CROSS-CRYSTALLIZATION AS A NEW OPTIMIZATION TOOL OF CRYSTALLIZATION PROCEDURES

Ivana Tomčová[1, 2] and Ivana Kutá Smatanová[1, 2]

[1]*Institute of Physical Biology, University of South Bohemia in České Budějovice, Zámek 136, CZ-373 33 Nové Hrady, Czech Republic*

[2]*Institute of Systems Biology and Ecology, Academy of Sciences of the Czech Republic, Zámek 136, CZ-373 33 Nové Hrady, Czech Republic*

ivas@greentech.cz

Keywords

Crystal morphology; Single crystal growth; X-ray diffraction; Biological macromolecules; Additives; Cupric compounds; Cross-crystallization

Abstract

The effect of several metal cations (Cu^{2+}, Cd^{2+}, Co^{2+}, Ba^{2+}) was tested in attempts to improve crystallization procedure and verify a newly discovered cross-crystallization method with two selected proteins; di-heme cytochrome c_4 from anaerobic purple sulphur bacterium *Thiocapsa roseopersicina* and sweet-tasting protein thaumatin from the African berry *Thaumatococcus daniellii*. Presence of Cu^{2+} ions promoted dramatic improvement in crystal morphology, internal packing and diffraction quality. This investigation qualitatively established the influence of cupric cations on the crystal growth by using the cross-crystallization procedure. It was found that influence of Cu^{2+} ions produced evidently different outer morphology and internal packing of thaumatin crystals (hexagonal prism). Usually their shape is presented as a tetragonal bipyramids. In the case of cytochrome, the good diffractable crystals were obtained only by using cross-crystallization method with metal-ion salts. Newly grown crystals (hexagonal prisms) of thaumatin and cytochrome displayed as the same primitive tetragonal system and diffracted up to 1.7 Å. Crystals were suitable for high-resolution structure analysis.

Introduction

The determination of successful crystallization conditions for a particular protein remains a highly empirical process. Screening procedures are rapid and economical means to determine preliminary crystallization conditions. During optimization a variable set of parameters (i.e. pH, precipitant type, precipitant/protein concentration, etc.) is screened to determine appropriate conditions for the nucleation and growth of single crystals suitable for X-ray diffraction analysis. Unfortunately, in many cases this strategy will not produce suitable single crystals. Empirically we have explored another tool used in optimization strategy described by Tomčová [1]. We developed and tested a crystallization procedure to modify crystal morphology, internal packing and also to influence crystal growth. For the first time the metal ion salts were added simultaneously to the protein drop and even to neighbouring drops to allow cross-influence during crystallization experiment. Here we report the effects of selected additives on crystallization of two different proteins; one well-known "model" protein thaumatin [2] and one crystallographicaly unexplored di-heme cytochrome c_4.

Methods

Description of cross-crystallization method

Cross-crystallization is a procedure applied to standard vapor diffusion sitting and/or hanging drop method. This procedure is based on using a set of additives that influence the quality of crystal growth. In principle, the inclusion of other droplets (containing chemical substances) against the same reservoir slightly changes the vapor pressure of water over the neighboring drop including protein. As described previously [1], the Emerald BioStructures CombiClover Crystallization Plate (EBS plate, Emerald BioStructures, Bainbridge Island WA, USA) with one central reservoir connected to four satellite drop-chambers (*a, b, c, d*) via dedicated vapor diffusion channels, was used in this procedure (Fig. 1a, 1b). Each of drop-chambers *a, b, c, d* was filled with different additive (in this case; chloride salts of copper, cadmium, cobalt and barium) and equal volume of the precipitating agent. The protein was only added into the one drop-chamber containing cupric chloride. Additives and reservoir solution, and not protein, were placed to the three remaining drop-chambers to promote crystallization in the fourth drop-chamber.

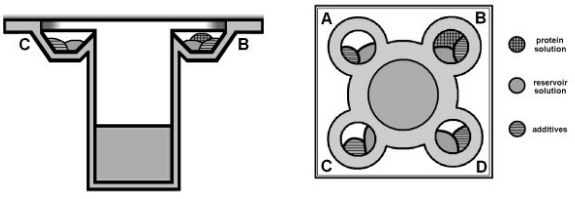


Figure 1a, 1b: Schematic side and top view of Emerald BioStructures CombiClover Crystallization Plate (EBS plate) for sitting drop experiments. Grey color presents reservoir solution, strap areas indicate each additives and grid represents protein-containing solution.

Results

Cytochrome crystallization

The cross-crystallization method was used to further improve quality of crystals by addition of additives. Deep red well-shaped cytochrome crystals grew within 3–4 days at 20 °C in the presence of 5 mM cupric chloride and ammonium sulfate in citric acid buffer at pH 5. Those crystals

were not reproducible unless the other metal salts ($CdCl_2$, $BaCl_2$, $CoCl_2$) were present in the remaining drop chambers as was described above. These cytochrome cross-crystallization experiments have been tested several times, and in all cases the cytochrome crystals grew only in hexagonal prism form. The same outer shape of crystals was observed when a cytochrome was cross-crystallized by hanging drop (Fig. 2).

Thaumatin crystallization

Thaumatin was crystallized using the standard sitting drop method [2, 3] with the polyethylene glycol (PEG) as a precipitating agent. Well-constructed tetragonal bipyramids were obtained from these crystallization conditions. The effect of metal salt ions on cross-crystallization was tested. Dramatic change in thaumatin crystal morphology and internal packing was observed when thaumatin was crystallized as hexagonal prisms (Fig. 2). In this case, cupric chloride caused the greatest change in crystal outer shape while the other additives showed no significant effect on crystal growth.

X-ray diffraction experiments

Both, cytochrome and thaumatin hexagonal prism crystals with dimensions of approximately 1.00 0.05 0.02 [mm] (Fig. 2) were tested at the synchrotron DESY/ EMBL. Complete data sets collections were executed at beamline X13 with tunable wavelength using Oxford cryo-system type magnets for crystal mounting. Crystals were removed from the drop with a loop and flash-cooled in a nitrogen stream (Oxford cryo-system) at 100 K at the goniometer part of beamline. A crystal to detector distance of 120 mm was used to collect at least 200 frames of each. The exposure time for each image was 30 sec and the oscillation angle was 1°. Diffraction data were collected to 1.72 Å resolution for cytochrome, to 1.70 Å for thaumatin (tetragonal bipyramid) and to 1.50 Å for thaumatin (hexagonal prism), using MAR CCD 165 mm detector at DORIS storage ring with triangular monochromator and bent mirror beam.

Discussion

The cross-crystallization method includes several factors that can facilitate protein crystallization, from the promotion of intermolecular contacts by divalent metal cations, stabilization of the protein with salts, to changing the aggregation state with precipitating agents. In fact, any addition of a new substance into a crystallizing mix resulting in crystallization is usually classified as a new crystallization technique and handled as a hot tip. However, the effectiveness of any newly discovered method could not be statistically determined. For example, from previous studies it was found that cupric ions in phosphate buffers have a tendency to produce heavy precipitate and even salt crystals [4, 5]. Another example of an additive effect, which can be explained on a molecular basis, is a formation of intermolecular contacts by intercalated divalent transition metal cations [6]. Cadmium (in sulfate solutions) was long known as a crystallization inducing agent of horse spleen

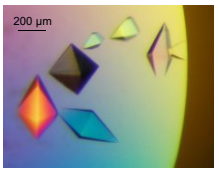
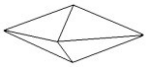
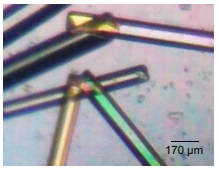
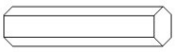
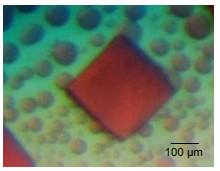
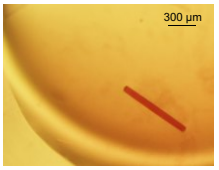
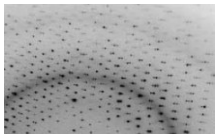
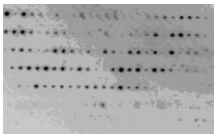
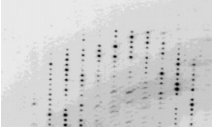
Protein	**Thaumatin**		**Cytochrome**	
Crystallization method	**Standard crystallization**	**Cross-crystallization**	**Standard crystallization**	**Cross-crystallization**
Crystallization conditions in protein solution	30-40% PEG 3350 15% PEG 6K 0.1 M TRIS pH 6.5	30-40% PEG 3350 15% PEG 6K 0.1 M TRIS pH 6.5 5 mM cupric chloride	3.2 M ammonium sulfate 0.1 M citric acid pH 5.0	3.2 M ammonium sulfate 0.1 M citric acid pH 5.0 5 mM cupric chloride
Crystal outer shape	 tetragonal bipyramids 	 hexagonal prisms 	 quasi crystals – plates	 hexagonal prisms 
Crystal system	orthorhombic	tetragonal	no diffraction	tetragonal
Space group	$P2_12_12_1$	$P4_12_12$	no diffraction	$P4_12_12$
Mosaicity	 0.89	 0.44	 no diffraction	 0.580
Resolution	1.7 Ĺ	1.5 Ĺ	no diffraction	1.72 Ĺ

Figure 2. Overview of thaumatin and cytochrome crystallization experiments show crystal morphology and internal packing influenced by metal-ion salts.

ferritin and has been re-discovered as a useful agent to promote crystallization or to increase diffraction quality in a number of cases [Trakhanov 1998]. However, even with a mechanistic explanation of this effect, no rational prediction regarding the probability of success – except statistical evidence – is available!

The specific morphology of thaumatin and cytochrome crystals may depend on factors such as the source of material used during crystal growth and chemicals in the crystallizing buffer in the mother liquor, or on the mother liquor itself. For a single crystal form the angles between the faces are constant, but this is not true if the crystals belong to the different crystal forms such as tetragonal bipyramids and hexagonal prisms as in thaumatin. Their appearance depends on the use of metal salt cations, such as cupric chloride, and partially on the buffer and the precipitating agent used. We assume these metal ions influence evaporation in the protein drop even if they are absent from that drop. As this effect was tested on two different proteins only, we cannot speculate about how universally applicable this will be. However, the influence of Cu^{2+} ions on cytochrome crystal growth appears to be specific, because no other successful combination of ion salts with cytochrome was found among these four salts singly or in pairs. A similar effect was observed even in thaumatin crystallization when conditions with cupric chloride produced thaumatin crystals with a different morphology. The combination of four particular salts that promote crystallization can be quite reproducible also with other chemicals or even other volumes of the same drop in the remaining drop chambers.

References

1. I. Tomčová, R.M.M. Branca, G. Bodó, Cs. Bagyinka, I. Kutá Smatanová, *Acta Cryst.* **F62** (2006) 820-824.
2. A. McPherson, Crystallization of biological macromolecules, Cold Spring Harbor laboratory press, New York, 1999.
3. T.M. Bergfors, Protein crystallization: Techniques, strategies and tips, International University Line, La Jolla, USA, 1999.
4. A. McPherson, J. Weickmann, *J. Biomolecular Structure & Dynamics* **7** (1990) 1053-1060.
5. J. Jancarik, R. Pufan, C. Hong, S.H. Kim, R. Kim, *Acta. Crystal. Sec.* **D60** (2004) 1670-1673.
6. H. Sigel, A. Sigel, Metal ions in biological systems, Marcel Dekker - Taylor & Francis - CRC, California, USA, 1990.

Acknowledgements

This work is supported by grants MSM6007665808 and LC06010 of the Ministry of Education of the Czech Republic and Institutional research concept AVOZ60870520 of Academy of Sciences of the Czech Republic to I.K.S.

CRYSTALLIZATION AND STRUCTURE - FUNCTIONAL STUDY OF PHOTOSYSTEM II FROM HIGHER PLANTS

Tatyana Prudnikova[1], Michal Kutý[1,2], José A. Gavira[3], Peter Palenčár[1], František Vácha[1,5], Pavlína Řezáčová[4], Juan M. García-Ruiz[3] and Ivana Kutá Smatanová[1,2]

[1]Institute of Physical Biology USB CB, Zamek 136, 373 33 Nove Hrady, Czech Republic
[2]Institute of Systems Biology and Ecology AS CR, Zamek 136, 373 33 Nove Hrady, Czech Republic
[3]Laboratorio de Estudios Cristalografico, Edf. Lopez Neira, P.T. Ciencias de la Salud, Avenida del Conocimiento, s/n, 18100 Armilla, Granada, Spain
[4]Institute of Molecular Genetics AS CR, Flemingovo n. 2, 16637 Prague, Czech Republic, current address: Dep. Biochemistry, UT Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, Texas 75390-8816
[5]Biological Centre IPMB AS CR, Branisovska 31, 370 05 Ceske Budejovice, Czech Republic
ivas@greentech.cz

Keywords

Photosystem II, reaction center, counter-diffusion method, vapor diffusion, membrane protein, absorption spectra, precipitation, exciton interaction, transition monopoles, transition dipole moments.

Abstract

Oxygen-evolving complex of photosystem II was crystallized using both counter-diffusion method and common vapor diffusion techniques. Absorption spectra of Photosystem II (PS II) reaction centres (RC) upon reduction of primary acceptor pheophytin of the D1 protein subunit were studied and compared with spectra calculated on the basis of Exciton Limit (Matrix Method).

Introduction

Photosynthesis realized by photosystem II (PSII) uses light energy to couple the formation of molecular oxygen to the fixation of carbon dioxide. This process simultaneously generates an aerobic atmosphere and produces a readily usable carbon source, both of which act to sustain almost all life on this planet. PS II is located in the thylakoid membrane of higher plants, algae and cyanobacteria. Its function to capture sunlight is realized by two antenna proteins CP47 and CP43. They transfer the excitation energy from the antenna system to the photochemical reaction center (RC) with primary electron donor P680, which is formed by chlorophyll *a* (Chl *a*) molecules is exited to P680*, followed by release of an electron that travels along the electron transfer chain [1, 2]. This large multisubunits

protein-pigment complex oxidizes water with releasing of oxygen molecule by a cluster of four manganese atoms. The Mn4-Ca cluster forming the catalytic site for water oxidation is located near the luminal side of PSII RC mostly surrounded by amino acid side chains from D1. One molecule of oxygen is accumulated in four successive charge separation steps and four positive charge equivalents, utilizing two molecules of water.

PS II consists of four membrane-internal subunits (D1, D2, CP43, and CP47), several smaller internal membrane (including PsbE and PsbF, constituting cyt *b*-559) and three external subunits (PsbQ, PsbP, PsbO in green algae and higher plants) [3]. PSII RC binds more than 50 co-factors and ion species. Cofactors contact to the geterodimetic D1/D2 system at the heart of PSII that include Chl *a*, Pheo *a*, plastoquinone, -carotene and one non-haem Fe^{2+}.

The aim of our experimental part was based on using advanced counter-diffusion and standard vapor-diffusion methods, to observe capability of individual precipitants to influence the crystals growth.

Our theoretical work was based on study of temperature dependence of the optical absorption changes upon reduction of primary acceptor pheophytin *a* (Pheo *a*) of the D1 protein subunit (D1-Pheo *a*) to find out whether pheophytin should be considered as a part of a multimer model.

Materials and methods

Experimental part

Crystallization of macromolecular complex such as PS II is influenced by many parameters. The most important is the protein itself, its purity, homogeneity and some other properties, but mainly its disposition to form crystals. This tendency to produce for diffraction measurement suitable crystals may be optimized combination of using different crystallization techniques and other physicochemical parameters (precipitants, additives, pH, etc.) influencing crystallization [4, 5].

Nowadays counter-diffusion method becomes very popular like substitution of common vapor diffusion techniques: sitting and hanging drops, microbatch under oil, etc. Capillaries of different length and diameters performed in the Granada Crystallization Boxes (GCB-Domino, Triana Sci&Tech, Granada, Spain) are a good choice for counter-diffusion technique. The capillaries (Fig.1) filled by protein {1} are placed into the different precipitant solutions {2} through the layer of 0.1% agarose {3} to fix them. Gradient of supersaturation appears along the length of the capillary due to diffusion of solutions against each other. Optimal condition for producing nice single crystals suitable for X-ray diffraction measurement can arise in some parts of capillary.

The sample with dimeric core complex of PS II (OEC PSII) was obtained form *Pisum sativum* (green pea), purified and isolated to concentration 2-3 mg/ml of chlorophyll *a* (~10-15 mg/ml of protein) and supplemented with 1mM $MnCl_2$ to crystallization test. 17.5% acrylamid gel was used to analyze protein composition of isolated trials. Fresh sample of OEC PSII was crystallized using counter-diffusion method as well as common vapor diffusion techniques.

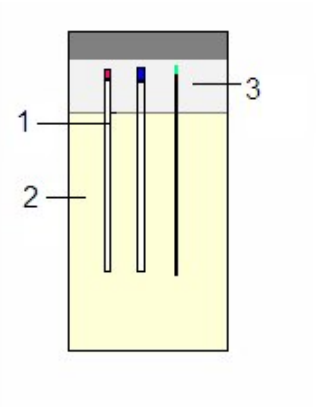


Figure 1. The Granada Crystallization Box with capillaries of different diameters (1 – capillary, 2 – precipitant, 3 – agarose).

Computational part

Exciton interaction between chlorophyll molecules is a general phenomenon in photosynthetic reaction centers. Strong interaction between closely located molecules of pigments leads to a situation where these molecules lose their individual identity and their spectral properties change significantly. The optical properties of chlorophyll-pheophytin complex are determined by the strong interactions between the pigments, which are nearly at Van der Waals' distance to each other.

Exciton interactions between pigments of PSII RC from higher plants upon oxidation or reduction of primary acceptor pheophytin have been studied by using experimental and molecular modeling techniques [7, 8]. For characterization of the spectral properties (absorption and CD spectra) of multimer protein-pigment system, Exciton Limit (Matrix Method) [9] was applied on crystal structure of PSII RC (accession code in protein databank 1S5L [10]). This method consists of two parts, in the first part, one finds the eigenvalues and eigenvectors of the matrix, and the off-diagonal elements are interaction energies between the molecules (pigments) and the diagonal terms represent individual transition energies of pigments. Interaction energies were calculated by two ways, the point dipole and also point monopole method. The point-dipole method [11] evaluates interaction energy between the transition dipole moments of pigments. The point-monopole method [12] describes the interaction energy between transition monopoles distributed on the pigments. This method is an improvement over the point dipoles method [14]. In order to obtain realistic light-adapted absorption spectra of PSII RC we modified site energies (individual transition energies of pigments) by so-called electrochromic shifts as interaction energies between atomic partial charges of D1-Pheo and differential dipole moments of the excited and ground states of PSII RC core pigments [13]. In the second part of calculation, the eigenvectors of this matrix were applied along with the molecular electric transition dipoles to calculate absorption intensity for each of the matrix eigenvalues. Each line of the line spectrum was replaced by a smooth Gaussians function to give the appearance of actual experimental spectra.

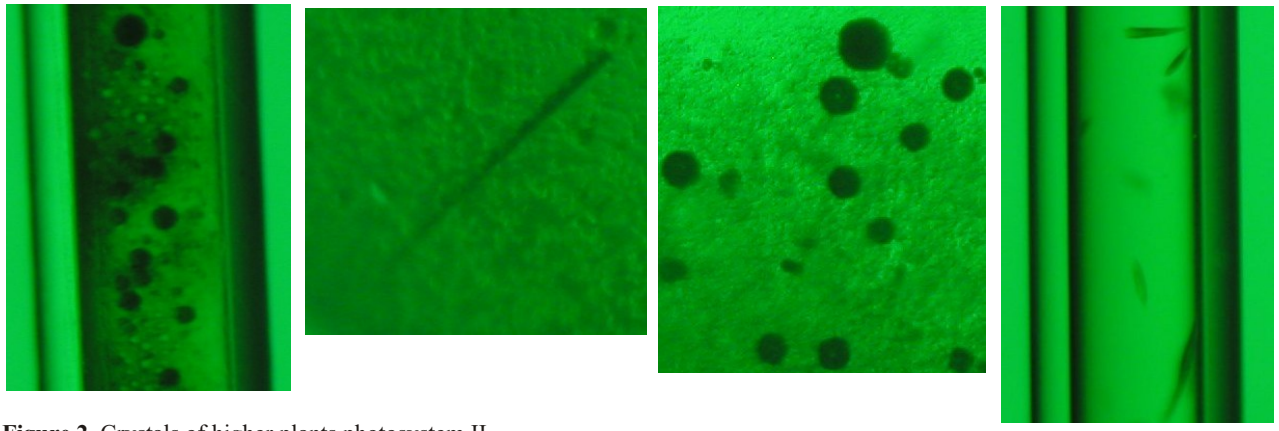


Figure 2. Crystals of higher plants photosystem II.

Results and discussion

Experimental part

Results describing our experimental work when several detergents for membrane protein (-DM, $C_{12}E_8$), buffers with different pH (MES, HEPES, Tris, KH_2PO_4), cryoprotectants (PEG with several molecular weight, glycerol, MPD) were used, were published by Kuta Smatanova et al. [6]. We also investigated the influence of several salt additives from Hampton Research screening test (Ba, Mg, Ca, Mn, Cd, Cu, Co, Cs, Zn, Y, Ni, Sr) (Hampton Research, CA, USA) to find suitable conditions to produce single crystals of diffraction quality. Crystals of hexagonal shape and needles obtained from different conditions will be measured at the source of synchrotron radiation in DESY, Hamburg (Germany) or ESRF, Grenoble (France).

Computational part

Analogous to our previous experiments we have obtained the temperature dependence of the light-induced difference spectrum under primary acceptor reduction (figure 3). Almost identical differences in intensities of 298 K and 77 K-calculated and 277 K and 77 K-experimental difference absorption spectra clearly supported earlier assumptions [7]. If the molecule of D1-Pheo *a* is a part of the multimer interaction, its reduction would lead to a change in the exciton interaction and consequently to a change in the optical absorption spectrum. Since the process of exciton interaction is not dependent on temperature and the D1-Pheo *a* reduction does not cause any change in the low temperature CD spectrum, we suppose that the D1-Pheo *a* molecule is not coupled in the multimer.

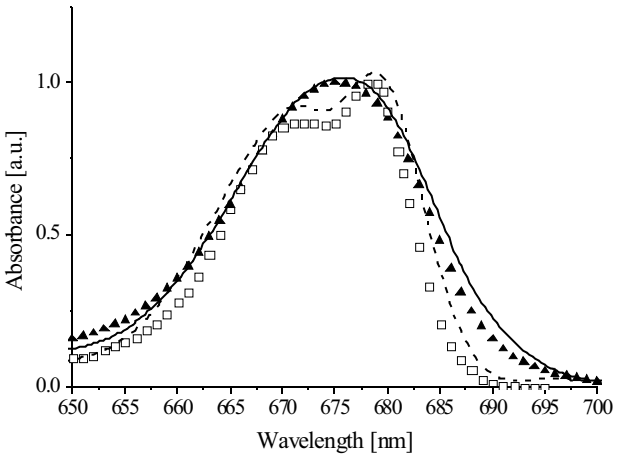


Figure 3. Comparison of calculated absorption spectra of PSII RC pigments calculated at 298K (solid line) and 77K (dashed line) with experimental absorption spectra measured at 273K (triangles) and 77K (squares) [8]. All spectra were normalized to their maxima using arbitrary units (a.u.).

References

1. J. Biesiadka, B. Loll, J. Kern, K.-D. Irrgang, A. Zouni: *Phys. Chem. Chem. Phys.*, **6** (2004) 4733-4736.
2. R. Anati, N. Adir: *Photosyntesis Research*, **64** (2000) 167-177.
3. J. Kern, B. Loll, C Luneberg, D.DiFiore, J. Biesiadka, K.-D. Irrgang, A. Zouni: *Biochimica et Biophysica Acta*, **1706** (2005) 147-157.
4. I. Kuta Smatanova, J.A. Gavira, P. Rezacova, F. Vacha, J.M. Garcia-Ruiz: *Acta Cryst*., **A61** (2005) 147.
5. K. N. Ferreira, T. M. Iverson, K. Maghlaoui, J. Barber, S. Iwata: *Science*, **303** (2004) 1831-1838.
6. I. Kutá Smatanová, J. A. Gavira, P. Řezáčová, F. Vácha, and J. M. García-Ruiz, accepted for publication in *Photosynthesis Research* (2007).
7. F. Vácha, M. Durchan and P. Šiffel: *Biochimica et Biophysica Acta*, **1554** (2002) 147.
8. F. Vácha, J. Pšenčík, M. Kutý, M. Durchan and P. Šiffel: *Photosynthesis Research*, 84 (2005) 297.
9. P. M. Bayley, *Prog. Biophys.,* **27** (1973) 3-76.
10. K. N. Ferreira, T.M. Iverson, K. Maghlaoui, J. Barber, S. Iwata: *Science*, **303** (2004) 1831-1838.
11. V. I. Prokhorenko, D.B. Steensgaard, A.R. Holzwarth: *Biophysical Journal*, **85** (2003) 3173-3186.
12. J. C. Chang: *Chem. Phys*., **67** (1977) 3901-3909.
13. G. Raszewski, W. Seanger, T. Renger: *Biophysical Journal*, **88** (2005) 986-998.
14. Jr. C. Weiss: *Spectrosc*., **28** (1972) 37.

Acknowledgements

This work is supported by grants NSM6007665808 and LC06010 of the Ministry of Education of Czech Republic and Institutional research concept AVOZ60870520 of Academy of Science of Czech Republic.

PHYLOGENETIC ANALYSIS OF EXTRINSTIC PROTEINS OF PHOTOSYSTEM II

Ž. Sovová and R. Ettrich

Laboratory of High Performance Computing, Institute of Physical Biology USB and Institute of Systems Biology and Ecology AS CR, Zámek 136, CZ-373 33 Nové Hrady, Czech Republic; email: ettrich@greentech.cz

Keywords
Photosystem II, extrinsic proteins, phylogeny analysis

Abstract
A phylogenetic analysis of extrinsic proteins associated with the oxygen evolving complex of Photosystem II was performed by generating trees for each extrinsic subchain of PSII. Subsequently these subtrees were written into one resultant tree. These investigations provide hints about the evolution of the oxygen evolving complex, helping in understanding functionally important and conserved parts of the protein structures. The detailed description of the evolution of oxygen evolving complex and the extrinsic proteins on basis of the presented data is a first step in a wider analysis to describe the interactions of the extrinsic proteins within the oxygen evolving complex and of the complex itself with PSII. In contrast with previous our study contains all extrinsic proteins including PsbP-like and PsbQ-like and we try to build resultant a tree containing all species, not just those having the same gene.

Introduction

Oxygenic photosynthesis is an energy-transducing process whereby light energy is trapped and converted into biochemical energy. This multi-step process encompasses a series of electron transfer reactions from water molecules to $NADP^+$, coupled to ATP synthesis [1]. Four types of protein complexes participate in this process going on in thylakoid membrane of cyanobacteria, red and green algae and in green plants. One of these complexes – photosystem II (PSII) – mediates electron transfer from water to plastoquinones, as a result of the photo induced charge separation between a primary chlorophyll donor P680 and a pheophytin acceptor molecule [1]. It forms a dimeric structure. Each of almost identical monomer units consist of more than 25 subunits.We focus on the extrinsic proteins of PSII that can be found in lumenal face of thylakoid membrane. PsbO protein – sometimes called 33 kDa protein according to its apparent molecular mass of spinach protein on SDS-gel – is common for all mentioned types of organisms. It is directly bound to intrinsic proteins of PSII, especially to CP47, but participation of other subunits can not be excluded. It is in charge of stabilization of manganese cluster and protecting it from attack by endogenous reductants. It also effects the conformation of the lumenal side of intrinsic PSII subunits which are responsible for binding PsbP and PsbQ [2]. Other extrinsic proteins differ among mentioned groups of organisms. Extrinsic subunits of green algae and green plants contains moreover PsbP (23 kDa protein) and PsbQ (16 kDa protein) subunits which are bound to PSII through PsbO protein, binding of PsbQ also requires presence of PsbP. These subunits create a high affinity Cl^- binding site, protect the manganese cluster from chemical attack and stabilize it. PsbP protein moreover prevents Ca^{2+} from being released from PSII during the S-state turnover.On the other hand extrinsic region of PSII of cyanobacteria contains proteins PsbO, PsbU (14 kDa protein) and PsbV (cytochrome c-550). PsbV has a function similar to that of O subunit in O_2 evolution. The function of PsbU remains unknown [1]. Recently it was shown [3] that cyanobacteria have also homologues of PsbP and PsbQ proteins of higher plants (homology of about 30 %). They take part in modifying the $CaCl_2$ requirements for PSII activity.Extrinsic proteins of PSII of red algae contain PsbO, PsbV, PsbU and protein which is called PsbQ like protein (20 kDa protein). The last protein does not function directly in oxygen evolution but is required for maximum binding of PsbV and PsbU protein which are required for optimum activity of oxygen evolution [4]. Our work was inspired by [5], some of their experiments were retaken with broader group organisms and we examined phylogeny of all extrinsic protein.

Methods

All available primary sequences of extrinsic proteins of PSII were extracted from databases using PubMed internet interface [6]. The abbreviation of used species can be seen in Table 1. If necessary, transit peptides were removed. Multiple alignment of sequences of each protein was done by ClustalX [7]. Later these alignments were used for computation of phylogenetic trees using PHYLIP software package [8] version 3.6. Two methods – Fitch-Margoliash with the assumption of molecular clock and protein parsimony – were used. To examine the stability of tree branches bootstrap for 1000 times was used; the order of protein sequences was jumbled for 50 times. Finally trees were rooted, the most ancient species was chosen as the root. For determining the relationship between different species trees calculated by Fitch-Margoliash with the assumption of molecular clock without branch length were used. Branches with bootstrap scores higher than 50 % were taken as conclusive. The resultant tree was drawn using CorelDraw software by adding branches to PsbO protein tree. If some species were presented at different places in different trees the one with the highest bootstrap value was used.To demonstrate the time distance of single species the same trees using the branch length were calculated. We used protein parsimony method to compare and confirm the results (especially „most important“ branches).

Results and Discussion

Our study is the first study where all available genes of extrinsic proteins of oxygen evolving complex were used. First we built phylogenetic tree for each gene separately and than we composed these trees into one containing all available genes.Our results clearly demonstrate that green plants (figures 1 to 6) form the closest group of species. This is in agreement with the fact that this group is evolutionary the youngest one. Also higher plants are multicellular organisms and thus less prone to mutation compared with unicellular organisms. On the other hand, the evolutionary oldest group of unicellular cyanobacteria is expected to be the most „diffuse" group of examined species. It can be divided into two branches from one of which the green plants had developed.Green algae form another evolutionary important group; they are relative close to green plants. These finding are supported by the composition of extrinsic proteins of PSII – both green plants and green algae have PsbO, PsbP and PsbQ. The group of green algae can be divided into two subgroups, a more consistent subgroup of multicellular green algae and more „diffuse" subgroup of unicellular green.Other unique group of genes are red algae that appear to be an interlink between the upper mentioned green algae and green plants and cyanobacteria. Extrinsic subpart of PSII contains chains PsbO, PsbU, PsbV and PsbQ homolog of green plants (homology of about 30 %). This means red algae is the only group of examined genomes (i.e. green plants and algae, red algae and cyanobacteria) lacking the gene encoding the PsbP chain. As can be seen in the phylogenetic trees of PsbO that red algae separated from the common ancestor at about the same time as green algae clearly demonstrating that red algae is not an ancestor of green algae. Also it seems that red algae did not develop from that time on. This supports the presumption that PsbP up rates the water oxidation and coupled reactions. Interestingly the subgroup of cyanobacteria adjacent to red algae lacks PsbQ proteins. So it seems that nature was trying many ways how to reduce protein chains necessary for water oxidation (the more protein the more energy that could be used more effectively is needed). Probably some when in the past there were some organisms having PsbO, PsbP, PsbQ

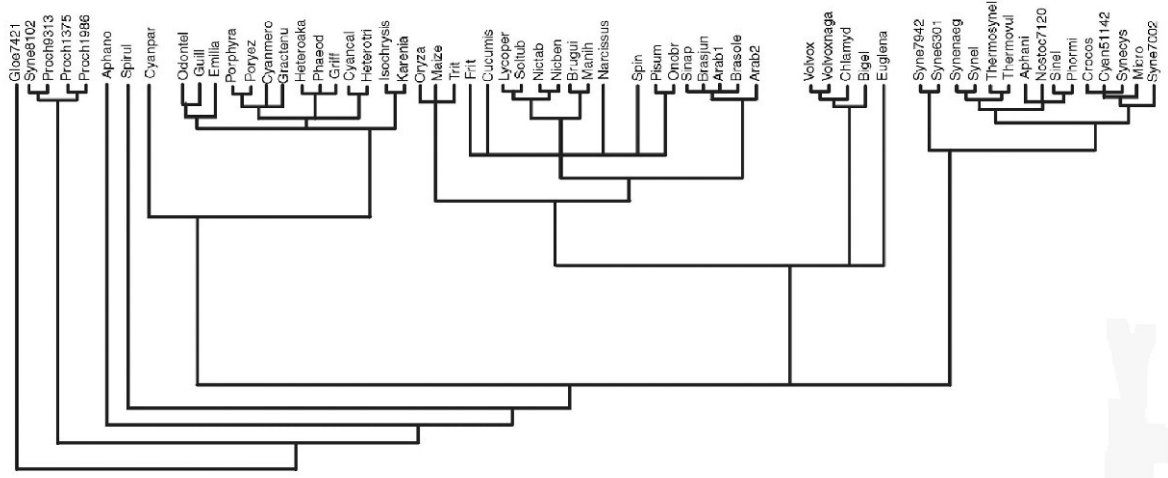


Figure 1. Resultant phylogenetic tree containing all 65 used species. As the root of this tree was used the most acient organism Gloeobacter violaceus PCC 7421.

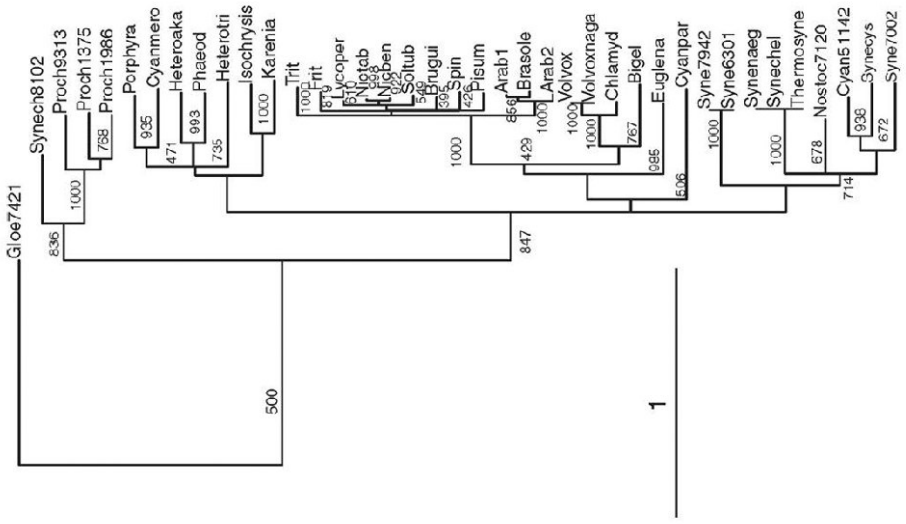


Figure 2. Phylogenetic analysis of 29 available PsbP sequences. Image shows the rooted tree using Gloeobacter as outgroup, giving the bootstrapping scores with scales.

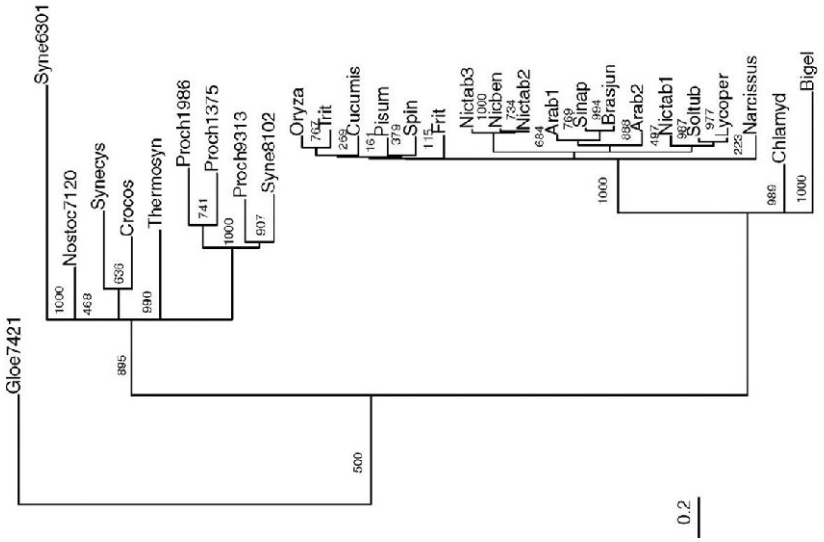


Figure 3. Phylogenetic analysis of 39 available PsbO sequences. Image shows the rooted tree using Gloeobacter as outgroup, giving the bootstrapping scores with scales.

Figure 4. Phylogenetic analysis of 15 available PsbU sequences. Image shows the rooted tree using Gloeobacter as out-group, giving the bootstrapping scores with scales.

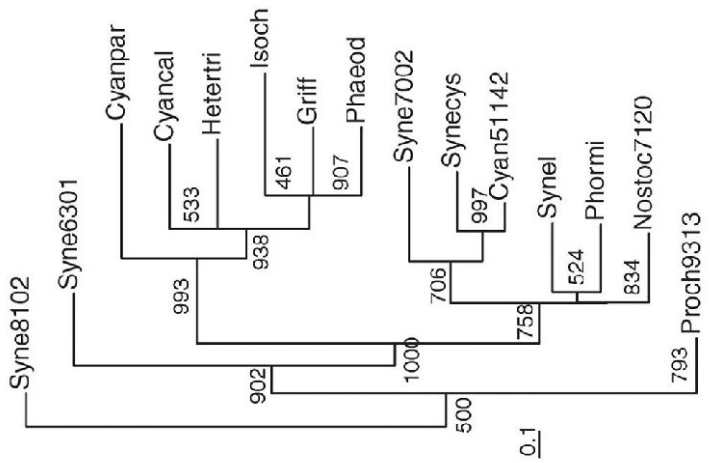


Figure 5. Phylogenetic analysis of 20 available PsbQ sequences. Image shows the rooted tree using Gloeobacter as outgroup, giving the bootstrapping scores with scales.

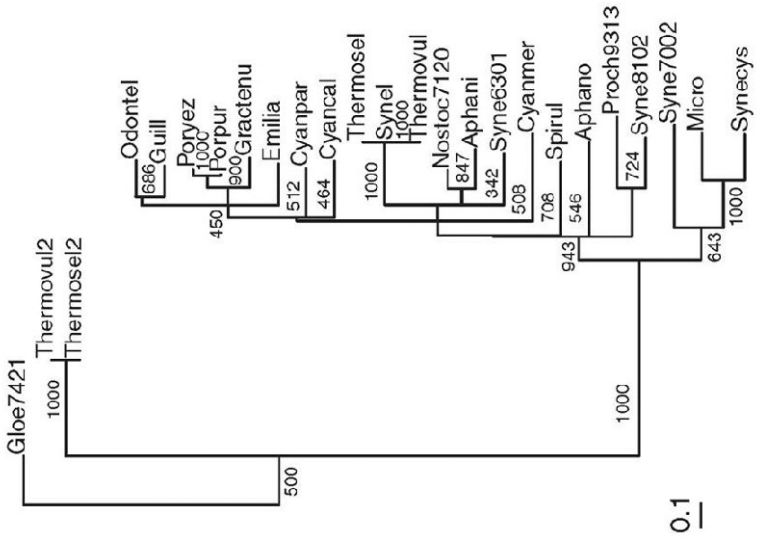


Figure 6. Phylogenetic analysis of 25 available PsbV sequences. Image shows the rooted tree using Gloeobacter as out-group, giving the bootstrapping scores with scales.

proteins and missing one of PsbU or PsbV proteins. There are known sequences of both PsbP and PsbQ proteins from the other subgroup of cyanobacteria; these cyanobacteria have also PsbU and PsbV proteins which play the crucial role in water oxidation. Later during the evolution (green algae and plants) the fundamental role of water oxidation was shifted to PsbP and PsbQ subunits and PsbV and PsbU subchains disappeared. In the case of PsbP it seems to be that first developed the PsbP-like protein and much later the PsbP protein in green algae and in higher plants. However, in case of PsbQ, the gene encoding the PsbQ chain in green algae is much closer to the PsbQ-like gene of cyanobacteria than to the higher plant one. Therefore it can be stated that PsbQ developed earlier, taking over the role of PsbU and PsbV, and then later PsbP developed to make oxygenic photosynthesis more efficient.

Acknowledgement

Support by the Grant Agency of the Czech Republic (grant number 206/03/D082) is gratefully acknowledged. Additional support from the Institutional Research Concept of the Institute of Systems Biology and Ecology (No. AVOZ60870520) and from the Ministry of Education of the Czech Republic (No. LC 06010, No. MSM6007665808) is acknowledged.

References

1. F.-A. Wollman et al., *Biochim. Biophys. Acta* **1411** (1999) 21-852.
2. A. Seidler, *Biochim. Biophys. Acta* **1277** (1996) 35-603.
3. L. E. Thornton et al, *Plant Cell* **1**6 (2004) 2164-21754.
4. I. Enami et al, *Biochemistry* **37** (1998) 2787-27935.
5. J. De Las Rivas et al, *Trends Plant Sci* **9** (2004) 18-256.
6. www.ncbi.nlm.nih.gov7.
7. J.D. Thompson et al, *Nucl Acids Res* **25** (1997) 4876-4884.

MATHEMATICALLY-BASED STUDIES OF COMPLEX SYSTEMS IN PHYSICS, MATHEMATICS, CHEMISTRY, BIOLOGY, ECONOMY AND POLITICS IN NOVÉ HRADY IN THE FIRST HALF OF 19TH CENTURY AND AT THE BEGINNING OF 21ST CENTURY

D. Štys [2]

[2]*Institute of Physical Biology, University of South Bohemia and Institute of Systems Biology and Ecology CAS, Zámek 136, 373 33 Nové Hrady, Czech Republic and institute of Microbiology CAS, 379 81 Třeboň, stys@jcu.cz*

author feels requisitiness to honour the co –authorship of
G.F.A. de Lonqueval von Buquoy, lord de Vaux [1],
[1]*Herrschaft Gratzen – panství Nové Hrady, Böhmen – Čechy*

Keywords
dynamic systems, heat transfer, glass technology, national economy, mathematical biology

Abstract
Georg Franz August de Lonqueval, count of Buquoy, lord de Vaux, in his approximately 200 texts, which include at least 24 separately printed books, paved the way to mathematically based study of complex systems. Count of Buquoy was, at the same time, able to test many of his conclusions experimentally at multiple scales and in multitude of areas; from production and examination of physical properties of new sorts of glass, through everyday use of new types of steam machines in mining operation up to testifying of his economical and nature-conservation theories in everyday routine of his possession. This article has two purposes: (i) to classify the work of Count of Buquoy and to indicate main fields of his interest, (ii) to announce the Buquoy symposium planned for summer 2011, the 200th anniversary of Buquoy's first books.

Introduction

Georg Franz August de Lonqueval, count of Buquoy, lord de Vaux, was born in Brussels in 1781 (GFAvB later in the text). He studied in Vienna and Prague. Since 1803 he governed the possession of Buquoy family in southern and western Bohemia (Nové Hrady and Červený Hrádek) until his death in 1851 in Prague. His certitude on the existence of mathematically based rules describing observable events in nature on one hand and his certitude of natural unity lead him to constant passion for search of the rules that govern the nature. At the same time, he was drawn to everyday economy of his possession for which he held responsibility in time of swift technological and economical paradigm change which had little parallel in the history of mankind.

Reviewed works of Georg Franz August de Lonqueval, count of Buquoy, lord de Vaux.

As is known to us now, the study of complex systems was to a large extent enabled only by the existence of computers. Many stories flitter around the word and found their way into non-fiction storybooks describing encounter between mathematicians and researchers in complex systems theory on validity of proof of their theories.

Modern, computer assisted, analysis of just one of the examples given in first works of GFAvB [1-3], the movement of a body with changing mass, lead to conclusions such as "Except for very special initial conditions it is difficult to obtain analytic solution to the equation of motion." [4]. Despite to the fact that only Newton mechanics rules were used, the system leads to oscillatory solution and the precise behavior of the system is highly sensitive to initial conditions, both properties typical to what has been named complex systems in modern terminology. The analytical mechanics is the subject of more than 20 further works of GFAvB, books as well as journal articles which have been comprehensively analysed in 1970´s and 1980´s in Russia [5-8].

It should be said that except the works in analytical mechanics, the only traceable reviewed work of GFAvB is that of mathematical economy. To list the most important contributions, we must consider his three consecutive books on the theory of national economy [9-11]. GFAvB´s conclusions in those books lead 140 years later to reflections like: "This analysis is so strikingly modern that it deserves a detailed report" [12]. The last reflection to GFAvB is that of the founding president of the Econometric society Joseph Schumpeter who comments GFAvB´s work by words "man and writings are forgotten unjustly, so I think" [13].

Other topics published in the work Georg Franz August de Lonqueval, count of Buquoy, lord de Vaux

The topics which may be traced in the work of GFAvB include: (1) the construction and theory of steam machine and theory of heat propagation, (2) theoretical mechanics, (3) mathematically-based theory of national economy, (4) analysis of common features of events in Nature, (5) theory of infinitesimal calculus and introduction of new methods for solution of oscillatory functions, integration and interpolation, (6) mathematically and physiologically – based theory of philosophy, government and discussion (debatieren), (7) glass technology and technological experiments, (8) theoretical and experimental optics, (9) theory of crystallisation and capillary events, (10) theory of stoichiometry and chemical reactivity, (11) theory of healing, (12) development of language and its forms, etc. GFAvB published most of his works as separate books, in Annalen der Physik and in the interdisciplinary journal Isis edited by Lorenz Oken. Other separately published works are reported but are not accessible to us. Numerous works,

and perhaps the most exciting, exist only in the form of manuscripts deposited in the Třeboň state archive.

Symposium on the occasion of 200th anniversary of publication of first work of Georg Franz August de Lonqueval, count of Buquoy, lord de Vaux in 2011

We announce hereby our intention to organise the GFAvB interdisciplinary symposium to be held in Nové Hrady in September 2011. Besides historical introduction, we wish (preliminarily) to follow the themes: (1) complex systems, (2) aspects in physical chemistry, (3) mathematical economy, (4) mathematical biology and medicine and (5) philosophy of nature. We plan for the meeting relatively early since we also want to reserve time for critical reading of at least some of the Buquoy's work and examination of the conclusions therein by modern methods. Please read the web-page *www. expertomica.cz/Buquoy* for further information.

References

1. G. J. F. von Buquoy , *Analytische bestimmung des Gesetzes der virtuellen Geschwindigkeiten in mechanischer und statischer Hinsicht* . Leipzig: Breitkopf und Härtel, 1812.
2. G. J. F. von Buquoy , *Weitere Entwickelung und Anwendung des Gesetzes der virtuellen Geschwindigkeiten in mechanischer und statischer Hinsicht* . Leipzig: Breitkopf und Härtel, 1814.
3. G. J. F. von Buquoy , *Exposition d´un noveau principe général de dynamique, dont le principe des vitesses virtuelles n´est qu´un cas particulier* . Paris: V. Courcier, 1815.
4. V. Šíma & J. Podolský, *Eur. J. Phys*., **26**, (2005), 1037.
5. G. K. Mikhailov, *Prepr. Instituta Probl. Mechaniki AN SSSR* **49,** (1975) 106.
6. G. K. Mikhailov, *Mechanika Tverdogo Tela Izv. AN SSSR* **5,** (1975), 41.
7. G. K. Mikhailov, *Georg Bukua i nachala dinamiki sistem s peremennymi massamy, Issledovanija po Istorii Fiziki i Mechaniki,* Moscow: Nauka, 1986. pp 191–238.
8. J. G. Panovko, *Mechanika deformirujemogo tverdogo tela: sovremennyje koncepcii, oshibki i paradoksy* Moscow: Nauka, 1985, pp 173–8.
9. G. J. F. von Buquoy , *Die Theorie der Nationalwirtschaft nach einem neuen Plane und nach mehreren eigenen nsichten dargestellt* . Leipzig: Breitkopf und Härtel, 1815.
10. G. J. F. von Buquoy , *Das nationalwitschaftliche prinzip oder was zuletzt alle nationalwitschaflichen Anstalten bezwecken müssen.*. Leipzig: Breitkopf und Härtel, 1816.
11. G. J. F. von Buquoy , *Erläuterung einiger eigenen Ansichten aus der Theorie der Nationalwirtschaft nebst tabellarischer Uebersicht des Zusammenhanges der wesentlichsterGewerbeunter einander und mehreren Beyträgen zum technischen Theile der Nationalwirtschaft. Zweiter Nachtrag zur Theorie der Nationalwirtschaft* . Leipzig: Breitkopf und Härtel, 1817.
12. R. Robertsson *Mathematical economics before Cournot, Journal of Political Economy LVII* (1949), 527.
13. J. Schumpeter: *History of Economic Analysis* (ed. by Elizabeth Boody-Schumpeter), NewYork: Oxford University Press, 1954.