Biosensors and Bioelectronics 24 (2009) 1490–1492

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Short communication

The phosphatidylinositol–protein nanocomplex as a new biosensor for ecological monitoring and clinical diagnostic

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ARTICLE INFO

Article history:
Received 19 February 2008
Received in revised form 4 May 2008
Accepted 20 May 2008
Available online 7 July 2008

Keywords:
Glutamate dehydrogenase
Nanocomplex
Biosensor
Ecological monitoring

ABSTRACT

Using chromatography on nanostructured carbon sorbent we had isolated an unusual nanocomplex from filling grains of wheat and maize, which consists of only phosphatidylinositol (PI) and one protein—glutamate dehydrogenase (GDh). It was very surprising that this nanocomplex shows activity of Nicotinamide adenine dinucleotide phosphate-GDh (NADP-GDh) without any treatment. Thus, the whole body of nanocomplex shows its activity without disturbing its integrity. This makes the nanocomplex very convenient for using it as a biosensor. The main feature of nanocomplex is its high sensitivity to ammonia ions. Linear response concentration for nanocomplex is from 0.5 μM to 10 μM ammonia ions. Due to these properties the nanocomplex may be very useful as nanobiosensor for ecological monitoring of pollution by sewer waters of natural reservoirs—lakes and rivers. Also this nanosensor can be applied for determination of ammonia ions, NADPH and 2-oxoglutarate in biological liquids for clinical diagnostic.

1. Introduction

Nanotechnology causes revolutionary influence in development of science and technology. Principles and methods of nanoscience and nanotechnology can give new approaches for the introduction of new biosensors. The development of biosensors is very perspective direction of biotechnology.

It is well known that anthropogenic pollution by waste waters causes increase in the contents of ammonia ions in natural water reservoirs—lakes and rivers, whereas in unpolluted reservoirs ions of ammonia are absolutely absent (Tsingarelli et al., 1987). Thus, the presence of ammonia ions is the evidence of pollution of water reservoirs. Therefore, highly sensitive and accurate method for the determination of ammonia ions concentration is necessary for ecological monitoring of natural waters. Existing methods have low sensitivity and are not precise. They allow determining the ammonia ions only in concentration range from 1 mM to 100 mM in the water. Therefore, for earlier prevention of water pollution there is high necessity for the development of new biosensor with high sensitivity to ammonia.

In this way the main task of our investigation is to find and characterize the new biosensor for ecological monitoring and clinical diagnostic. The basis of our investigation was the discovery of the phospholipid complex in dry wheat grains (Gilmanov et al., 1982). The complex consists of only one phospholipid: phosphatidylinositol (PI) and only one protein—glutamate dehydrogenase (GDh) (Al-Sohaimy, 2004).

While GDh may be released only by PI specific phospholipase C there is only one opportunity to explain this phenomenon that GDh is attached to PI membrane by its own covalently bound glycosylphosphatidylinositol anchor which is anchored to PI membrane by mechanism which is described by Low (Low, 1987) as illustrated in Fig. 1.

Thus, the GDh is attached to PI membrane by its own covalently bound PI anchor. The molecules of GDh form the dense protein covering of the complex.

We isolated the nanocomplex from dry wheat grains but it had not any catalytic activity.

In this reason the question arises: is there catalytically active nanocomplex in plants? For this purpose it is necessary to find such complex with high GDh activity and study its convenience for using as biosensor.

1.1. Purification of nanocomplex

While the active biochemical processes take place in maturing ears of the cereals we decided to isolate active nanocomplex from filling grains of wheat (*Triticum aestivum*) Steklovidnaya-24 cultivar and maize taken in milk-wax stage.

For isolation we cut off embryo parts of the taken seeds and then unembryonated filling grains were homogenized in porcelain mortar with 0.05 M morfoline ethane sulfonate (MES) buffer pH ~ 7.4,

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doi:10.1016/j.bios.2008.05.009

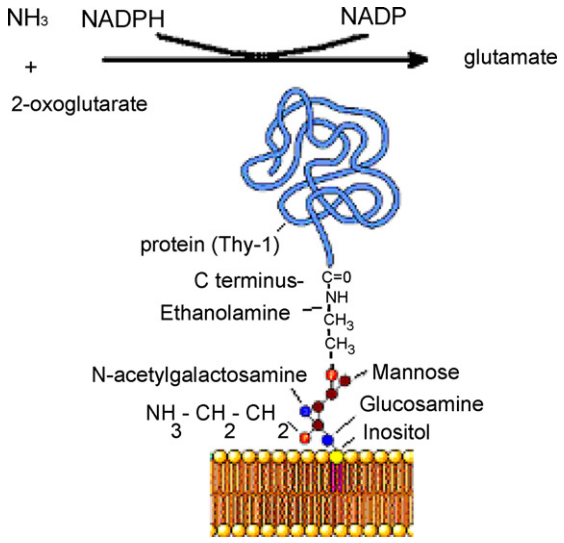

Fig. 1. The scheme of attachment of GPI protein to the PI membrane. GDh is attached to PI membrane of nanocomplex by its own covalently bound glycosylphosphatidylinositol anchor (Moran et al., 1991; Fergusson, 1999).

and the obtained homogenate was centrifuged at 10000 × *g* for 10 min on refrigerating centrifuge type K-24 (Germany).

For purification of active nanocomplex from filling seeds of wheat and maize for the first time we have used the nanostructured carbon sorbent which was developed by supervisor professor Z.A. Mansurov in Combustion problem Institute of Al-Farabi Kazakh National University (Mansurov and Zhubanova, 2008). This carbon sorbent contains carbon nanostructured elements which provide rigid carcass of the sorbent. Using this sorbent allows us to purify nanocomplex which is 2 to 3 times faster and which has 4 to 5 times higher activity than Sepharose 4B column chromatography.

Then supernatant was purified by chromatography on column with "Nanocarbosorb" ARK type which is produced by scientific-industrial technological center "Zhalyn" (Almaty, Kazakhstan). Results of purification of nanocomplex from filling grains of wheat and maize are presented in Fig. 2. The fraction of nanocomplex was eluted in the first peak after chromatography on column with "Nanocarbosorb" ARK type.

1.2. Characterization of nanocomplex

We determined natural catalytic activity of Nicotinamide adenine dinucleotide phosphate-GDh (NADP-GDh) of nanocomplex from filling grains of wheat and maize in reaction of reductive amination of 2-oxoglutarate by the spectrophotometry at 340 nm on the spectrophotometer Ultrospec-1100 pro, Amersham Bioscience (UK). Reaction mixture contained 15.4 mM 2-oxoglutarate, 0.1 mM NADPH, 0.05 M MES buffer pH ~ 8.3, and different concentrations of $(NH_4)_2SO_4$. The concentration of 2-oxoglutarate and NADPH was taken at full saturation concentration. The excess of these substrates did not inhibit the reaction velocities. The total volume of reaction mixture was 2 ml. The controls were without 2-oxoglutarate or ammonia. The specific activity of NADP-GDh is expressed as μM of formed NADP per mg protein in 1 min. The quantity of protein was determined by microbiuret method which is described by Bailey (Bailey, 1967).

It is necessary to note that the studied fraction of nanocomplex shows high activity of NADP-GDh without any treatment with detergents, phospholipases and other agents. Thus, the whole body of nanocomplex shows up its activity without disturbing its integrity. The specific activities of NADP-GDh of both fractions are equal: 148 ± 3 μM formed NADP per 1 mg protein in 1 min for nanocomplex fraction from filling wheat seeds and 155 ± 4 μM formed NADP per 1 mg protein in 1 min for nanocomplex fraction from filling maize seeds.

First of all it is necessary to study the dependence of activity of wheat nanocomplex anchored NADP-GDh on ammonia concentrations. The obtained results of these experiments are presented in Fig. 3. The same dependence was obtained for maize nanocomplex.

It is necessary to note that after achievement of maximal activity for both cases the further increase in ammonia concentration above optimal leads to inhibition of NADP-GDh activity by ammonia.

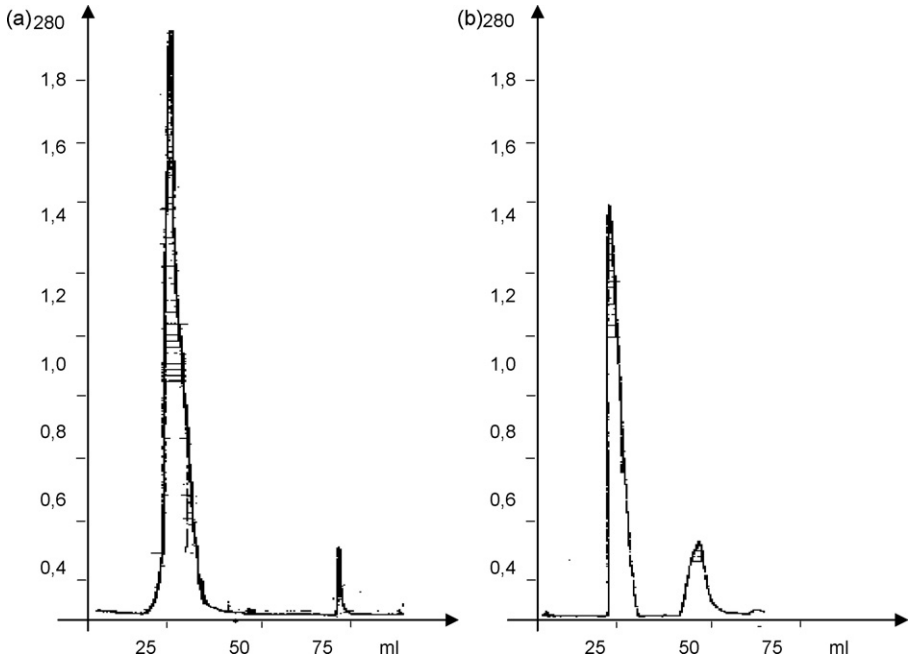

Fig. 2. Separation of cell-free extracts obtained from filling seeds of wheat (a) and maize (b). Column size is Ø3 cm × 20. Column was filled with "Nanocarbosorb" ARK type and washed with 0.05 M MES buffer, pH ~ 7.4.

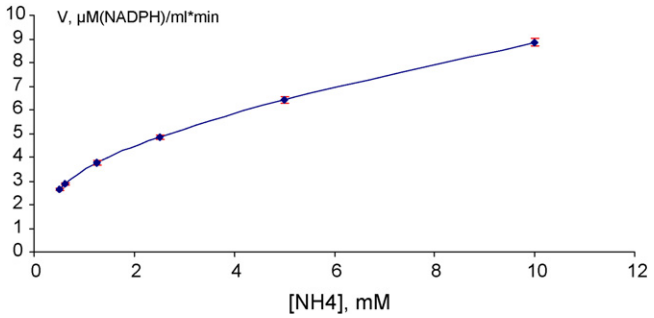

Fig. 3. The dependence of activity of NADP-GDh of nanocomplexes from filling grains of wheat. (RSD = 1.44%).

Thus, the NADP-GDh of nanocomplex of both fractions has very high affinity to ammonia. These results show that this nanocomplex plays key role in ammonia assimilation in filling grains of the cereals. So the high sensitivity of nanocomplex to ammonia allows it to serve as nanobiosensor to determine very low concentrations of ammonia.

In this reason this property of nanocomplex opens the perspective of its practical application.

It was shown that use of nanocomplex allows us to determine the concentration of ammonia ions with high accuracy in range from 0.5 μM to 10 μM. Thus, we developed highly precised and sensitive nanobiosensor for determination of ammonia ions, which has no analogues. Thus, we developed effective method of purification of nanocomplex from filling seeds of cereals. On the basis of this nanocomplex the method of determination of low concentrations of ammonia for ecological monitoring was developed. And this method is also convenient for determination of ammonia, 2-oxoglutarate and NADPH in biological liquids, which is very important in clinical diagnostic of different diseases.

Application of nanobiosensor allows determining the concentration of 2-oxoglutarate in range from 1 mM to 10 mM and concentration of NADPH in range from 0.5 μm to 100 μM.

Thus, using the nanostructured carbon sorbent we developed simple method of isolation of high precise nanosensor for ecological monitoring and clinical diagnostic. This investigation was supported by grant from Centre of Biological Investigation of Ministry of Education and Science of the Republic of Kazakhstan, and by the grant of International Science and Technology Centre (ISTC) project K297.

2. Conclusions

The nanocomplex that consists of phosphatidylinositol and NADP-glutamatedehydrogenase was isolated from the filling grains of wheat and maize by chromatography on nanostructured carbon sorbent. This nanocomplex shows high sensitivity to ammonia ions in linear range from 0.5 μM to 10 μM. This nanocomplex may be very useful as highly sensitive biosensor for determination of ammonia for aims of ecological monitoring, and for the determination of ammonia ions, 2-oxoglutarate and NADPH in clinical diagnostic. Due to low price and high sensitivity of this biosensor it can find wide application for prevention of pollution of natural water reservoirs by waste waters and for diagnostic of pathological states in clinical diagnostic.

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