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A novel organic-inorganic hybrid polyoxometalate for the selective adsorption/isolation of β-lactoglobulin

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A novel polyoxometalate (POM)-based organic-inorganic hybrid $[C_{33}H_{24}O_4]H_3PMo_{12}O_{40}$, namely TPPA-$PMo_{12}$, is prepared via one-pot hydrothermal reaction between Keggin POM ($H_3PMo_{12}O_{40}$, PMo_{12}) and star-like N-donor ligand (tri(4-pyridylphenyl) amine, TPPA). The hybrid polyoxometalate is confirmed by characterizations with XRD, FT-IR, TGA, SEM and EDS. It exhibits excellent adsorption performance towards β-lactoglobulin, and thus a solid-phase extraction procedure is established for the efficient and selective isolation of β-lactoglobulin from complex sample matrices. At pH 5.0, an adsorption efficiency of 99.2% is achieved for processing 100 μg mL^{-1} β-lactoglobulin in 1.0 mL aqueous solution with 0.5 mg TPPA-PMo_{12} as adsorbent. The adsorption behavior of β-lactoglobulin fits Langmuir model, corresponding to a theoretical adsorption capacity of 1428 mg g^{-1}. The retained β-lactoglobulin could be readily recovered by 0.05 mol L^{-1} Tris-HCl buffer, giving rise to a recovery of 91.5%. The hybrid polyoxometalate is practically applied to the selective isolation of β-lactoglobulin from milk whey, and SDS-PAGE assay results clearly indicate that β-lactoglobulin of high-purity is obtained.

1. Introduction

Polyoxometalates (POMs) are classic metal-oxygen clusters comprising of large diversities of nano-structures[1,2]. Due to their versatile physical and chemical properties, POMs have attracted much interest and gained extensively applications in various fields including catalysis[3], electricity and magnetism[4], photochromism[5], biology[6] and medicine[7]. POMs have also been used as anticancer and anti-viral agents[8] as they can selectively bind to the positive regions of proteins, which lead to increasing research interest on the molecular interactions between different POMs and protein species[9,10]. It is reported that POMs ($Na_2H[PW_{12}O_{40}]$) is able to selectively precipitate the prion protein PrP 27-30, and the negative charge of Keggin-type POMs pose decisive effect on the quaternary structure of PrP 27-30[11]. The Anderson-Evans-type POMs $Na_6[TeW_6O_{24}]\cdot 22H_2O$ (TEW) has been employed as additive for the crystallization of lysozyme, wherein the negatively charged TEW polyoxotungstate binds to sites with a positive electrostatic potential located between two (or more) symmetry-related protein chains[12]. The molybdenum storage protein (MoSto) is also reported to covalently interact with octamolybdate ($[Mo_8O_{26}]^{4-}$) owing to the strong coordination capability of Mo atom in the octamolybdate towards the $N_{\varepsilon 2}$ nitrogen of histidine (His156) and the $O_{\varepsilon 1}$ oxygen of glutamic acid (Glu129) in MoSto[13].

The well-defined size and layered structure of POMs make then excellent inorganic building blocks for the fabrication of novel multi-dimensional frameworks by rational regulation with suitable inorganic or organic components[14,15], breeding the studies on inorganic-organic hybrids an emerging multidisciplinary research field focus on POM chemistry[16]. In the last two decades, a large number of POMs-based inorganic-organic hybrids have been reported[17,18]. Most of them are obtained by means of electrostatic interactions, hydrogen bonds and van der Waals interactions between the organic and inorganic components[19]. The POMs-based hybrids, e.g., $(Mb)_4[PMo_{12}O_{40}]\cdot 2DMF$, $(Mb)_4[GeMo_{12}O_{40}]\cdot 2DMF$ and $(Mb)_5[S_2Mo^{IV}Mo_{17}{}^{VI}O_{62}]CH_3CN$, are demonstrated to be very stable and exhibit excellent electro-catalytic activities toward the reduction of chlorate, bromate, nitrite and hydrogen peroxide[20,21]. Amino acids including proline, glycine, leucine and asparagine have been combined with POMs to produce POMs-amino acid hybrids with promising biological activities, and these hybrids show enhanced targeting inhibition effect on amyloid-peptide aggregation associated with Alzheimer's disease[22]. The preparation of flowerlike hierarchical nanostructures have been reported by the co-assembly of dopamine and phosphotungstic acid, which exhibit great potential in drug-delivery[23]. Most recently, POMs-based hybrids prepared via strong covalent coordination between the organic and inorganic moieties are also reported, including POMs-based metal organic frameworks (POMOFs), exhibit appealing structural motifs with improved properties[24,25]. It is known that the N-donor ligands are crucial for the design and synthesis of inorganic-organic hybrids[26]. Compared with the benzene-centered triangular ligands, N-centered ligands such

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as tri(4-pyridylphenyl) amine (TPPA) are more "ductile" in tailoring to the shape of metal cations[27-29].

β-lactoglobulin (β-Lg) is a major milk allergen in bovine milk[30,31], the removal/isolation of β-Lg is frequently required in the dairy manufacturing process, where ion exchange, ultra-filtration and selective precipitation are among the common methodologies[32]. In the present work, we prepare a novel organic-inorganic hybrid polyoxometalate (TPPA-PMo_{12}) via hydrothermal reaction between Keggin-type POM ($H_3PMo_{12}O_{40}$, PMo_{12}) and the star-like N-donor ligand TPPA. The obtained TPPA-PMo_{12} hybrid exhibits favorable adsorption selectivity towards β-lactoglobulin. In practical application, the isolation of β-lactoglobulin from bovine milk whey is achieved by using the obtained TPPA-PMo_{12} hybrid as adsorbent.

2. Experimental

2.1 Materials and reagents

All the chemical used in the present study are at least of analytical reagent grade unless otherwise specified. Bovine serum albumin (BSA, pI 4.90, Mr 66.0 kDa; A1933, ≥98%), β-lactoglobulin from bovine milk (β-Lg, pI 5.30, Mr 18.4 kDa; L39008, ≥90%), and α-lactalbumin from bovine milk (α-La, pI 4.50, Mr 14.2 kDa; L5385, ≥85%), tris(hydroxymethyl) aminomethane (Tris, >99.9%), glycine ($C_2H_5NO_2$; ≥99%), N,N,N′,N′-tetramethylethylenediamine (TEMED, 99%), ammonium persulphate (APS, 98%), acrylamide N,N′-Methylene-bis-acrylamide, acetonitrile (ACN, ≥99.9%), trifluoroaceticacid (TFA, ≥99.9%), and formicacid (FA, ≥99.9%) are purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. The protein molecular weight marker (low, 3595A, Takara Biotechnology Co., Ltd., Dalian, China) is a mixture of six purified proteins (phosphorylase b, Mr 97.2 kDa; serum albumin, Mr 66.4 kDa; ovalbumin, Mr 44.3 kDa; carbonic anhydrase, Mr 29.0 kDa; trypsin inhibitor, Mr 20.1 kDa; lysozyme, Mr 14.3 kDa). Tri(4-bromophenyl) amine ($C_{18}H_{12}Br_3N$, 98%), pyridine-4-boronic acid ($C_5H_8BNO_2$, 98%), and tetrakis(triphenylphosphine) pallasium(0) ($Pd(pph_3)_4$, 99.8%) are obtained from J&K Scientific Co. Ltd. (Beijing, China). Keggin-type structure phosphomolybdic acid $H_3PMo_{12}O_{40}{\cdot}xH_2O$ (PMo_{12}), Coomassie Brilliant Blue G-250, K_2CO_3, $CuCl_2{\cdot}H_2O$, HCl, H_3PO_4, HAc, H_3BO_3, NaCl, NaOH, KH_2PO_4, Na_2HPO_4, and sodium dodecyl sulphate (SDS) are acquired from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). N-dimethylacetamide (DMAC), dichloromethane, ethyl acetate, n-hexane, methylalcohol, and ethanol (Bodi Chemical Holding Co., Ltd., Tianjin, China) are used as received. Deionized water (ddH_2O) of 18 MΩ cm is used throughout. Raw bovine milk is obtained from a local dairy farm.

2.2 Instrumentation

Elemental analysis is carried out on a LECO CS230 infrared carbon-sulfur analyzer (LECO, USA) and a Optima 8300DV ICP-OES spectrometer (PerkinElmer, USA). FT-IR spectra are recorded on a Nicolet 6700 spectrometer (Thermo Electron, USA) using a KBr disk from 400 to 4000 cm^{-1} with a resolution of 2.0 cm^{-1}. XRD patterns are taken on a Rigaku D/max-a X-ray diffractometer (Rigaku, Japan) with graphite-mono-chromatized Cu-Kα radiation (k=1.54056 Å), with a step size of 0.03°. The thermal stability of the product is analyzed by using a TG-DSC simultaneous thermal analyzer (Mettler Toledc, Switzerland) in a temperature range of 25-1000℃ in oxygen atmosphere by heating at 10℃ min^{-1}. SEM images are taken on a ZEISS Ultra/Plus scanning electron microscope (ZEISS, Germany) and energy dispersive spectrometer (EDS) analysis results are obtained by Oxford instrument X-Max^N 50 (Oxford Instrument, England). U-3900 UV-vis spectrophotometer (Hitachi, Japan) with a 1.0 cm quartz cell is used for the quantitative detection of protein. Circular dichroism (CD) spectra in the wavelength range of 200-260 nm are obtained on a MOS-450 automatic recording spectropolarimeter (Bio-Logic, France) at 293K, with nitrogen protection by using 1-mm cell with a scan rate of 0.5 s nm^{-1}. An Agilent 6540 UHD Accurate-Mass Q-TOF LC/MS mass spectrometer (Agilent Technologies) equipped with an orthogonal ESI source is used in the positive ionization mode for protein identification. A PB-10 pH Meter (Beijing Sartorius Instruments Co., Ltd., China) is used for pH measurement.

2.3 Preparation of the TPPA-PMo_{12} hybrid

TPPA is firstly prepared as following: tris(4-bromophenyl) amine (482 mg, 1 mmol), pyridin-4-boronic acid (369 mg, 3 mmol), potassium carbonate (4.14 g, 30 mmol), $[Pd(PPh_3)_4]$ (5.8 mg, 0.005 mmol), N,N-dimethylacetamide (70 mL) and ddH_2O (20 mL) are mixed together and the mixture is refluxed under nitrogen protection for 10h. Thereafter the mixture is extracted by dichloromethane and the extract is evaporated under reduced pressure. After purification with column chromatography on silica gel (n-hexane/EtOAc, 1:3 v/v) a yellow solid product of TPPA is achieved[33].

A mixture of $CuCl_2{\cdot}H_2O$ (0.085 g, 0.5 mmol), TPPA (0.02g, 0.05 mmol), $H_3PMo_{12}O_{40}$ (0.009 g, 0.05 mmol), and ddH_2O (8 mL) is then sealed into a 15-mL Teflon-lined stainless steel autoclave and heated at 180℃ for 7 days. After cooling to room temperature, a brown solid product of TPPA-PMo_{12} hybrid is collected.

2.4 Adsorption/desorption of proteins by TPPA-PMo_{12} hybrid

The adsorption behaviors of the main protein species in bovine milk whey, i.e., α-La, β-Lg and BSA, onto the TPPA-PMo_{12} hybrid are studied. pH value of the protein sample solutions are controlled by Britton-Robinson (BR) buffer within a range of 5.0-9.0.

In general, 1.0 mL of protein sample solution is mixed with 0.5 mg of the TPPA-PMo_{12} hybrid and the mixture is shaken vigorously for 30 min to facilitate the adsorption of protein species. After centrifugation at 8,000 rpm for 3 min, the supernatant is collected to quantify the residual protein content by monitoring the absorbance at 595 nm after binding with the Coomassie Brilliant Blue (Bradford method). The adsorption efficiency (*E*) is calculated by the following

equation, where C_0 and C_1 represent the original and the residual protein concentrations, respectively.

$$E = \frac{(C_0 - C_1)}{C_0} \times 100\%$$

After adsorption, the TPPA-PMo_{12} hybrid is pre-washed with 1.0 mL of ddH_2O to remove any loosely retained species on the surface of the adsorbent. Afterwards, 1.0 mL of 0.05 mol L^{-1} Tris-HCl buffer at pH 8.0 is added and the mixture is oscillated for 10 min to strip the adsorbed protein from the surface of the TPPA-PMo_{12}. The supernatant after centrifugation at 8,000 rpm for 3 min is collected for evaluating the elution efficiency or for ensuing investigations.

2.5 Protein identification with HPLC-MS after processing by TPPA-PMo_{12} hybrid

For HPLC-MS analysis, nitrogen is used as sheath gas and drying gas with flow rates of 10 L min^{-1} and 8 L min^{-1}, and their temperatures are set to 350℃ and 200℃, along with a nebulizer pressure of 35 KPa. The capillary and nozzle voltages are set at 2.5 kV and 1.25 kV, respectively. The fragmentor and skimmer voltages are 175 V and 65 V, respectively. The flowing liquid solvent is a mixture of ACN/H_2O (70:30, v/v) containing 0.1% trifluoroacetic acid. It is delivered at 0.1 mL min^{-1} by a 1260 infinity series isocratic pump (Agilent Technologies).

2.6 Sample pretreatment of bovine milk sample

The fresh bovine milk is centrifuged at 5,000 rpm for 30 min at room temperature and then adjusted to pH 4.6 with diluted HCl. The obtained solution is kept at 35℃ and stirred for 50 min to precipitate casein. Milk whey is thereafter obtained by removing casein and milk fat globules by centrifugation for further 30 min at 5,000 rpm, followed by filtration with 0.45 μm microfiltration membrane

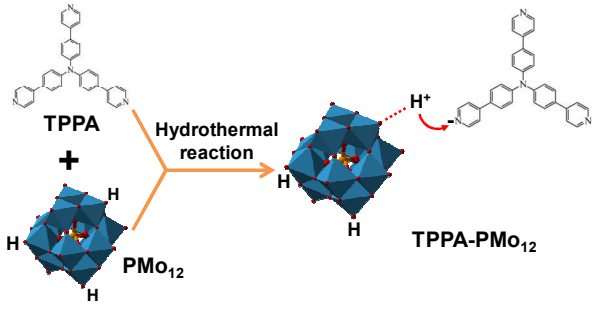
3 Results and Discussion

3.1 Characterization of the TPPA-PMo_{12} hybrid

The POM-based organic-inorganic hybrid is prepared via hydrothermal reaction of Keggin-type PMo_{12} and N-donor ligand TPPA. As is known that PMo_{12} is a soluble polyoxometalate and its anionic moiety facilitates its association with protonated organic ligand TPPA through directional hydrogen bonds (N–H···O) to produce insoluble compounds, which further stack into the layered hybrid driven by non-covalent interactions between oxygen atoms of PMo_{12} and pyridine groups of TPPA[34].

FT-IR spectra of the TPPA-PMo_{12} hybrid, TPPA and PMo_{12} are shown in Figure 1A. The characteristic absorption bands of Keggin structure[35], i.e., υ_{as}(P-O_a) at 1062 cm^{-1}, υ_{as}(Mo-O_d) at 958 cm^{-1}, υ_{as}(Mo-O_b-Mo) at 876 cm^{-1} and υ_{as}(Mo-O_c-Mo) at 797 cm^{-1} are all clearly identified in the spectrum of TPPA-PMo_{12} hybrid. At the same time, the featured bands of pyridyl and phenyl are also observed, wherein the absorptions within 1600-1450 cm^{-1} are assigned to aromatic ring skeleton vibrations, and those within 1330 to 1200 cm^{-1} are attributed to the deformation vibrations of the C-H and C-N bonds[12]. These observations well demonstrate the presence of TPPA and PMo_{12} in the final product of the TPPA-PMo_{12} hybrid.

In Figure 1B, the Bragg peaks within 5-50° in the XRD pattern of the source material PMo_{12} corresponds to the characteristic diffraction peaks of Keggin-type structures, attributed to (001), (110), (140) and (014) planes[36]. The XRD pattern of the as-synthesized TPPA is identical to the patterns for phenyl and pyridyl at about 8.1° and 13.5°-35.7°. In the XRD pattern for the TPPA-PMo_{12} hybrid, a broad band is observed in the wide-angle range of 15° to 40°, which should be ascribed to the phenyl and pyridyl region. The sharp peaks in small-angle region are the characteristic peaks of Keggin structure, arising from the regular layer arrangement[37]. It should be noticed that no change of peak position is observed, suggesting that the crystalline structures of PMo_{12} and TPPA are well maintained in the TPPA-PMo_{12} hybrid.



Scheme 1. Schematic illustration of the preparation of TPPA-PMo_{12} hybrid via one pot hydrothermal reaction between Keggin POM and star-like N-donor ligand.

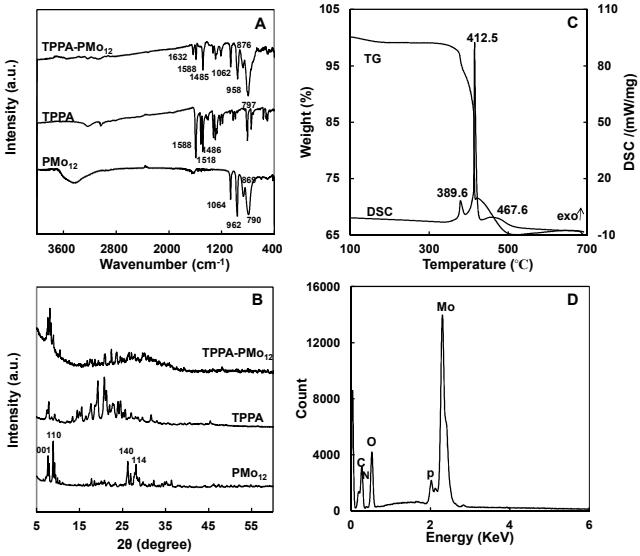


Figure 1. (A) FT-IR spectra of TPPA-PMo_{12}, TPPA and PMo_{12}. (B) XRD patterns of TPPA-PMo_{12}, TPPA and PMo_{12}. (C) TGA and DSC of TPPA-PMo_{12} hybrid at O_2 atmosphere. (D) EDS analysis results for TPPA-PMo_{12} hybrid.

TGA and DSC analysis results in Figure 1C illustrated that a total mass loss of 33.68 wt% is recorded for the TPPA-PMo_{12} hybrid from 350℃ to 500℃. In detail, there are three weight-loss stages corresponding to the three exothermic peaks in the DSC curve. The peak at 389.6℃ is ascribed to the oxidizing reaction of organic ligand TPPA, while those at 412.5℃ and 467.6℃ are both contributed by the decomposition of the polyanion $[PMo_{12}O_{40}]^{3-}$[38].

The EDS analysis results not only identify C, N, and O in the TPPA-PMo_{12} hybrid, but also confirm the presence of P and Mo (Figure 1D). Meanwhile, however, no Cu in detected in the EDS. This observation indicates that the role of $CuCl_2$ is solely serving as a template and not participating in the hydrothermal reaction for the preparation of the TPPA-PMo_{12} hybrid. Elemental analysis results indicate that the percentages of the elements in the final product TPPA-PMo_{12} are 16.3% for C and 46.6% for Mo, respectively. These results are consistent with the theoretical values, i.e., 17.2% for C and 49.9% for Mo in TPPA-PMo_{12}. Thus, a purity of 93.4% for the TPPA-PMo_{12} hybrid is derived.

3.2 Adsorption of proteins by the TPPA-PMo_{12} hybrid

As is known that α-La, β-Lg and BSA are the main whey protein species in bovine milk, and their isoelectric points are very close at around pI 5.0[39]. Figure 2 shows the pH dependent adsorption behavior of these proteins onto the TPPA-PMo_{12} hybrid. The whey protein species exhibit poor stabilities under strong acidic and alkaline media, the investigations are confined within a narrow range of pH 5.0-9.0. It is seen that the adsorption of α-La and BSA is very limited within the whole pH range tested. In the case of β-Lg, a maximum adsorption efficiency of 99.2% is achieved at pH 5.0, while a further increase of pH results in a sharp decline of the adsorption efficiency, and virtually no adsorption is observed at pH>7.

BSA and α-La are two kinds of albumins and there are lots of α-helices included in their secondary structure, usually regarded as "rigid" protein regions, while β-Lg is a globular protein belonging to lipocalin family and composed of 10-15% of α-helix and 50% of anti-parallel β-sheet[40]. The 8-stranded anti-parallel β-sheets usually fold into a calyx, at the entrance of which the hydrophobic residues located as "flexible" protein regions[41]. It has been demonstrated that small hydrophobic ligands such as aroma compounds are prone to bind inside the central calyx via hydrogen bonding interaction[41,42]. Thus, pyridyl and phenly groups of TPPA-PMo_{12} hybrid occupy the β-Lg's hydrophobic cavity directed to the calyx entrance, leading to a high adsorption efficiency of 99.2% for at pH 5.0. PMo_{12} in the hybrid provides charge and O-containing active sites to facilitate the adsorption of protein species. That is to say protein retention by the hybrid material is not due to coagulation of the proteins[16].

The surface charge analysis results suggest that the TPPA-PMo_{12} hybrid becomes negatively charged at pH>5.8 (Figure S1). With the increase of pH value of the sample solution, electrostatic repulsion between β-Lg and the hybrid becomes more prominent, resulting in the decline for β-Lg adsorption efficiency.

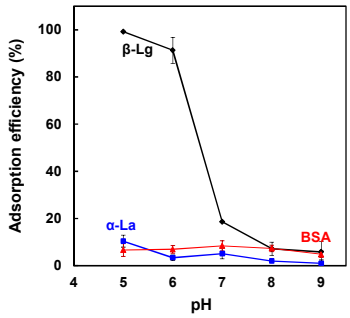


Figure 2. pH dependent adsorption behaviors of BSA, α-La and β-Lg onto the surface of TPPA-PMo_{12} hybrid. Protein solution: 100 mg L-1, 1.0 mL; TPPA-PMo_{12} hybrid: 0.5 mg; adsorption time: 30 min.

The significant difference of adsorption behaviors for BSA, α-La and β-Lg at pH 5.0 provides a practical strategy for the selective isolation of β-Lg in the presence of other protein species by simply controlling the pH value of the sample solution. For this purpose, the effect of ionic strength on the adsorption of β-Lg is investigated by the addition of certain amount of NaCl into the protein sample solution, and the results are shown in Figure S2A. It can be seen that the increase of ionic strength poses virtually no effect on the adsorption of β-Lg. This observation further demonstrates that it is the hydrogen bonding interaction, rather than electrostatic interaction, facilitates the adsorption of β-Lg. In this respect, it is not necessary to control the ionic strength in practical adsorption of β-Lg, a 0.04 mmol L^{-1} B-R buffer at pH 5.0 is adopted for the preparation of protein sample solutions. An obvious improvement on the adsorption efficiency for β-Lg is observed with the increase of adsorption time up to 30 min (Figure S2B). In fact, an adsorption efficiency of >90% could be readily achieved within 20 min. In practice, a 30 min adsorption time is eventually adopted.

Figure S3 illustrates the dynamic adsorption isotherm of β-Lg onto the TPPA-PMo_{12} hybrid at room temperature within 100-1400 mg L^{-1}. It fits well with *Langmuir* adsorption model as expressed in the following equation, suggesting that the adsorption of β-Lg onto the TPPA-PMo_{12} hybrid is monolayer coverage and it further demonstrated that the protein adsorption is not caused by the coagulation of the protein. *Ce* (mg L^{-1}) is the protein concentration, *Q** (mg g^{-1}) denotes the amount of adsorbed protein at equilibrium, Q_m (mg g^{-1}) is the adsorption capacity and K_d is the adsorption constant. By fitting the experimental data to Langmuir adsorption model, an adsorption capacity of 1428 mg g^{-1} is derived, which agrees well with the actual adsorption capacity of 1309 mg g^{-1}.

$$Q^* = \frac{Q_m \times C_e}{K_d + C_e}$$

3.3 Recovery of the adsorbed β-Lg from TPPA-PMo_{12} hybrid

In practice, it is important to transfer the adsorbed/isolated protein species from the adsorbent, TPPA-PMo_{12}, into an appropriate aqueous medium to facilitate further biological investigations. In this respect, various buffer solutions have

been investigated for the stripping/recovery of the adsorbed β-Lg from the hybrid, including Tris-HCl, BR, PBS and glycine buffer. As illustrated in Figure 3, favorable recovery of β-Lg could be achieved by employing Tris-HCl buffer as stripping reagent. This might be ascribed to the competitive participation of the hydroxyl groups of Tris in the formation of hydrogen bond between the hybrid and the protein β-Lg. Further experiments indicate that Tris-HCl buffer at pH 8.0 provides a recovery of 91.5% for the adsorbed β-Lg.

To further ascertain the adsorption selectivity and elution efficiency, a mixture of 100 mg L^{-1} α-La and 250 mg L^{-1} β-Lg is prepared to perform the adsorption/desorption processes as described in the experimental section. The protein mixture, the residual solution after adsorption and the eluent after stripping are collected to undergo HPLC-MS analysis. α-La and β-Lg are both observed in the total ion chromatogram of the original protein mixture (Figure 4A), with retention time of 3.62 and 4.95 min, respectively. After adsorption by the TPPA-PMo_{12} hybrid, only α-La is left and β-Lg disappears completely, indicating the favorable adsorption selectivity of TPPA-PMo_{12} hybrid towards β-Lg. In the eluent, only β-Lg is observed. Figure 4B-C illustrate the mass spectra recorded for the fractions of the supernatant after adsorption by the TPPA-PMo_{12} hybrid, and those for the eluent by stripping the TPPA-PMo_{12} hybrid with Tris-HCl. The molecular weight of α-La and β-Lg is deduced to be 14178 and 18363 Da respectively, which agree well with the theoretical average molecular weight[43].

The reusability of the TPPA-PMo_{12} hybrid for the adsorption/desorption performance for β-Lg is investigated and the results are given in Figure S4. It can be seen that an adsorption efficiency of >90% and an elution efficiency of >80% could be achieved after six continuous operation runs of adsorption/desorption.

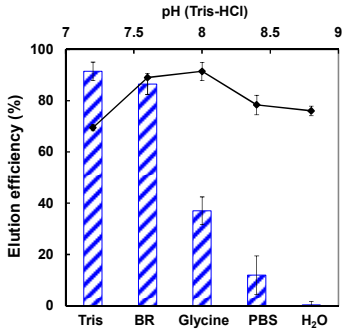


Figure 3. The recoveries of the adsorbed β-Lg from TPPA-PMo_{12} hybrid by using various buffers as stripping reagents and the effect of pH value of Tris-HCl buffer on the elution efficiency. β-Lg solution: 100 mg L^{-1}, 1.0 mL; pH: 5.0; TPPA-PMo_{12} hybrid: 0.5 mg; adsorption time: 30 min; stripping reagent: 0.05 mmol L^{-1}, 1.0 mL; stripping time: 10 min.

In order to evaluate the effect of TPPA-PMo_{12} hybrid on the secondary structure of β-Lg during the adsorption/desorption process, circular dichroism (CD) spectra are recorded for both standard β-Lg and that part of the recovered β-Lg after adsorption by the hybrid followed by stripping with Tris-HCl (0.05 mol L^{-1}, pH 8.0). As illustrated in Figure 5, CD spectrum of the recovered β-Lg coincides well with that of the β-Lg standard, both exhibiting a minimum ellipticity at 216 nm, derived from the β-sheet structure[44]. These observations indicate that the adsorption/desorption process onto TPPA-PMo_{12} hybrid poses virtually no effect on the secondary structure of β-Lg, suggesting the favorable biocompatibility of the hybrid material.

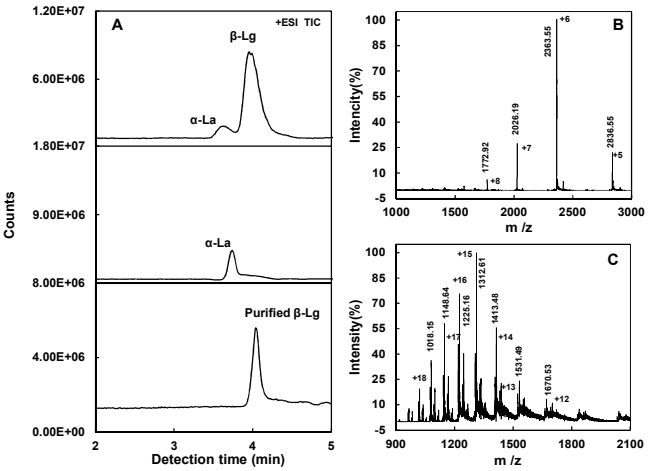


Figure 4. The total ion chromatograms of proteins. (A) A mixture of 100 mg L^{-1} α-La and 250 mg L^{-1} β-Lg; (B) mass spectra of the supernatant fluid after adsorption by the TPPA-PMo_{12} hybrid; (C) mass spectra of the recovered protein from the TPPA-PMo_{12} surface.

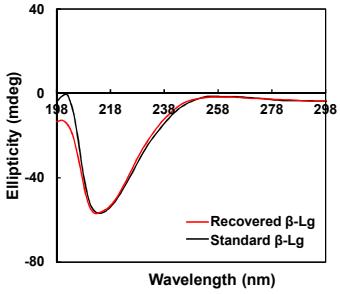


Figure 5. CD spectra of β-Lg standard and that recovered by stripping with Tris-HCl buffer.

3.4 Isolation of β-Lg from bovine milk whey

The practical applicability of the TPPA-PMo_{12} hybrid in the selective adsorption of protein is demonstrated by the isolation of β-Lg from a complex biological sample matrix, i.e., bovine milk whey. The average content of β-Lg is 2.0-3.0 g L^{-1} in bovine milk whey proteins. The whey sample is first diluted for 5-fold with 0.04 mol L^{-1} B-R buffer (at pH 5.0) followed by centrifugation at 5000 rpm for 5 min. The supernatant is then collected to undergo the adsorption/desorption processes with the TPPA-PMo_{12} hybrid as described in the experimental section. A standard SDS-PAGE assay is afterwards performed to evaluate the efficiency of the entire isolation process.

The protein (β-Lg) standard solution and that collected after undergoing the adsorption/desorption process are first mixed

with the loading buffer (a mixture of 0.25 mol L^{-1} Tris-HCl (pH 6.8), 10% (w/v) SDS, 0.5% (w/v) bromophenol blue, 50% (v/v) glycerine, and 5% (w/v) β-mercaptoethanol). The mixture is boiled for 3 min and then subject to electrophoresis. SDS-PAGE is carried out on a vertical polyacrylamide gel system at a constant voltage of 100 V until the protein bands reach the interface between the stacking and separating gels. The obtained electrophotogram is illustrated in Figure 6. The diluted whey sample exhibits a few major bands within a range of molecular weight from 14.3-97.2 kDa (Lane 2), attributed mainly to α-La (14.2 kDa), β-Lg (18.4 kDa), together with certain amount of BSA (66.4 kDa). These protein bands are still observable in the residual solution after treatment by the TPPA-PMo_{12} hybrid, while the intensity of the β-Lg (18.4 kDa) band becomes weaker (Lane 3). As for the recovered solution, only a band at 18.4 kDa is observed (Lane 5), which is consistent with that for the β-Lg standard (Lane 4). This suggests high purity for the recovered β-Lg.

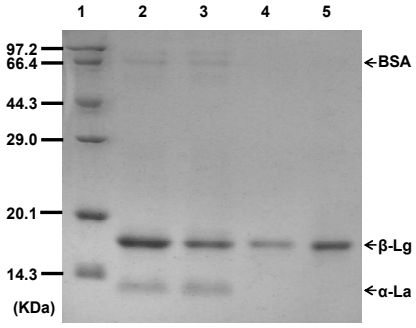


Figure 6. SDS-PAGE assay results. Lane 1: Molecular weight standards (marker in kDa); Lane 2: 5-fold diluted bovine milk whey; Lane 3: 5-fold diluted bovine milk whey after adsorption by the TPPA-PMo_{12} hybrid under experimental conditions as described in the text; Lane 4: β-Lg standard solution of 100 mg L^{-1}; Lane 5: the collected β-Lg from TPPA-PMo_{12} surface with 1.0 mL 0.05 mol L^{-1} Tris-HCl buffer at pH 8.0.

Conclusions

A new organic-inorganic hybrid polyoxometalate (POM) is prepared via one-pot hydrothermal reaction. The hybrid POM possesses excellent biocompatibility and exhibits high selectivity for the adsorption of β-lactoglobulin with a favorable adsorption capacity of 1428 mg g^{-1}. It thus provides practical feasibility for the selective isolation of β-lactoglobulin from bovine milk whey. Considering the structural versatility of POMs, it is quite feasible to develop/fabricate novel multi-functionalization organic-inorganic hybrid by rational selecting the inorganic and organic components, which might provide attractive and efficient adsorbent in the bioseparation filed, especially in the isolation of protein species of interest from complex biological sample matrices. Additionally, the abundant active oxygen atom involved in POMs would offer great potential in achieving excellent adsorption selectivity by regulating the interactions of POMs-based materials with the targets.

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Graphical Abstract

A novel polyoxometalate (POM)-based organic-inorganic hybrid is prepared via one-pot hydrothermal reaction between Keggin POM and a star-like N-donor ligand. The hybrid exhibits excellent adsorption performance towards β-lactoglobulin with a favorable sorption capacity.