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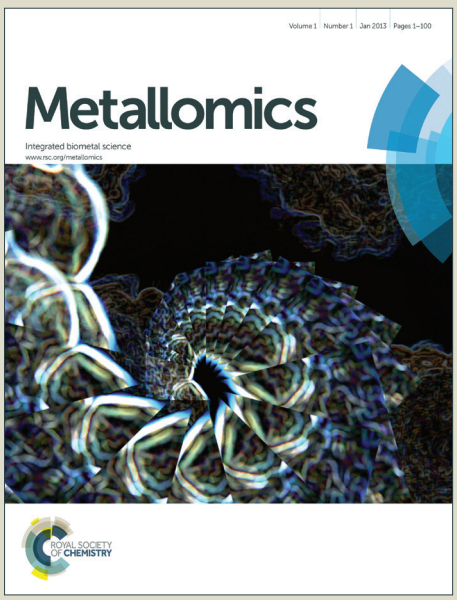

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ARTICLE TYPE

In-vitro induction and proteomics characterisation of an uranyl-protein interaction network in bovine serum

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Uranyl ions (UO_2^{2+}) were shown to interact with a number of foetal serum proteins, leading to the formation of a complex that could be isolated by ultracentrifugation. The molecular weight of the complex was estimated based on size-exclusion chromatography as 650,000 Da. Online ICP AES detection indicated that UO_2^{2+} in the complex co-eluted with minor amounts calcium and phosphorous, but not with magnesium. A 1D gel electrophoresis of the U-complex produced more than 10 bands of similar intensity compared with only 2-3 intense bands corresponding to the main serum proteins in the control serum, indicative of the specific interaction of UO_2^{2+} with minor proteins. A proteomics approach allowed for the identification of 74 proteins in the complex. Analysis of the protein-protein interaction network in the UO_2^{2+} complex identified 32 proteins responsible for protein-protein complex formation and 34 with demonstrated ion-binding function, suggesting that UO_2^{2+} stimulates the formation of protein functional networks rather than using a particular molecule as its target.

Introduction

As a consequence of an increasing number of industrial and military applications, the environmental burden of UO_2^{2+} (mostly depleted) has been steadily increasing.[1,2] Uranium shows a weak natural radioactivity, but its principal toxicity is chemical as demonstrated based on various biological models in a number of recent studies.[3,4,5,6] The most common in biological systems U species is UO_2^{2+}. Its main carrier in mammals blood serum, which is responsible for the delivery of U(IV) to different target tissues such as bone[7,8], kidney[9,10], and brain.[11,12] Therefore, the reactivity of UO_2^{2+} in serum and its interactions with molecular targets, especially proteins, are important to understand its toxicity and tissue uptake, and have been the topic of several studies.[13,14,15,16,17,18]

In serum, UO_2^{2+} ions are involved in numerous exchange reactions, leading to equilibria among low molecular species and proteins.[15] The highly soluble UO_2^{2+} carbonate complex is readily removed from an organism, which is the basis of detoxification treatments.[19] In less soluble forms, potentially in phosphate and/or protein complexes, UO_2^{2+} can be accumulated in the kidney and cause toxic effects. [9,20]

The equilibria among UO_2^{2+} low molecular weight and protein complexes depend on the concentration of carbonate and phosphate, and also on that of calcium.[14,15,21,22] The relatively insignificant differences between the binding constants of UO_2^{2+} complexes with several small ligands and proteins are responsible for the dynamics and complexity of the reactivity of UO_2^{2+} in serum.[15]

Analogies between the UO_2^{2+} and calcium ions, which both show a high affinity to phosphate, were reported in terms of biocoordination reactions and subsequent tissue distribution.[15,14] The similarity of the Ca^{2+} and UO_2^{2+} biomineralisation pathways was highlighted based on UO_2^{2+} accumulation in bones.[7,8,23,24] The formation of calcium phosphate aggregates in serum has been observed,[25] as has the formation of ternary calcium-phosphate-protein complexes that form spontaneously to inhibit the calcification of soft tissues.[25,26] Studies on the potential involvement of UO_2^{2+} in these processes led to the observation of a relatively soluble ternary Ca-UO_2^{2+}-phosphate or UO_2^{2+}-phosphate species[27,28] that could further interact with serum proteins involved in biomineralisation processes.

Studies on UO_2^{2+} interactions with proteins are typically performed in binary systems. Fluorescence spectroscopy was used to explore the stoichiometry of UO_2^{2+} binding with apotransferrin, serum albumin, metallothionein, and ferritin,[29,30] and contributed to studies on the U(VI) speciation in human serum[30]. Surface plasmon resonance (SPR) was used to determine the apparent Kd of UO_2^{2+} with the most abundant serum proteins (FETUA, CO3, A2MG, etc.).[17] Studies on the binary system UO_2^{2+} revealed the formation of a UO_2^{2+} binding pocket with carboxylic or phosphorylated residues in some proteins such as calmodulin,[31] and the possible stabilisation of UO_2^{2+} ions by a hydrogen bond.[32] The fundamental limitation of binary studies was the lack of consideration for the role of low molecular inorganic anions. A study on ternary systems including carbonate in HSA–UO_2^{2+}, HSTF-UO_2^{2+}, or FETUA-UO_2^{2+} -complex formation studies indicated a high probability of carbonate/protein ligand exchange.[30,33]

The degree of complexity of UO_2^{2+} interactions in serum

exceeds that of binary or ternary systems. At this time, most advanced studies on the molecular targets for UO_2^{2+} ions in serum were based on mimicking UO_2^{2+} interactions with multiprotein standards[18] or on the analysis of protein fractions isolated from serum based on their affinity to surface immobilised UO_2^{2+}.[16]

It was shown that principal UO_2^{2+} ion anchoring motifs on the protein side chains include carboxylic[15,34] and phosphorylated groups,[31] and the complexes were incorporated into the bone.[35] Similar reactions can potentially occur in serum, although the calcification process occurs spontaneously and involves some biomineralisation inhibitors such as albumin or fetuin.[25,26] The substitution of Ca^{2+} by UO_2^{2+} has been explored in previous studies,[36] and is a potential explanation for the incorporation of UO_2^{2+} into Ca^{2+}-dependent steps[37] of the coagulation cascade.

Studies performed to date have probed interactions with proteins after being separated from the biological matrix. The diversity of literature data and different facets of UO_2^{2+} interactions indicate that these interactions are more complex than those involving a single protein or one particular ligand in a binary or ternary system.

The objective of this work was to explore interactions of UO_2^{2+} with serum proteins on a global scale including the whole serum proteome and carbonate and phosphate species, and to detect and characterise UO_2^{2+}-dependent multiprotein systems in serum. For this purpose, the concentration of UO_2^{2+} was increased to a point a U-protein complex could be isolated by chromatography and characterized.

Experimental

Sample preparation

Foetal bovine serum with two different LOT numbers was received from Gibco, (Gibco, Saint Aubin, France). The reported Ca^{2+} concentration for FBS is 14.7 mg/dl.[38] Because different sera with different lot numbers can differ in terms of the protein level, experiments were performed with three different lots.[39]

The protocol used to isolate UO_2^{2+}-dependent proteins in serum was based on that used for calcium phosphate-dependent proteins described previously.[40] A serum enriched with UO_2^{2+} phosphate/carbonate species were prepared accordingly. A specific amount of $UO_2(NO_3)_2{\times}6H_2O$ was mixed with Na_2CO_3 to reach a final stoichiometry of 1 : 2. An aliquot of this solution was added to 0.5 ml of serum. A further 0.5-ml aliquot of serum was mixed with Na_3PO_4 solution (pH 7.4 set with conc. HCl). Both serum aliquots were immediately combined. The final concentrations of UO_2^{2+} and phosphate species were 1 mM and 2.0 mM, respectively. All steps were performed at 4°C with attention to avoid degassing. To obtain calcium enriched serum the procedure described elsewhere was followed.[40] The final concentrations of Ca^{2+} and phosphate were 1 mM and 2.0 mM, respectively. Control serum was prepared by mixing one 0.5-ml aliquot of serum with $NaNO_3$ and another one with Na_3PO_4 (pH 7.4 set with conc. HCl). The final concentrations of added $NaNO_3$ and Na_3PO_4 were 2.0 and 2.0 mM. After combining both serum aliquots, samples were incubated at 37°C in 5.5% CO_2 for 3 h and analysed immediately. All inorganic reagents were purchased from Sigma Aldrich (Lyon, France).

Chromatography

Size-exclusion chromatography was performed using an Agilent 1200 system with the Superdex 200TM 10/30 column. The eluent was 50 mM 2-amino-2-hydroxymethyl-propane-1,3-diol in 0.1 M NaCl at pH 7.4 at 0.5 ml/min. Elution was monitored at 280 nm based on inductively coupled plasma emission spectrometry (ICP-AES). An ICP-AES model SPECTRO (Kleve, Germany) instrument fitted with a Sea-spray nebuliser and a cyclonic spray chamber was used. The conditions were: power 1450 W, coolant argon gas flow 12/min, and auxiliary argon gas flow 1 l/min. Ca (315.89, 317.93), Mg (279.55, 280.27, 285.21), P (177.49, 178.29), and U (385.96, 409.14) were monitored.

Gel electrophoresis

SDS-polyacrylamide gel electrophoresis of the UO_2^{2+}-enriched serum fraction was performed using NuPAGE Novex Bis-Tris Midi Gels (Life Technologies, SAINT AUBIN, France). Samples were prepared according to the gel supplier. Collected solutions were mixed with the sample buffer (NuPAGE sample buffer, NP0008) and the reducing agent (NuPAGE Sample Reducing Agent, NP0004), and were heated for 10 min at 70^0 C. The gradient (4-12%) pre-cast polyacrylamide gels were run using a NuPAGE MOPS (3-(N-morpholino)propanesulphonic acid) SDS running buffer system. The process was performed at a voltage up to 80 V. The gels were stained overnight in a Page Blue Protein Staining Solution (Thermo Scientific).

Protein identification

Protein identification was performed using nanoLC-ESI-MS/MS at Proteome Factory (Berlin, Germany). The MS system consisted of an Agilent 1100 nanoLC system (Agilent, Waldbronn, Germany), PicoTip electrospray emitter (New Objective, Woburn, MA), and an Orbitrap XL or LTQ-FT Ultra mass spectrometer (ThermoFisher, Bremen, Germany). Protein bands were in-gel digested by trypsin (Promega, Mannheim, Germany) and applied to nanoHPLC-ESI-MS/MS. Peptides were trapped and desalted on the enrichment column (Zorbax SB C18, 0.3 × 5-mm, Agilent) for 5 min using 2.5% acetonitrile/0.5% formic acid as eluent. The peptides were separated on a Zorbax 300 SB C18, 75 µm × 150-mm column (Agilent) using an acetonitrile/0.1% formic acid gradient from 5% to 32% acetonitrile. MS/MS spectra were recorded in data-dependent mode according to the manufacturer's recommendations. Proteins were identified using MS/MS ion search of the Mascot search engine (Matrix Science, London, England) and nr protein database (National Center for Biotechnology Information, Bethesda, MD). The ion charge in search parameters for ions from ESI-MS/MS data acquisition were set to "1+ 2+ or 3+" according to the instrument's and method's common charge state distribution.

Protein characterisation

Protein characterisation was performed using STRING v10 (Search Tool for Retrieval of Interacting Genes/Proteins, http://string-db.org/) software based on the functional association network principle. Information regarding the interactions was derived from different sources including experimental interactions, pathway knowledge obtained from manually curated

databases, text-mining based on abstracts and full-text articles, de novo interaction prediction based on the genomic information, and co-expression analysis.[41] The statistical enrichment functions provided the protein's functional annotations from the Gene Ontology (GO), KEGG, PFAM, and INTERPRO databases. Identified proteins were characterised based on the GO enrichment tool for biological processes and molecular functions, and the KEGG pathway was manually curated for enrichment. Co-occurrence, co-expression, experiments, and database prediction methods were applied for PPI characterisation.

Results and Discussion

The gist of the experiment was based on the protocol described elsewhere in order to find Ca^{2+} phosphate species dependent proteins e.g. fetuin or albumin.[40] It consisted in increasing serum Ca concentration to 5 mM[40] at which it became possible to observe a high molecular mass complex with calcium phosphate species in serum.[34] We justified the use of this approach for U because of the "bioinorganic" similarity of UO_2^{2+} to Ca^{2+} corroborated by the similarity of proteins evoked as targets for both metal ions, e.g. albumin or fetuin. As U, even in toxic conditions, is not as ubiquitous as Ca, it was decided to work with concentrations a factor of 10 lower, the lowest, i.e. 0.5 mM at which the formation of a macromolecular complex appeared. Such a concentration may still appear high but concentrations up to 1mM of UO_2^{2+} were frequently applied in case of cell culture incubation for biochemical assays.[28,42–45] Immobilized affinity chromatography (IMAC) also required similar concentrations (>100 mmol/L of beads).[16,46]

The proposed approach can not only reduce the amount of UO_2^{2+} added but also allow a longer incubation of proteins with UO_2^{2+} protected from the hydrolysis by an excess of carbonate or/and phosphate species.

Effect of UO_2^{2+} on the protein molecular weight distribution in serum: comparison with calcium

Size-exclusion chromatography (Fig. 1) shows that the addition of UO_2^{2+} leads to the formation of an additional intense peak eluting close to the exclusion volume with an approximate molecular weight of 660,000 Da. There is observed difference between a marker at 2,000,000 and this one which means the elution volume although close to the complex cannot be assimilated with it. The formation of this peak does not result in the modification of the chromatogram morphology compared with that of the control serum, which indicates that the major serum containing proteins (albumin, transferrin, and fetuin) do not play an important role in the interaction with UO_2^{2+} species.

The increase in the sum of the peak areas in the UV chromatogram upon the addition of UO_2^{2+} ions is a result of absorption of UO_2^{2+} complexes at 280 nm. Note that formation of high molecular weight complex is interesting as proteins are picked up selectively from the foetal serum as demonstrated by the comparison of the gel electropherograms discussed below. Note thatn SEC-ICP MS of a serum sample with the baseline U concentrations does not show the formation of such a complexes.

Interestingly, a similar peak is not induced in the presence of Ca. Indeed, the morphology of the chromatogram of serum doped with calcium is identical to that of the control serum (Fig. 1).

The elemental composition of the formed compound was studied under the same chromatographic conditions using SEC with ICP-AES detection. Fig. 2 demonstrates the presence of the large majority of UO_2^{2+} (91% of U) in this peak co-eluting with phosphorous and calcium, but not with magnesium. The second observed U signal around 6% of total U correlates, in terms of the elution volume, with the U-fetuin complex reported in the literature.[17,33] The presence of the minor peaks (Fig. 2a) shows that traces of UO_2^{2+} may be bound to the major serum proteins, but this is not a major interaction. The molar ratio UO_2^{2+} : phosphate species was set up to 1 : 2 by addition of phosphate to incubated foetal serum system in order to avoid unspecific reaction of UO_2^{2+} present only in carbonate complex. SEC-ICP-MS chromatograms show that P coelutes with U in the large molecular weight complex (Fig. 2). Uranyl ions not protected as carbonate/phosphate species added to serum as nitrate immediately cause unspecific precipitation.

Total U recovery from the spiked serum in these experimental conditions was 60 - 80%. As UO_2^{2+} ions are retained by the gel surface, such a high recovery indicates the high stability of the U-protein complex formed.

Characterisation of the UO_2^{2+}-induced compound

The uranium-containing compound, which was thought to be a UO_2^{2+}-protein complex (note that both UO_2^{2+} species and proteins absorb UV light at 280 nm), was separated by ultracentrifugation. This process leads to the formation of a yellow-transparent pellet (Fig. 3ab) with a gel-like consistency. Such a pellet was not observed during ultracentrifugation of control serum (Fig. 3c). SDS-PAGE analysis of the pellet showed that it contained a considerable amount and variety of proteins (Fig. 3d). Note that the formed gel was fairly unstable and spontaneously solubilised after five hours in the supernatant. A comparison of the electropherogram (Fig. 3d) with that of the control serum (Fig. 3e) indicated that a specific protein enrichment process was triggered by UO_2^{2+} addition rather than a random protein precipitation. Indeed, the major serum proteins (even if present in the U-protein complex) are not more abundant than other proteins detected.

A comparison of gel electropherograms of UO_2^{2+} enrich fraction from two sera systems from different batches showed that protein pattern is virtually identical in both samples (Fig. S1)

Almost half of the proteins isolated elsewhere on the basis of the affinity to immobilized UO_2^{2+} supplied at more than 100 mmol/L of beads[16] were identified here (Fig. S2ab). However, a comparison of protein distribution pattern in the U-rich fraction from foetal and bovine (adult) serum differ significantly (Fig. S2c).

To characterise the protein composition, the fraction corresponding to the U peak (Fig. 4a) apex was collected and subjected to SDS-PAGE (Fig. 4b,c). No proteins were detected in control serum in the same fraction. In the case of the UO_2^{2+}-doped serum, 14 protein bands ranging from 10 kDa to 250 kDa could be observed. The proteomics approach allowed for the identification of 74 proteins, for which at least four peptides were identified after tryptic digestion and nanoHPLC-MS/MS (Tab. S1). The identified proteins are summarised in Fig. 4. In some

cases, the position in the gel of identified proteins does not correlate with the determined molecular weight. This could be the reason of (i) residual proteolytic activity that results in the observation of fragments of proteins in the gel that are interpreted as intact proteins, and (ii) the presence of UO_2^{2+} which may not allow the complete denaturation and thus presence at higher molecular masses. These caveats do not alter the sense of the qualitative composition of the U complex which is the gist of the manuscript. The detailed characteristics, molecular functions, and biological processes related to these proteins, with a particular emphasis on coagulation and biomineralisation, are highlighted in Table 1.

Table 1. The results of analysis of identified proteins in prism of biological processes KEGG pathways and molecular function by STRING program.

Biological processes

Term	Number	p-val.
Response to stress	26	$1.22 \cdot 10^{-7}$
Regulation of protein metabolic process	25	$9.67 \cdot 10^{-8}$
Regulation of proteolysis	24	$1.69 \cdot 10^{-17}$
Regulation of hydrolase activity	23	$1.91 \cdot 10^{-23}$
Blood coagulation	15	$3.62 \cdot 10^{-17}$

Molecular function

Term	Number	p-val.
Ion binding	34	$7.8 \cdot 10^{-3}$
Protein binding	32	$6.42 \cdot 10^{-3}$
Cation binding	21	$3.25 \cdot 10^{-1}$
Anion binding	20	$1.57 \cdot 10^{-2}$
Enzyme regulator activity	21	$2.54 \cdot 10^{-13}$
Peptidase regulator activity	19	$3.77 \cdot 10^{-19}$
Heparin binding	8	$1.08 \cdot 10^{-6}$

KEGG pathways

Term	Number	p-val.
Complement and coagulation cascades	26	$4.59 \cdot 10^{-45}$
Staphylococcus aureus infection	7	$1.36 \cdot 10^{-7}$
ECM-receptor interaction	5	$4.7 \cdot 10^{-4}$

The identification of several identical proteins within the different bands (e.g. ITIH1, ITIH3, CO3, ALBU, etc.) may be explained in several ways. Primarily, the amount of proteins in the analysed lane was very high, which could result in their overlapping with each other, as well as the formation of stable protein-protein complexes that survived denaturation conditions. The identification of high-MW proteins in the bands corresponding to a low MW may be the result of protein denaturation of a small set of peptides, which allowed for their further migration in gel. The proteins found in several bands are also known to be abundant in serum, and may prevent other proteins from being separated properly according to their masses. It remains unknown how the UO_2^{2+} ions influence protein separation in the SDS environment.

In summary, the U-protein complex contains a protein network as a ligand, which is discussed further in terms of protein-protein interactions.

Characterisation of the UO_2^{2+} protein ligands in terms of protein-protein interactions

The obtained data indicate the importance of a protein network as a UO_2^{2+}-binding ligand(s) rather than individual proteins, as postulated in previous studies. Therefore, proteins identified in the UO_2^{2+} protein complex were investigated in terms of molecular and biological functions, including metal-binding affinities, involvement in coagulation, mineralisation, and complement activation cascades using a tool to study protein-protein interactions (STRING). Data analysis showed a network rich in interactions (observed 144, expected 91) (Fig. 5).

Table 1 focuses on the most important molecular and biological protein-protein interactions, and shows the presence of 32 proteins (Fig. S3a) responsible for protein binding, explaining the formation of high-molecular-weight complexes. The ion-binding function was recognised for 34 proteins including 20 for anions and 21 for cations (Fig. S3b). This finding is in good agreement with previous studies on the mechanism that UO_2^{2+} could be bound to proteins as a metal cation, but also in an anionic complex.[15] To better characterise the metal binding function of these proteins, the UniProt database was searched for other metals bound by the identified proteins. The results shown in Fig. 5 indicate that large number of the proteins found were mentioned previously in the context of binding calcium. Interactions of UO_2^{2+} with haemoglobin (a known Fe-binding protein) was also reported previously.[47]

Table 1 shows the impact of UO_2^{2+} on heparin binding proteins (eight proteins) (Fig. S3c). In terms of biological processes, molecular functions, and Kyoto Encyclopedia of Genes and Genomes pathway (KEGG),[48] 26 of the observed proteins are responsible for response to stress, as well as blood coagulation processes (15 proteins; Fig. S3de, respectively). On the one hand, UO_2^{2+} ions may impact the coagulation cascade by interfering with Ca^{2+} ions due to the similar biocoordination properties. It was shown that UO_2^{2+} can substitute for the Ca^{2+} ions in native proteins.[36] On the other hand, it is possible that UO_2^{2+} ions impact the cascade through some "key" ligands, such as heparin. Interestingly, a large group of proteins were shown to be involved in regulating protein processing (25 proteins; Fig. S3f), such as

regulation of proteolysis (24 proteins) and hydrolase activity (23 proteins).

KEGG pathways analysis indicated that 26 proteins were involved in the complement and the coagulation cascades (Fig. S3g). The molecular impact of UO_2^{2+} on this process could be explained by the impact on the GLA domains. These domains act as natural Ca^{2+} binding motifs[49] of coagulation; namely, F2, PROC, and F10. Previous reports suggest that calcium could be substituted by UO_2^{2+} [36] or Mg.[50]

Conclusions

While most of the in-vitro studies concerned isolated proteins, the described case addresses for the first time U-protein interactions in serum in their globality, including protein-UO_2^{2+}-protein complex interactions, UO_2^{2+}, added to foetal serum simultaneously with carbonate and phosphate protecting it from rapid unspecific reaction with proteins, activates a protein network. The interaction results in the formation of a poorly soluble complex that can be separated by centrifugation and accounts for more than 95% of the UO_2^{2+} present. This behaviour of UO_2^{2+} seems to be unique since Ca^{2+} supplemented in same concentrations does not form such a complex. A number of proteins involved in the reaction with calcium ions suggest that UO_2^{2+} ions affect Ca^{2+}-dependent processes in serum.

Demonstrated strategy illustrate possibility to isolate the UO_2^{2+} dependent proteins starting from a very complex biological medium, such as serum. Proteins were isolated not only based on the affinity to UO_2^{2+} species to single proteins but also potentially based on affinity of these ions to serum protein complexes.

Analysis of the protein network using STRING indicates that several serum processes may be affected, such as response to stress, blood coagulation processes, and complement and coagulation cascade.

Presented UO_2^{2+} dependent proteins isolation method is a interesting alternative to IMAC due to the fact that equilibrium between the serum proteins and UO_2^{2+} carbonate and phosphate species is more close to native serum processes that interactions of proteins with immobilised UO_2^{2+} on surface in much higher concentrations during the IMAC. Although the formation of the complex was demonstrated for bovine (foetal and adult) sera only, a similar reaction is likely to be observed with sera of other organisms.

Disclosure

The authors report no conflicts of interest.

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Figure captions

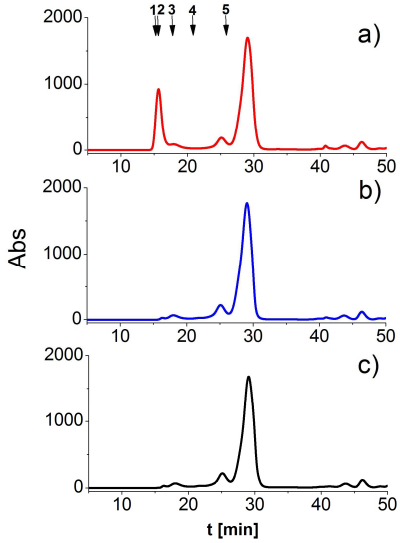
Figure 1. Effect of UO_2^{2+} and calcium on the protein molecular weight distribution in serum. Size-exclusion LC chromatography (UV detection) of: a) serum with UO_2^{2+} (1.0 mM), b) serum with calcium (1.0 mM), c) control serum. MW standards were marked with numbers: 1) BlueDextran 2000, 2) 669 kDa, 3) 440 kDa, 4) 158 kDa, and 5) 75 kDa.

Figure 2. Size-exclusion LC-ICP AES of serum incubated with UO_2^{2+} (red) and control serum (black): a) uranium (385.96 nm); b) phosphorous (177.49 nm); c) calcium (315.89 nm), and d) Mg (279.55 nm). MW standards were marked with numbers: 1) BlueDextran 2000, 2) 669 kDa, 3) 440 kDa, 4) 158 kDa, and 5) 75 kDa.

Figure 3. Formation of a pellet during centrifugation (12,000 rpm at 20°C) of serum incubated with: a) UO_2^{2+} species (after 180 min), b) higher-magnification view of the pellet; c) control serum; d) SDS electropherogram of the pellet (washed with 0.15 M NaCl at 4°C); e) SDS electropherogram of control serum (the serum was diluted until the intensity of thee albumin bands reached that in Fig. 3d).

Figure 4. a) Zoom of the SEC molecular weight range showing the UO_2^{2+} - protein complex fraction heartcut (shadowed) for proteomics analysis (1 and 2 present molecular markers BlueDextran 2000 and 669 kDa, respectively). Panel: b) UV-Vis chromatograms for control (grey) and UO_2^{2+}-doped serum (red): c) ICP AES chromatograms of U (385.96 nm), P (177.49 nm), and Ca (315.89 nm) (panels c), d) and e), respectively). F) SDS-PAGE of the heartcut fraction. Left: M_w markers, middle: control serum, right: UO_2^{2+}-doped serum. List of proteins identified using the proteomics approach and assigned to respective bands. A detailed description of the detected proteins is provided in the Supplementary Information (Table S1).

Figure 5. STRING mapping profiles of protein interaction network identified in complex with UO_2^{2+} species in foetal serum. Protein marked with circle are known for metal ions binding, based on information from UNIPROT database (http://www.uniprot.org). Symbol M^{n+} shown proteins known for interactions with variety of metal ions.



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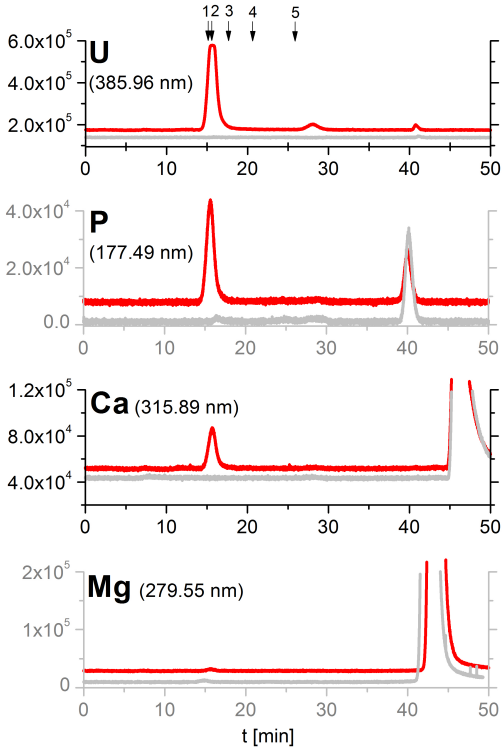

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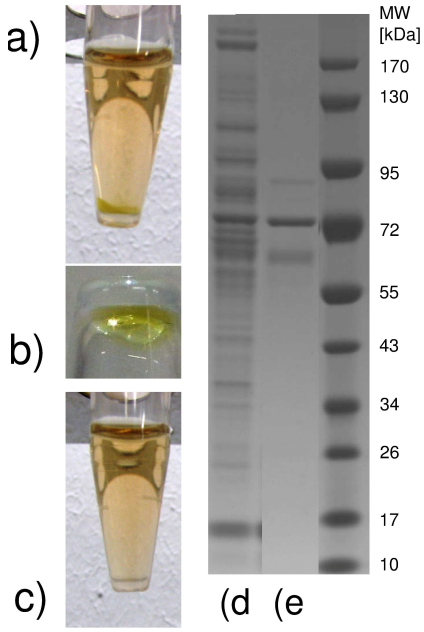

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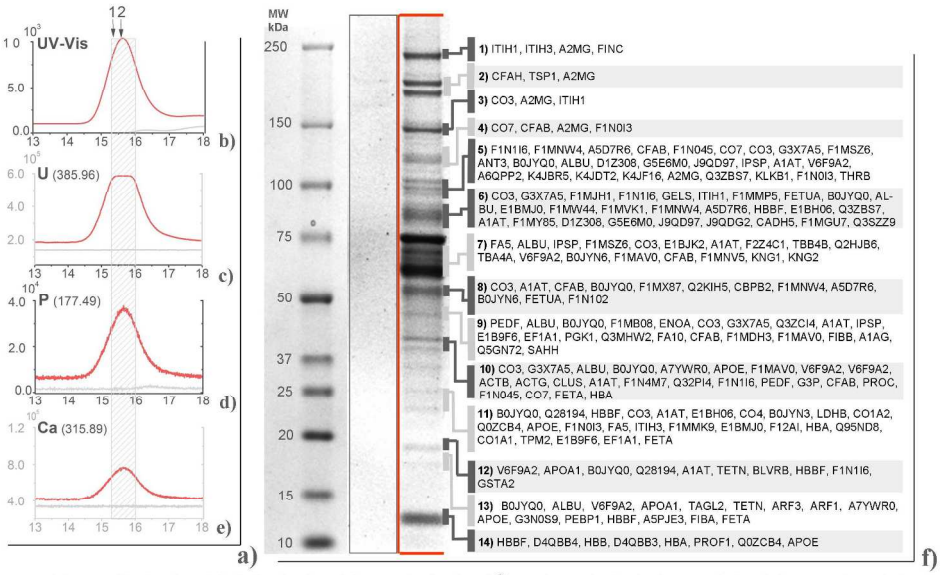

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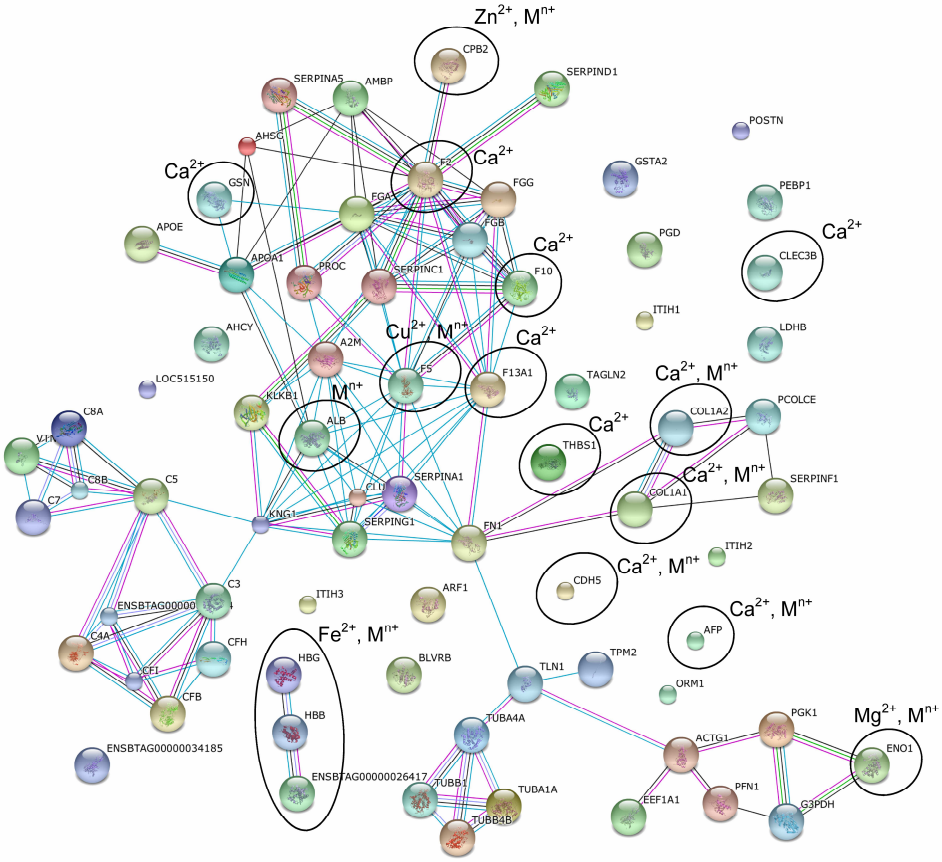

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