Effect of anoxia and *Polyscias filicifolia* Bailey biomass tincture on the activity of tRNA and aminoacyl-tRNA synthetases in isolated pig heart

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Key words: *anoxia; pig heart; Polyscias filicifolia Bailey; tRNA; aminoacyl-tRNA synthetase.*

Summary. *Objective. The aim of this study was to investigate effect of anoxia and Polyscias filicifolia Bailey biomass tincture on the activities of different tRNA and aminoacyl-tRNA synthetases in isolated pig heart.*

Material and methods. The isolated pig heart was perfused according to the modified method of Langendorf, using an artificial blood circulation apparatus. Anoxia 20 min in duration was performed by perfusion of isolated heart with Krebs-Henseleit bicarbonate buffer saturated with gas mixture (95% N_2 and 5% CO_2). Control heart was perfused with the same buffer saturated with gas mixture (95% O_2 and 5% CO_2). Effect of Polyscias filicifolia Bailey biomass tincture was evaluated by perfusion of isolated heart with a buffer containing tincture. Total tRNA and aminoacyl-tRNA synthetases were isolated from pig heart. Activities of tRNA and aminoacyl-tRNA synthetases were measured by the aminoacylation reaction using C^{14}-amino acids.

Results. Anoxia 20 min in duration has caused a decrease in the acceptor activity of tRNA and increase in the activities of aminacyl-tRNA synthetases. Polyscias filicifolia Bailey tincture did not affect the acceptor activity of tRNA and activities aminacyl-tRNA synthetases. After 20-min anoxic perfusion with the buffer containing Polyscias filicifolia Bailey biomass tincture, the acceptor activities of tRNA increased to the control value and activities of aminacyl-tRNA synthetases reached the control value.

Conclusions. The acceptor activity of tRNA from isolated pig heart decreased and activities of aminacyl-tRNA synthetases increased under anoxia. Perfusion with buffer containing tincture of Polyscias filicifolia Bailey biomass restored acceptor activities of tRNA and activities of aminacyl-tRNA synthetases.

Introduction

During the past several years, much new evidence has accumulated regarding the molecular and biochemical mechanisms underlying cardiac responses to hypoxia. Ischemia and hypoxia cause alterations in different tissues and organs (1–3). The heart is an organ with particular susceptibility to hypoxia (4). The oxygen deprivation for 20–40 min induces irreversible histochemical and functional changes in the myocardium (5). Protein synthesis system is especially sensitive to the shortage of oxygen in cells (1, 3, 6). Changes in protein synthesis under ischemia and anoxia may be determined by the alterations in energetic state of cells and/or functional activity of translation system components (1, 7). The lack of oxygen affects different levels of the protein synthesis system: regulation of mRNA translation (3, 6) as well as aminoacyl-tRNA formation (8, 9).

Pharmaceutical industry has been offering various herbal preparations that along with adaptogenic properties have protective power against harmful effects of ischemia and anoxia. Preparations from *Aralaceae* family herbs (*Panax ginseng*, *Panax notoginseng*, *Panax quinguefolium*, *Eleutherococcus senticosus*, *Aralia manshurica*, *Polyscias filicifolia*) have antimutagenic, antioxidative activity and protect against ischemia and anoxia. Moreover, these preparations influence enzymatic activity (2, 10–14). Ginseng root is one of the most popular herbs throughout the world and is believed to be a panacea and to promote longevity. It has been used as a medicine to protect against cardiac ischemia, a major cause of death in Western countries. It was shown that *Panax ginseng* prevents myocardial ischemia-reperfusion injury induced by

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ischemia (12, 15). However, high prices of ginseng root limit its usage. Preparations of other plants from *Aralaceae* family are often used instead.

One of such preparations is *Polyscias filicifolia* Bailey biomass. It was shown that the preparation of cultured *Polyscias filcifolia* Bailey cells normalized the intensity of protein synthesis, duration of synthesis of the average polypeptide chain, and activities of aminoacyl-tRNA synthetases in rabbit liver under experimental myocardial ischemia (16). *Polyscias filicifolia* Bailey biomass tincture showed a protective effect on the total protein synthesis and some components of translation system in isolated anoxic pig myocardium (17, 18). However, we need more detailed investigations to prove possible protective effect of *Polyscias filicifolia* Bailey biomass tincture on the activity of tRNA and aminoacyl-tRNA synthetases under anoxia.

The aim of this study was to investigate the effect of *Polyscias filicifolia* Bailey biomass tincture on the acceptor activities of tRNA and activities of aminoacyl-tRNA synthetases specific to alanine, glutamic acid, leucine, and serine in isolated pig heart under normoxia and anoxia.

Material and methods

Experiments were done on isolated pig hearts weighing 100–150 g. Pig hearts were obtained from a slaughterhouse. Preparation, control and anoxic perfusion were performed immediately after slaughter. The hearts were perfused according to the modified method of Langendorf (19), using an artificial blood circulation apparatus. Anoxia was performed by the perfusion of isolated pig heart with Krebs-Henseleit bicarbonate buffer saturated with gas mixture (95% N_2 and 5% CO_2). Control hearts were perfused with the same buffer saturated with gas mixture (95% O_2 and 5% CO_2). Effect of anoxia was evaluated after 20-min anoxic perfusion. With the aim to determine effects of *Polyscias filicifolia* Bailey biomass, pig heart was perfused under normoxic and anoxic conditions with a buffer containing tincture of *Polyscias filicifolia* Bailey (0.5 mL tincture /1000 mL buffer).

Polyscias filicifolia Bailey biomass was obtained from Dr. V. A. Kunakh, Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine. The tincture of *Polyscias filicifolia* Bailey was prepared according to the requirements for preparations of tinctures (17).

Preparations of total tRNA were obtained by phenol deproteinization of pig heart extract and by further DEAE-cellulose column chromatography according to Brungraber (20) with the subsequent deacylation as described earlier (21). Preparations of total aminoacyl-tRNA synthetases were isolated by the DEAE-cellulose column chromatography of pig heart postribosomal supernatant as described in (22). Acceptor activities of tRNA in total tRNA preparations isolated from the control and anoxic myocardium were measured and renaturation of inactive tRNA conformers was performed as described in (23). Activities of aminoacyl-tRNA synthetases in preparations of total aminoacyl-tRNA synthetases were measured by the initial rate of aminoacylation (18). Activity of inorganic pyrophosphatase was measured colorimetrically as described in (9). Significance of difference between groups was estimated using Student's *t* test. Changes are statistically significant when $P<0.05$.

Results

It was shown that 20–40-min anoxia caused significant histochemical and functional changes in the myocardium (5). Therefore, we chose 20-min anoxic perfusion to investigate the effect of anoxia on the activities of $tRNA^{Ala}$, $tRNA^{Glu}$, $tRNA^{Leu}$, $tRNA^{Ser}$ and alanyl-, glutamyl-, leucyl-, and seryl-tRNA synthetases. Pig hearts from the control animal group were perfused under normoxic conditions in adequate timespan. With the aim to evaluate the effect of *Polyscias filicifolia* Bailey tincture on the activities of tRNA and aminoacyl-tRNA synthetases, this preparation was added to the perfusion buffer. In our previous study, we have shown that activities of tRNA and aminoacyl-tRNA synthetases did not change after adding ethanol into perfusion buffer under normoxic and anoxic conditions. Concentration of ethanol was the same as in tincture of *Polyscias filicifolia* Bailey (18).

Acceptor activity of total tRNA preparations isolated from the control (normoxic) and anoxic myocardium was measured by the ability to accept the following C^{14}-amino acids: alanine, glutamic acid, leucine, and serine. These amino acids were included in different quantities in the composition of proteins synthesized in the cells of myocardium (23). The results of a comparative study in vitro of the acceptor activity of tRNA are summarized in Table 1.

The data obtained show that the acceptor activity of tRNA specific for alanine, glutamic acid, leucine, and serine decreased by 32–58% after 20-min anoxic perfusion in comparison with the control. After 20-min anoxic perfusion with the buffer containing *Polyscias filicifolia* Bailey biomass tincture, the acceptor activities of these tRNA increased to the control value. However, *Polyscias filicifolia* Bailey biomass tinc-

Table 1. **Effect of *Polyscias filicifolia* Bailey biomass tincture on the acceptor activities of tRNA in preparations of total tRNA from control pig myocardium and myocardium after 20-min anoxia**

[^{14}C] amino acid	Control	Control + *Polyscias*	Anoxia	Anoxia + *Polyscias*
Alanine	3.03±0.17	3.04±0.27	1.28±0.07*	2.82±0.20
Glutamic acid	3.73±0.34	4.41±0.65	2.00±0.16*	4.55±0.60
Leucine	2.75±0.19	3.17±0.18	1.86±0.18*	3.02±0.23
Serine	2.57±0.18	2.55±0.22	1.23±0.06*	2.46±0.09

*Difference between the control and experimental groups is statistically significant.
(nmol aminoacyl-tRNA/per mg of tRNA; mean±SE; n=8)

ture did not affect the acceptor activities of $tRNA^{Ala}$, $tRNA^{Glu}$, $tRNA^{Leu}$, $tRNA^{Ser}$ in preparations of total tRNA from the control pig heart.

As it was reported earlier, the treatment of total tRNA preparations with magnesium ions did not affect the acceptor activity of $tRNA^{Leu}$ from the normoxic myocardium. However, addition of magnesium ions resulted in recovery of $tRNA^{Leu}$ acceptor activity to the control level after 20-min anoxia (9, 23).

Results in Fig. 1 show that the acceptor activity of $tRNA^{Leu}$ from the control and anoxic myocardium that was perfused with the buffer containing *Polyscias filicifolia* Bailey biomass tincture did not change after treatment with magnesium ions and remained at the control level. On the other hand, the acceptor activity of $tRNA^{Leu}$ after anoxic perfusion with the buffer containing *Polyscias filicifolia* Bailey biomass tincture was the same as in the control.

We have studied the effects of anoxia and *Polyscias filicifolia* Bailey biomass tincture on the activities of alanyl-, glutamyl-, leucyl-, and seryl-tRNA synthetases. Results in Table 2 demonstrate that the activities of these aminoacyl-tRNA synthetases in the preparation of total aminoacyl-tRNA synthetases from pig myocardium after 20-min anoxia increased by 66–77% in comparison with the control.

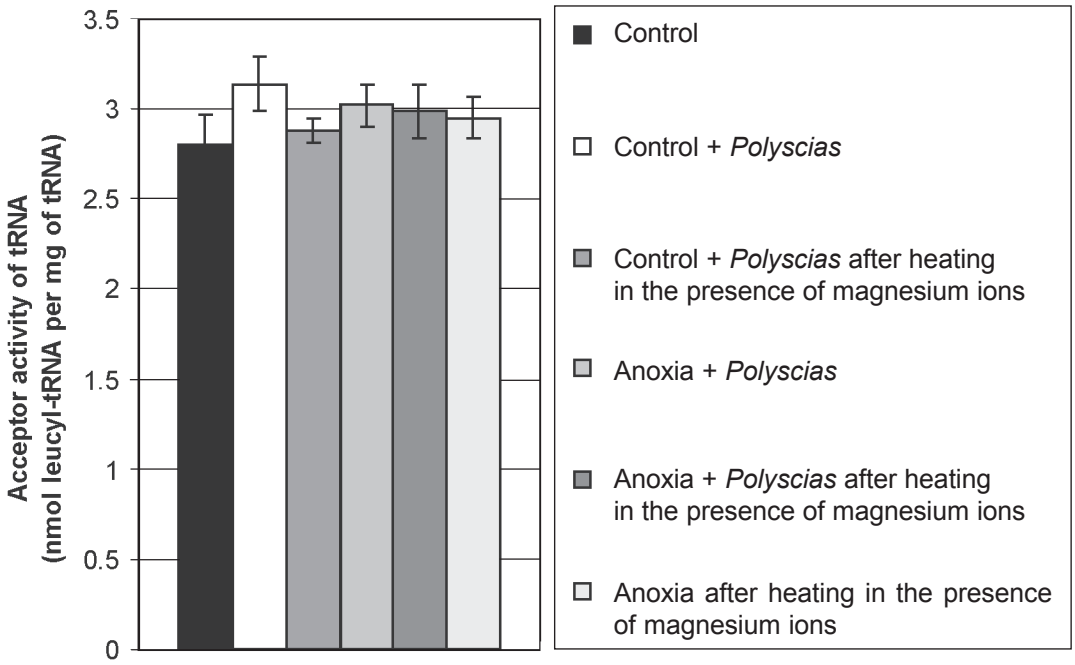

Fig. 1. **Effect of *Polyscias filicifolia* Bailey biomass tincture on the acceptor activity of $tRNA^{Leu}$ from control and anoxic (20 min of anoxia) myocardium and after heating in the presence of magnesium ions**
Control (normoxia) represents acceptor activity of $tRNA^{Leu}$ in preparations of total tRNA obtained from pig hearts after perfusion under normoxic conditions with buffer without *Polyscias filicifolia* Bailey tincture. Data represent results of 8–10 separate experiments.

Table 2. **Effect of *Polyscias filicifolia* Bailey biomass tincture on activities of aminoacyl-tRNA synthetases in preparations of total aminoacyl-tRNA synthetases from control pig myocardium and myocardium after 20-min anoxia**

[^{14}C] amino acid	Control	Control + *Polyscias*	Anoxia	Anoxia + *Polyscias*
Alanine	40.61±3.60	37.80±3.50	68.72±4.70*	37.02±2.40
Glutamic acid	42.60±4.62	36.62±4.00	71.80±6.41*	43.00±6.10
Leucine	71.82±6.50	63.21±6.21	127.42±15.12*	74.02±7.53
Serine	44.10±3.51	44.60±3.11	73.60±2.10*	39.04±2.30

*Difference between the control and experimental groups is statistically significant.
(pmol/min per mg of protein; mean±SE; n=8)

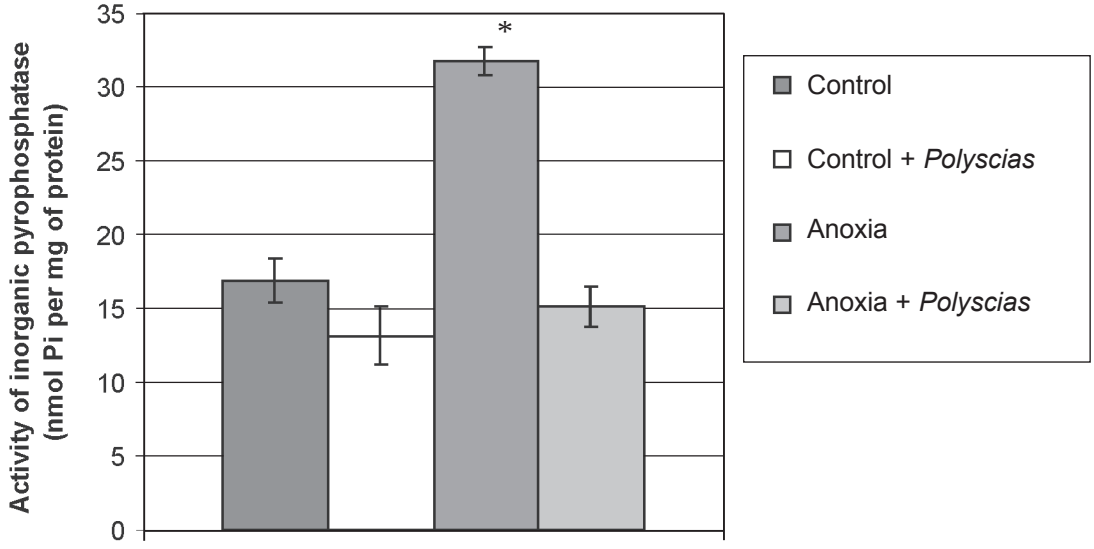

Fig. 2. **Effect of *Polyscias filicifolia Bailey* biomass tincture on the activity of inorganic pyrophosphatase in preparations of total aminoacyl-tRNA synthetases from control pig myocardium and myocardium after 20-min anoxia**

Control (normoxia) represents activity of inorganic pyrophosphatase in preparations of total aminoacyl-tRNA synthetases obtained from pig hearts after perfusion under normoxic conditions with buffer without *Polyscias filicifolia* Bailey tincture. Data represent results of 8–10 separate experiments.
*Difference between the control and experimental groups is statistically significant.

Polyscias filicifolia Bailey biomass tincture did not affect the activities of these aminacyl-tRNA synthetases from normoxic pig heart. The activities of aminoacyl-tRNA synthetases after 20-min anoxic perfusion with the buffer containing *Polyscias filicifolia* Bailey biomass tincture reached the control value.

Results in Fig. 2 show the effect of anoxia and *Polyscias filicifolia* Bailey biomass tincture on the activity of inorganic pyrophosphatase in total preparations of aminoacyl-tRNA synthetases from normoxic and anoxic pig myocardium.

The activity of inorganic pyrophosphatase after 20-min anoxia increased by 87% compared to the control. *Polyscias filicifolia* Bailey biomass tincture did not affect the activity of inorganic pyrophosphatase from control pig heart but after 20-min anoxic perfusion with the buffer containing *Polyscias filicifolia* Bailey biomass tincture, the activity of inorganic pyrophosphatase decreased to the control value.

Discussion

The obtained data indicate that acceptor activity of pig myocardium tRNA specific for alanine, glutamic acid, leucine, and serine after 20-min anoxic perfusion decreased as compared to the control. Perfusion of pig heart with the buffer containing *Polyscias filicifolia* Bailey biomass tincture did not have influence on the acceptor activities of tRNA from control pig

heart. Activities of these tRNA after 20-min anoxic perfusion with the buffer containing *Polyscias filicifolia Bailey* biomass tincture were the same as in the control. These results confirm reports about the protective action of *Polyscias filicifolia* Bailey biomass on rabbit liver tRNA, when preparations of biomass were orally administered before experimental myocardial ischemia (24). The decrease in the acceptor activity of tRNA may be associated with conformational changes of some molecules of tRNA under anoxia (9, 23). Reversible alterations of the three-dimensional structure of tRNA molecules resulting in the formation of functionally inactive conformers can be considered one of the possible reasons for the changes in the acceptor activity of tRNA. Magnesium ions are essential for the tertiary structure of tRNA (25). Short-term treatment (5 min at 60˚C) of tRNA preparations with magnesium ions led to renaturation of tRNA molecules (9, 23). It was shown that the acceptor activity of tRNA from control and anoxic myocardium, which was perfused with the buffer containing *Polyscias filicifolia* Bailey biomass tincture, did not change after treatment with magnesium ions and remained at the control level. Moreover, after anoxic perfusion with the buffer containing *Polyscias filicifolia* Bailey biomass tincture, the acceptor activity of tRNA was the same as in the control. Therefore, it may be assumed that *Polyscias filicifolia* Bailey biomass tincture is the factor that protects tRNA molecules against conformational changes.

Aminoacyl-tRNA synthetases play a central role in translation by providing the aminoacyl-tRNA used in protein biosynthesis (26). The data on the activity of alanyl-, glutamyl-, leucyl-, and seryl-tRNA synthetases showed that the activity of these enzymes under anoxia significantly increased as compared to the control. The activities of the aminacyl-tRNA synthetases after normoxic and anoxic perfusion with the buffer containing *Polyscias filcifolia* Bailey biomass tincture did not change and was the same as in the control. The obtained data showed that *Polyscias filicifolia* Bailey biomass tincture had a protective effect on aminoacyl-tRNA synthetases under anoxia. These results confirm the data that preparations of *Polyscias filicifolia* Bailey biomass have a protective effect on activities of aminoacyl-tRNA synthetases from rabbit liver under experimental myocardial ischemia (16, 24).

Catalytic activity of aminoacyl-tRNA synthetases depends on various cytoplasmic factors such as inorganic pyrophosphatase that catalyses cleavage of inorganic pyrophosphate, a potent inhibitor of tRNA aminoacylation (27). The activity of inorganic pyrophosphatase, which is found in preparations of total aminoacyl-tRNA synthetases, after 20-min anoxic perfusion significantly increased as compared to the control. However, after 20-min anoxic perfusion with the buffer containing *Polyscias filicifolia* Bailey biomass tincture, the activity of inorganic pyrophosphatase reached the control values. Comparison of the *Polyscias filicifolia* Bailey effect on the activities of aminoacyl-tRNR synthetases and inorganic pyrophosphatase under anoxia showed that changes in the activities of aminoacyl-tRNA synthetases are associated with the alterations in the activity of inorganic pyrophosphatase, which is found in preparations of total aminoacyl-tRNA synthetases. It may be concluded that changes in the activity of inorganic pyrophosphatase are one of the reasons of altered functional activity of aminoacyl-tRNA synthetases under anoxia. One of the possible mechanisms of the protective action of *Polyscias filicifolia* Bailey biomass tincture on the activity of aminoacyl-tRNA synthetases might be its effect on inorganic pyrophosphatase, which is important in the regulation of the aminoacyl-tRNA synthetase activity.

Conclusions

1. Acceptor activities of pig myocardium tRNA specific for alanine, glutamic acid, leucine, and serine after 20-min anoxic perfusion decreased as compared to the control.

2. Activities of tRNA specific for alanine, glutamic acid, leucine, and serine after 20-min anoxic perfusion with the buffer containing *Polyscias filicifolia* Bailey biomass tincture did not change and were as in the control.

3. Activities of alanyl-, glutamyl-, leucyl-, and seryl-tRNA synthetases isolated from pig heart after 20-min anoxic perfusion significantly increased as compared to the control.

4. The activities of alanyl-, glutamyl-, leucyl-, and seryl-tRNA synthetases, after anoxic perfusion with the buffer containing *Polyscias filicifolia* Bailey biomass tincture, did not change and were as in the control.

5. Changes in the activities of aminoacyl-tRNA synthetases after anoxic perfusion with the buffer containing *Polyscias filicifolia* Bailey biomass tincture are associated with alterations in the activity of inorganic pyrophosphatase.

Anoksijos ir *Polyscias filicifolia Bailey* tinktūros poveikis tRNR ir aminoacil-tRNR-sintetazių aktyvumui kiaulės širdyje

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Raktažodžiai: anoksija, kiaulės širdis, *Polyscias filicifolia Bailey*, tRNR, aminoacil-tRNR-sintetazė.

Santrauka. *Tyrimo tikslas.* Ištirti anoksijos ir *Polyscias filicifolia Bailey* tinktūros poveikį įvairių tRNR ir aminoacil-tRNR-sintetazių aktyvumui suminiuose preparatuose, išskirtuose iš kiaulių širdžių.

Tyrimo medžiaga ir metodai. Kiaulių širdys buvo perfuzuotos pagal modifikuotą Langendorf metodą naudojant dirbtinės kraujotakos aparatą. 20 min. anoksija buvo sukeliama perfuzuojant kiaulės širdį Krebs-Henseleit bikarbonatiniu buferiu, įsotintu dujų mišiniu (95 proc. N_2 ir 5 proc. CO_2). Kontrolinės gyvūnų grupės širdys buvo perfuzuotos tokios pačios sudėties buferiu, įsotintu dujų mišiniu (95 proc. O_2 ir 5 proc. CO_2). *Polyscias filicifolia Bailey* biomasės tinktūros poveikis įvertintas perfuzuojant širdį buferiu, į kurį buvo įpilta tinktūros. Iš kiaulių širdžių išskirti suminiai tRNR ir aminoacil-tRNR-sintetazių preparatai. tRNR ir aminoacil-tRNR-sintetazių aktyvumas nustatytas pagal aminoacilinimo reakciją naudojant C^{14}-aminorūgštis.

Rezultatai. Po 20 min. perfuzijos anoksijos tRNR akceptinis aktyvumas sumažėjo, o aminoacil-tRNR-sintetazių aktyvumas padidėjo. Įpylus į perfuzijos buferį *Polyscias filicifolia Bailey* tinktūros, akceptinis tRNR aktyvumas ir aminoacil-tRNR-sintetazių aktyvumas nekito. Po 20 min. perfuzijos anoksijos buferiu, kurio sudėtyje buvo *Polyscias filicifolia* tinktūros, tRNR akceptinis aktyvumas ir aminoacil-tRNR-sintetazių aktyvumas normalizavosi iki kontrolės dydžio.

Išvados. Anoksijos metu kiaulių širdžių akceptinis tRNR aktyvumas sumažėjo, o aminoacil- tRNR-sintetazių aktyvumas padidėjo.

Perfuzija buferiu, kurio sudėtyje buvo *Polyscias filicifolia Bailey* biomasės tinktūros, normalizuoja akceptinį tRNR aktyvumą ir aminoacil-tRNR-sintetazių aktyvumą.

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