Protein as Possible Bioactive Principle

Active Sites for Sorption

Various mechanisms for the sorption of metal ions onto biomaterials have been discussed in the literature based on the presence of different functional groups like polyphenols, carbohydrates, polypeptide hydroxyl groups, sulfonic acid groups, nitro, carboxyl acid groups, and proteinaceous amino acids. Among the above functional groups, proteinaceous amino acids have been found to play an important role in metal sorption mechanism. The proteinaceous amino acids have a variety of structurally related pH-dependent properties of generating appropriate atmosphere for attracting the cationic and anionic species of metal ions simultaneously. This chapter is devoted to provide experimental proof that carboxyl and amino ligands present in the biomaterial are responsible for metal binding.

The above facts have been established on the basis of the following experiments.

Esterification

The biomaterial was subjected to esterification using standard practices:

$$\text{Biomaterial} - \text{COOH} + \text{CH}_3\text{OH} \overset{(\text{CH}_3\text{O})_3\text{CH}}{\rightarrow} \text{Biomaterial} - \text{COOCH}_3 + \text{H}_2\text{O}$$

The esterification of naturally occurring carboxylic acids resulted in the formation of ester. The esterified biomaterial was monitored for its biosorption efficacy, exhibiting decreasing trend of sorption for cationic metals. The decreasing trend of sorption of the esterified biomass is likely due to blocking of COOH group resulting in the formation of ester linkage (-COOR-) which is not supposed to show any affinity for the attraction of M^+ ion. This fact indirectly highlights the role of COO^- ligand in M^+ binding.

S. Srivastava, P. Goyal, *Novel Biomaterials*, Environmental Science and Engineering,
DOI 10.1007/978-3-642-11329-1_15,

Propylamination

The biomaterial was subjected to propylamination using standard practices:

$$\text{Biomaterial} - \text{COOH} + \text{CH}_3\text{CH}_2\text{CH}_2\text{NH}_2 \rightarrow \text{Biomaterial} - \text{COONH}_2 + \text{C}_3\text{H}_6$$

Similarly carboxylic acid group was converted into amide by reaction with propylamine. Propylamination of the biomaterial has again resulted in the decrease of sorption efficiency highlighting the role of carboxylic acid moiety for cationic metal sorption. Blocking the carboxylic groups with propylamine, which neutralizes these anions, considerably decreases the cationic metal ion uptake, indicating that negatively charged carboxylic groups play an important role in biosorption due to electrostatic attraction.

The observed decrease in sorption of the cationic metals after neutralizing the carboxylate anion of the biomaterial clearly indicates that negatively charged carboxylate anions play an important role in biosorption phenomenon.

Tables 1, 2, and 3 include the decreasing trend of esterified and propylaminated biomaterial with respect to unmodified representative biomaterial *Leucaena leucocephala seed powder* (*LLSP*) *and Zea mays cob powder* (*ZMCP*).

Table 1 Decrease in sorption efficiency of different chemically modified LLSP in case of single metal solution

Nature of biomaterial	Cd(II)	Cr(III)	Ni(II)
Unmodified (%)	97.25	88.65	76.23
Esterified biomaterial (%)	91.46	81.17	71.67
Propylaminated biomaterial (%)	89.23	73.35	64.78

Table 2 Decrease in sorption efficiency of different chemically modified *Z. mays* cob powder in case of single metal solution

Nature of biomaterial	Cd(II)	Cr(III)	Ni(II)
Unmodified (%)	79.36	76.43	71.98
Esterified biomaterial (%)	71.46	81.17	71.67
Propylaminated biomaterial (%)	89.23	73.35	64.78

Table 3 Enhancement of sorption efficiency of acetylated ZMCP

	Sorption efficiency (%)
Type of biomaterial	Cr(VI)
Unmodified ZMCP	78.12
Acetylated biomaterial	71.76

The role of amino group present in the biomaterial for metal sorption can also be highlighted based on the following chemical reaction:

$$\text{Biomaterial} - NH_2 + (CH_3CO)_2O \rightarrow \text{Biomaterial} - NHCOCH_3 + CH_3COO^- + H^+$$

Acetylation of the biomaterial containing amino groups with acetic anhydride results in the blocking of available amino ligands and decreases the number of positively charged sites on the biomaterial surface. The resulting acetylated biomaterial exhibited decrease of sorption of anionic metal, exhibiting equally important role of amino group in the sorption of anionic metal.

Tables 4 and 5 present the chemical composition of major constituents with special reference to the free and bound amino acids in the representative plant biomasses of shelled *Moringa oleifera* and *L. leucocephala* seed powder.

CELLULAR CONSTITUENTS OF SMOS

Heterogeneous complex mixture having various functional, importantly low molecular weight amino acids	
Crude protein	432.5 g/kg DM
Crude fat	312.0 g/kg DM
Carbohydrate	211.2 g/kg DM
Ash	44.3 g/kg DM

CELLULAR CONSTITUENTS OF LLSP

Heterogeneous complex mixture having various functional, importantly low molecular weight amino acids	
Fat	7.50%
Ash	0.78%
Protein	32.90%
Calcium	0.42%
Phosphorus	0.64%

Sincere efforts have been made to isolate the protein content of the seed biomaterial and to further fractionate the various protein bands with the determination of their molecular weight. It is also reported that major fraction of the protein is likely to be responsible for metal sorption efficacy and considered as bioactive principle.

Table 4 Amino acid composition of *L. leucocephala* seed powder (Padmavathy and Shobha 1987)

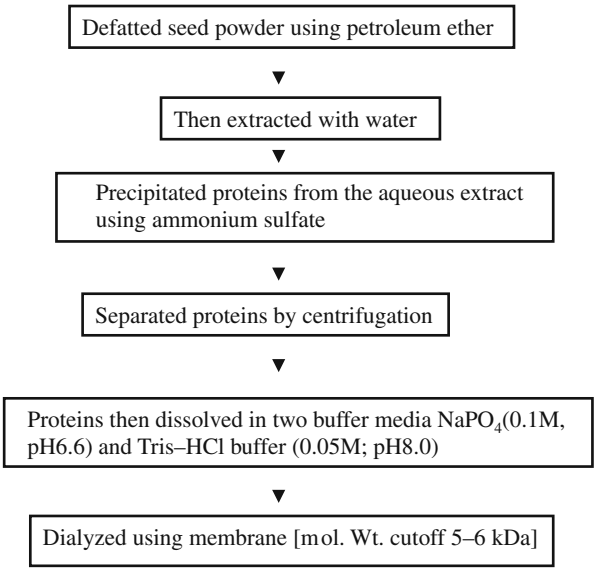
Amino acid	*L. leucocephala* seed powder (g/100 g)
Isoleucine	15.88
Trypsin	15.90
Glutamic acid	11.35
Arginine	6.17
Leucine	4.84
Lysine	4.21
Glycine	3.68
Serine	3.36
Valine	3.02
Phenylalanine	3.02
Alanine	3.00
Proline	2.78
Histidine	2.57
Threonine	2.26
Methionine	0.85
Cystine	0.87
Tyrosine	2.42

Table 5 Amino acid composition of shelled *Moringa* oleifera seed powder (Bachewel et al. 1995)

Amino acid	Shelled *M. oleifera* seed powder (g/100 g)
Isoleucine	12.81
Trypsin	11.56
Glutamic acid	9.15
Arginine	5.27
Leucine	5.54
Lysine	6.11
Glycine	5.18
Serine	2.36
Valine	2.02
Phenylalanine	2.02
Alanine	2.56
Proline	3.13
Histidine	2.98
Threonine	2.53
Methionine	0.81
Cystine	0.76
Tyrosine	2.44

Isolation and Characterization of Protein

Protein content of seed powder (SMOS and LLSP) was isolated using standard practices. The various steps involved in the isolation of protein are the following:



Quantification of Protein

The active proteins are to be bioassayed (Bradford 1976).

Different dilutions of the protein standard (bovine serum albumin) are to be prepared. Protein sample solution is to be taken in a clean dry test tube and mixed with dye reagent (Coomassie brilliant blue solution) and vortex. The solutions are incubated at room temperature. The absorbance is to be measured against blank (dye reagent) at 595 nm using UV spectrophotometer. The concentration of the recovered protein content from dialysis is calculated (Fig. 1).

Fig. 1 UV spectrophotometer

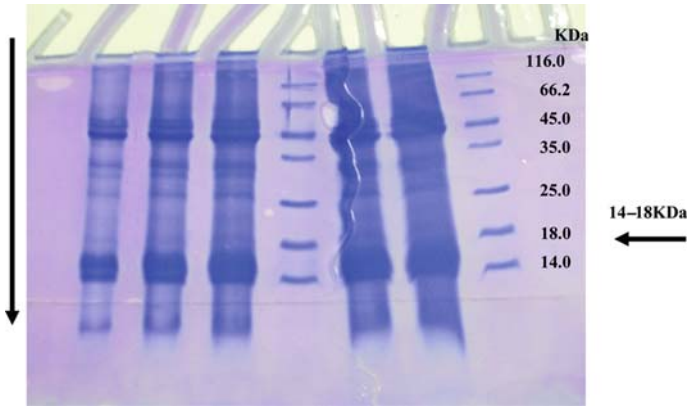
Molecular Weight Determination (Gel Electrophoresis)

Molecular weight of the proteins is to be determined using SDS-PAGE, 15% gels (Laemmli 1970). Active protein, flowthrough, washings, and elutions are to be subjected for gel elctrophoresis. Samples (10 μL) are to be loaded on each well and run on 15% polyacrylamide gels using a Mini Protean II. A wide-range molecular weight marker (10–20% Biomarker, BioRad) is used as a size maker. Polyacrylamide gels (7 × 10 cm) containing 30% acrylamide are to be prepared (Laemmli 1970). The separating gel (15%) contained 30% acrylamide, 0.8% bisacrylamide, 4× Tris–Cl\SDS, pH 8.8, H_2O, 10% ammonium persulfate, and TEMED. The stacking gel (5%) contained 30% acrylamide + 0.2% Bis, 4× Tris buffer (pH 8.8), 4× Tris buffer\SDS (0.5 M), pH 6.8, 10% ammonium persulfate, TEMED, and H_2O. Electrophoresis is to be performed at 30 mA and 150 V per gel until the dye reached the bottom of the gel. After electrophoresis the gels are to be stained in Coomassie Brilliant Blue (contains 0.1% Coomassie brilliant G-250, 0.2% phosphoric acid, 10% ammonium sulfate, and 20% methanol) overnight and destained with a solution containing 1% acetic acid and 1% glycerol.

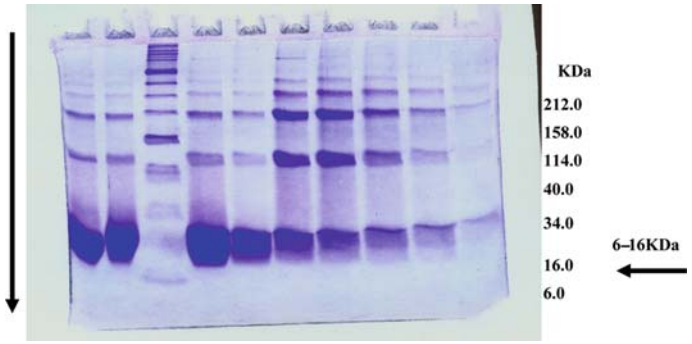
Characterization of Protein

A gel chromatogram of any protein sample represents the presence of prominent protein bands of different intensities which are to be further fractionated in terms of molecular weight.

Representative gel chromatograms of the protein content of the LLSP and SMOS are presented in Photographs 1 and 2.



Photograph 1 Electrophoretic nature and mol. wt. determination of LLSP protein. The *arrow* indicates direction of protein migration



Photograph 2 Electrophoretic nature and mol. wt. determination of SMOS protein. The *arrow* indicates direction of protein migration

The fractions of proteins having different molecular weights are collected, dried, and assayed for sorption efficacy to trace the exact protein content showing maximum sorption efficacy and then predicted as bioactive principle.