

REVIEW

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of the materials submitted for participation in a competition for the academic position "Professor" in the field of higher education 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological sciences, scientific specialty "Biochemistry"

Common part

The competition for "Professor" is in the field of higher education 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological sciences, scientific specialty "Biochemistry". It was announced for the needs of the Faculty of Medicine, Sofia University "St. Kliment Ohridski" in SG no. 63 of 30.07.2021 (correction in SG No. 65 of 06.08.2021). The only candidate is Assoc. Prof. Albena Georgieva Jordanova from the same faculty. The review of the documents shows that the procedure for opening and announcing the competition has been followed. The presented set of materials on electronic media have been prepared in accordance with the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its application and the internal regulations of Sofia University "St. Kl. Ohridski" and meet the criteria for acquiring the academic position "Professor".

Brief biographical data

Dr. Albena Jordanova graduated in "Biochemistry and Microbiology" (specialization Biochemistry) in the Biological faculty of Sofia University "St. Kliment Ohridski" in 1992. In the period 2004 - 2007 she was a PhD student at the Institute of Biophysics, BAS. In 2007, after successfully defending a dissertation on "Surface properties and behavior of lipid liquid-crystalline phases", the Higher Attestation Commission awarded her the scientific and educational degree "PhD" in the scientific specialty of biophysics. Dr. Albena Jordanova has 26 years of experience in the specialty, holding successively different positions: 1995 -1997 - biologist in the Department of Biochemistry at Sofia University "St. Kliment Ohridski"; 1997-2002 - biologist at the Institute of Biophysics, section "Model Membranes", BAS; 2002- 2006 – res. ass. at the Institute of Biophysics, section "Model Membranes", BAS; 2006 - 2013 - consecutively as a senior lecturer and chief assistant at the Institute of Biophysics, section "Lipid-protein interactions", BAS, and since 2012 she has been an Associate Professor of biochemistry at the Faculty of Medicine of Sofia University. Since 2019 she is the Head of the Department of Chemistry and Biochemistry, Physiology and Pathophysiology.

General presentation of scientific papers

Assoc. Prof. Jordanova has published a total of 95 scientific papers in the field of the competition. In 32 of them, she is a lead author. 32 of the publications are in journals with IF and have a total

IF of 52,661. The data from Scopus show her works are cited 266 times with h-index 7 (Google scholar h-index - 9). She has participated in 158 national and international scientific forums.

In the current competition, apart from the PhD thesis and the competition for Associate Professor, Dr. Jordanova has presented 42 scientific papers: 10 publications in refereed journals with IF, 1 monograph, 4 publications in refereed scientific journals without impact factor, 14 publications in collections of scientific forums and 13 textbooks for students. The publications submitted for the competition have a total IF of 15,876 and are cited 202 times in referenced lists. Assoc. Prof. Jordanova has participated in 72 scientific forums after holding the academic position of "Associate Professor"

According to indicator B of the Law for the Development of the Academic Staff in the Republic of Bulgaria, a monograph is presented, with which Assoc. Prof. Jordanova covers the required 100 points. The indicators from group D are as follows: 3 publ. with Q1 = 75 p.; 4 publ. with Q2 = 80 p.; 3 publ. with Q3 = 45, 2 publ. with Q4 = 24 points, collecting a total of 224 points (minimum 200 points required). According to the citation indicator, there are 404 points (minimum 100 required).

Scientific contributions

The main contributions of the research of Assoc. Prof. Jordanova are aimed at analyzing the structure and properties of alveolar surfactant in norm and pathology and assessment of the functional state of the lung in various diseases, as well as study of intermolecular interactions in model biological membranes through modern highly informative laboratory methods.

Some of the publications in the period before her habilitation as an Associate Professor have a similar scientific orientation, but the publications presented for participation in this publication expand and upgrade the research, both in the field of basic and in the field of applied scientific activities.

Contributions under indicator B3 - Habilitation work

In the attached report on Indicator B 3. is presented a monograph on "Alveolar surfactant - norm metabolism and pathology. *In vitro* methods for assessing its functional status." The monographic work describes in detail the composition, structure, properties and molecular mechanisms of interaction of the components of the alveolar surfactant for the implementation of physiological processes in the lung in norm and pathology. The mechanisms of development, symptoms and modern therapeutic approaches in the treatment of various lung diseases, which occur as a result of lack of "mature" surfactant or its inactivation under the influence of various factors, are discussed in detail. Among the main contributions of the monograph is the in-depth analysis of the data published so far, as well as the numerous author's results on the application of laboratory methods for testing the functionality of the components of the alveolar surfactant and assessment of lung status. The monograph contains, describes and richly illustrates with mainly author's results 7 biochemical and 7 biophysical methods used in testing the condition and activity of the alveolar

surfactant in various lung pathologies. In addition to the classical methods, including determination of phospholipid and protein concentration, identification of individual lipid components, determination of the lecithin / sphingomyelin ratio, counting of lamellar bodies, test for formation of stable foam, etc. Modern methods are also described, which give an ambiguous answer about the condition and functional properties of the lung: TDx-FLM II, Langmuir and Wilhelmy monolayer technique, axisymmetric drop shape analysis of a pending drop, analysis of the shape of hysteresis curves in dynamic compression of the studied clinical trials, etc. The presented *in vitro* methods are convenient, fast and highly informative, and the clinical samples tested through them are diverse - tracheal and gastric aspirates, amniotic and lavage fluids, etc. Of particular importance for the rapid and adequate determination of pulmonary maturity is the use of gastric aspirates, as a reliable clinical sample for analysis of the quantity and quality of surfactant in at-risk newborns. The main contribution of the monograph, in addition to comprehensive information about the metabolism of surfactant components in norm and pathology, is the emphasis on the practical orientation of the described informative laboratory methods, which is important for the relevance of the monograph as a modern guide to biochemistry, neonatology and pediatrics. It would be useful both in the training of medical students and in the clinical practice of neonatologists, physiologists and pulmonologists.

Contributions under indicator G7 of fundamental nature

Assessment of the functional state of the lungs in various diseases.

The biochemical and biophysical properties of gastric aspirates (GA) from preterm infants with neonatal respiratory distress syndrome (NRDS) and healthy term infants were analyzed in order to establish a rapid and convenient method for assessing surfactant maturity. A comparative analysis of the concentration of proteins and lipids in GA was performed. Phospholipid and protein concentrations in preterm infants were found to be lower than in preterm infants. The dynamic surface characteristics of GA show significantly higher mean values of the minimum surface tension (γ_{\min}) in premature infants than in full-term infants. It has been shown that γ_{\min} values are an important parameter for assessing the maturity of the surfactant in the lung. For the first time, a combination of modern innovative techniques such as axisymmetric analysis of a pending drop shape and Brewster-angle microscopy (BAM) was used (publications 55, 70, 78). The maturity of the alveolar surfactant in at-risk newborns was tested by the combined application of the methods of thin liquid films and BAM. It has been shown that the probability of thin black film formation, as well as the morphology of gastric aspirate monolayers observed by BAM, differ significantly between the tested groups of children, which is important for undertaking the correct strategy in neonatology units (publication 80). Different isoforms of the specific surfactant proteins SP-A, SP-B and SP-C in gastric aspirates were detected for the first time by SDS-PAAGE and Western blot analysis. Expression of different isoforms of individual specific proteins was found in all GA samples studied depending on the stage of maturation of the alveolar surfactant (publications 77, 81).

The efficacy of betamethasone corticosteroid therapy routinely administered to at-risk pregnant women after *in vitro* fertilization and multiple pregnancies was analyzed. It has been found that corticosteroid therapy improves the surface characteristics of aspirates and leads to visible changes in the morphology of the monolayer films formed by them. The results of the biophysical analysis confirm that the administration of betamethasone activates the biosynthesis of the components of the alveolar surfactant (publications 78, 81).

In the treatment of pulmonary alveolar proteinosis to remove the intraalveolar accumulation of lipoproteins, complete pulmonary lavage was performed, and samples taken at each stage of the procedure were analyzed by biochemical and biophysical methods. Laboratory analysis of lavage fluids shows that at each subsequent stage of the procedure, the concentrations of proteins and phospholipids decrease and the values of the equilibrium surface tension increase, which confirms the effectiveness of the applied procedure (publication 63).

The biochemical and biophysical characteristics of the alveolar surfactant in the non-ventilated (operated) and ventilated lobe of the lung in patients with non-small cell lung cancer were studied. Phospholipid and protein concentrations as well as surface tension values under equilibrium and dynamic conditions were determined. There was a negative trend in the equilibrium values of surface tension in cancer patients compared to the control group (publication 64).

Investigation of intermolecular interactions in model biological membranes through modern highly informative laboratory methods

It has been found that the presence of hydrophilic polymers prevents the inactivation of alveolar surfactant by plasma proteins by creating an attractive osmotic pressure in the surface film, leading to displacement of unwanted inhibitors from the surface and improving the quality and effectiveness of the surfactant. The surface behavior of exogenous surfactants used in clinical practice - Curosurf and Survanta (in the absence and presence of albumin) and the effect of the addition of hydrophilic polymers (polyethylene glycol, dextran, polyvinylpyrrolidone, hyaluronic acid) to monolayer and bilayer were studied. It has been found that the addition of polymers in the presence of albumin causes a restoration of the surface activity of surfactants and their ability to maintain low surface tension values under dynamic conditions of compression / decompression. The incorporation of polymers into bilayer thin liquid films has also seen the elimination of the inhibitory effect of albumin and the formation of stable thin films, analogous to films of pure surfactants (publication 54).

Using the Langmuir monolayer technique in combination with Wilhelmy's method for measuring surface pressure, the interaction of synthetic methionine-enkephalin and its amidated analogue with the characteristic membrane lipids 1-palmitoyl-2-oleoyl-3-glycerophosphocholine, sphingomyelin and cholesterol, as well as the synthetic leucine-enkephalin and leucine-enkephalinamide was studied. The compression modulus of the monolayer films was determined and it was found that the addition of synthetic enkephalins leads to a change in their surface characteristics, which is more pronounced with enkephalinamide. The surface morphology of the

monolayer films before and after the interaction with the enkephalins was visualized by BAM. The obtained images show an increase in the surface density of the formed mixed films, especially in two- and three-component lipid mixtures, as the effect is more pronounced in enkephalinamide. Observations confirm the presence of interactions between peptides and lipids in the membrane, which is more pronounced with the amidated peptide and suggests differences in the folding of the two enkephalins (publications 62, 68).

Thin liquid films stabilized with palmitoylisophosphatidylglycerol were examined by the Sheludko and Exerova method. The relationship between the film thickness and the electrolyte concentration in the solution, as well as the pressure / thickness proliferation isotherms in the presence of Na^+ and Ca^{2+} ions were studied. It was found that at low concentrations of NaCl thick films are formed, and with increasing concentration of Na^+ the films thin to ordinary black films. Thin films in the presence of Ca^{2+} follow the same dependence, but at higher electrolyte concentrations form Newtonian black films, most likely due to the strong interactions between ions and polar lipid heads. The disjoining pressure/thickness isotherms for both electrolytes showed a gradual decrease in film thickness without observing transients (publication 69).

The molecular mechanism of the antimicrobial action of newly synthesized benzantrone on model membranes resembling bacterial ones was studied. The strongest interaction was found with negatively charged phosphatidylglycerols and dipalmitoylphosphatidylethanolamine. It has been hypothesized that electrostatic interactions with polar lipid heads and the formation of hydrogen bonds between the ethanolamine amino group and keto groups in the benzoquinone structure are important for the antimicrobial activity of the test compound (publication 79).

It has been confirmed that Langmuir monolayers are a convenient and informative model system for studying the molecular interactions and properties of biological membranes (publications 54, 62, 68, 79).

Contributions under indicator G7 with applied character

Gastric aspirates (GA) obtained immediately after birth with a nasogastric tube has been shown to be an appropriate clinical sample for rapid assessment of surfactant maturity. The analysis of gastric aspirates would limit the application of the invasive techniques used so far in clinical practice (publications 55, 66, 70, 73, 76, 78, 80).

It has been proven that the minimum value of surface tension, determined by axisymmetric analysis of the shape of a pending drop (ADSA), is a reliable parameter by which to quickly and unambiguously determine the lung maturity of newborns. The measurement of this important surface characteristic is performed within 20 minutes and requires 50 microliters of the tested clinical sample (publications 55, 66, 70, 73, 76, 78).

A fast, convenient and safe laboratory approach based on the combined use of ADSA, BAM and the thin liquid film method for assessing the functional status of the lungs in patients with

respiratory diseases is proposed, which could be introduced and reliably used in clinical practice with a view to applying life-saving therapy (publications 55, 66, 70, 73, 76, 78, 80).

The analysis of clinical samples from patients with pulmonary alveolar proteinosis contributes to the improvement of the applied procedure for conducting complete pulmonary lavage, the main contribution being the reduction of the amounts of saline used (from 20 liters to 15 liters), which shortens the duration of the procedure and is already actually applied in clinical practice (publications 58, 63, 73).

Studies of the surface properties of alveolar surfactant in lavage samples taken from patients with alveolar proteinosis and non-small cell lung cancer prove the effectiveness and potential of the ADSA method using a minimal amount of lung lavage for rapid diagnosis of lung function and prompt therapy (publications 58, 63, 64, 66, 73).

In conducting experiments to establish the effects of hydrophilic polymers on restoring the surface properties of albumin-inhibited exogenous surfactants, it was found that in the presence of polyethylene glycol, polyvinylpyrrolidone and hyaluronic acid, Survanta reaches low values of minimum surface tension. In the case of Curosurf, a similar positive trend was observed with the addition of dextran, polyvinylpyrrolidone and hyaluronic acid. The results obtained may find application in clinical practice in the treatment of acute respiratory distress syndrome in adults, which may occur as a result of the inhibitory action of albumin in the lung (publication 54).

The results of the study of the interaction between synthetic enkephalins with model membrane systems show that the combination of the Langmuir monolayer model with BAM can be successfully used in preclinical studies to test the biological activity and membrane penetration of newly created analogs of natural analogues (publications 59, 62).

The increasing resistance of many pathogens to many of the antibiotics used in clinical practice requires the development of new antimicrobial substances with better therapeutic activity. Studies of the interaction between newly synthesized benzantrone and bacterial-like membranes contribute to the discovery of the mechanism of the potential antimicrobial action of newly synthesized antibacterial compounds with a view to their future application in pharmacology and clinical practice (publication 79).

Project activity

According to the completed reference for fulfillment of minimum national requirements, Assoc. Prof. Jordanova is a participant in the development of 8 research projects funded by the Ministry of Education and Science and 2 departmental. She is the leader of a project funded by the Ministry of Education and Science. The total value of the funds raised from the project amounts to BGN 120,000, which according to indicator E (14-18) brings a total of 144 points. She was also the leader of 9 scientific projects financed by the Research Fund of Sofia University.

Pedagogical activity

Assoc. Prof. Jordanova is the holder of the compulsory lecture courses: for students majoring in "Medicine" and "Medicine in English" at the FM - Sofia University and for students majoring in "Nurse" - "Clinical Laboratory, Biochemistry and Immunology". She is also the holder of the elective courses for students majoring in "Medicine" and "Medicine in English" "Characteristics of Metabolism in Norm and Pathology" and "Phytoproducts and their application in medicine", proposed and approved on her initiative respectively in 2014 and 2019.

Assoc. Prof. Jordanova was a scientific supervisor of a successfully defended diploma thesis for a master's degree at the Faculty of Biology at Sofia University and co-supervisor of a doctoral student expelled with the right to defense. She was an academic mentor in the practical training of students in a real work environment in the period 2016-2018 in the implementation of project BG02M2OP001-2.002-0001, "Student Internships".

Assoc. Prof. Jordanova is a co-author of 5 published textbooks in Bulgarian and English, prepared in accordance with the approved curriculum in biochemistry for students at the Faculties of Biology and Medicine, Sofia University, 6 Collections of theoretical tests and practical tasks from the Regional and National Rounds of The Olympiad in Biology and Health Education and 1 textbook containing richly illustrated theoretical material on human anatomy and physiology and various tests for student exams in biology for the Medical Universities.

Expert activity

Assoc. Prof. Jordanova has been a member of the National Commission for Organizing and Conducting the National Olympiad in Biology and Health Education since 2008, and since 2011 she has been its Chairman. Since 2010 she is the head of the National Biology Team and a member of the international jury of the International Biology Olympiad (IBO), and during this period the National Team of the Republic of Bulgaria has won 3 gold medals, 7 silver medals, 24 bronze medals and 8 honorary diplomas for worthy performance at the International Olympiads. She was a member of the National Commission for organizing and conducting the National Competition "Young Talents", MES (2015-2019); member of the National Commission for conducting and evaluating the participants in the festival "Science on Stage" (2021); member of the National Commission for Evaluation of Matriculation Examination in Biology (2013 till date). She is a member of the USB, of the Management Board of the Association of Olympic Teams in Natural Sciences, of the Association of Biochemists in Bulgaria.

Personal impressions

I have known Albena since her student years and I have witnessed her growth as a scientist and teacher. She has in-depth knowledge in the field of biochemistry and biophysics, both theoretically and practically. The professionalism she possesses, as well as her personal qualities such as perseverance, diligence, correctness and exceptional ability for work and motivation of the students, define her as a candidate, meeting the requirements for the position of "Professor".

Conclusion:

The documents and materials submitted by A. Jordanova meet all the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria (RASRB), the Regulations for its implementation and the internal regulations of Sofia University “St. Kl. Ohridski”, and exceed the criteria for acquiring the academic position of “Professor”. The presented materials and personal impressions give me reason to express my positive opinion regarding the candidacy of Assoc. Prof. Dr. Albena Georgieva Jordanova for the academic position "Professor". She is an established specialist and lecturer, with proven authority in the scientific community and among the student community. As a member of the Scientific Jury for the announced competition, I give a positive assessment and recommend to the members of the esteemed Faculty Council of the Faculty of Medicine to vote positively for the election of Assoc. Prof. Dr. Albena Georgieva Jordanova to the academic position "Professor" in professional field 4.3. Biological sciences, specialty Biochemistry.

24.11.2021 / prof. Dr. Mariela Odjakova /