Inc., GB) and Statistica 8.0 software packages (StatSoft, USA). All the data were expressed as means ± standard deviation and were subjected to regression analysis and one-way analysis of variance ($p < 0.05$). All sample preparations as well as all determinations were repeated at least three times.

3. Results and discussion

ATPS formed from polyethylene glycol and hydroxyethyl starch are quite new and have not been analyzed thoroughly. Zielińska-Dawidziak et al. [19] and Scheibe and Daniels [20] analyzed protein migration and basic properties of these systems. It was shown that the concentration of water is always higher in the PEG-rich phase and time of separation is inversely proportional to the PEG concentration.

In the presented experiment the tendency towards dilution of the PEG-rich phase causes shrinking of the HES-phase and big disproportions in volumes of analyzed phases are observed. The volume of the bottom phase was importantly reduced especially in the system obtained by mixing of HES with highly concentrated PEG 6000 (e.g. the volume ratio of the phases after separation of the system composed of 1% HES and 5% PEG 6000 was 3:97). These observations made it possible for us to limit the concentration of polymers used in the separation of ferritin to max. 6%. It also persuaded us to analyze ferritin concentration only in the PEG-rich phase.

The authors of the experiment observed before some interaction between PEG and ferritin. The interactions between PEG and proteins as well as metals were noted before. It was observed that PEG molecules in contact with some proteins acts as chaperons [21–23]. Topchieva et al. [23] suggested that PEG may be aggregated on the surface of protein and Vincentelli et al. [24] observed the influence of PEG on surface properties of some protein. Furthermore, PEG is used for metal extraction because it chelates metals acting as ether 18-crown-6 [25–27]. This interaction has a strong effect on the measurement of ferritin concentration. Determination of ferritin concentration in the PEG-rich phase was not possible with ELISA, Bradford or BCA methods, even after the precipitation of protein with the TCA method. The only method, in which these interactions were insignificant, was UV spectroscopy. Fig. 2 presents the spectrum of ferritin in 50 mM Tris–HCl buffer (pH 8.0) and in PEG. The relationship between absorption and concentration of ferritin in buffer and in 5% PEG 3000 was also independent of the dilution medium. These results convinced us to use UV spectroscopy