JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

COMPOSITION AND PROPERTIES OF HIGH-POLYMER DNA FROM GONADS OF DIFFERENT SPECIES OF HYDROBIONTS

*Yulia M. Pozdnyakova, Tatiana N. Pivnenko**

Address(es):
Research Institute of Innovative Biotechnologies, FSBEI HPE Far Eastern State Technical Fisheries University, Lugovaya 52 B, Vladivostok, Russia, 690087.

*Corresponding author: tnpivnenko@mail.ru

https://doi.org/10.15414/jmbfs.3082

ARTICLE INFO

Received 7. 5. 2020
Revised 8. 1. 2021
Accepted 8. 6. 2021
Published 1. 12. 2021

Regular article

OPEN ACCESS

ABSTRACT

The method was elaborated for obtaining high-polymer DNA from male gonads of hydrobionts as a potential macromolecular matrix for production of preparations for various purposes. A comparative analysis of chemical composition and activities of endogenous proteases and deoxyribonucleases was performed for four fish species and a sea scallop and bovine spleen (as the main source of raw materials) in order to identify factors that affected the degree of DNA depolymerization in the process of its isolation. The DNA content of male gonads of the hydrobionts exceeded that in the bovine spleen 4-11 times. It was found that the activity profiles of endogenous proteases in gonads of all studied hydrobionts were generally similar (with the exception of that in pollock). Nucleases of various species were characterized by a wide range of activity values. The degree of DNA depolymerization, qualitative and quantitative composition of protein and lipid components of the obtained preparations determined the possibility of their use as medical drugs. The method of obtaining the target product with the use of salt extraction and ethanol precipitation was supplemented by proteolysis in order to remove residual protein to a value of no more than 5% of dry weight. A stepwise change in the composition of the preparations and in the DNA characteristics in the process of isolation and purification was shown. The obtained preparations were evaluated by spectral analysis with comparison of the obtained data with phage λ DNA, by electrophoresis for determination of molecular mass and by estimation of hyperchromic effect.

Keywords: gonads of aquatic organisms, DNA, proteases, deoxyribonucleases, degree of depolymerization, hyperchromic effect

INTRODUCTION

Preparations of nucleic acid isolated from animal, plant and microbial sources are used in the manufacture of medicines, cosmetics, dietary supplements, specialized foods (for children, sports, and therapeutic purposes), as well as for the production of nucleotides, nucleosides, purines and pyrimidines (**Yamamoto *et al.*, 1997; Kim, 2014; Chiesa *et al.*, 2016; Attarwala *et al.*, 2018; Besednova *et al.*, 2018**).

Currently, DNA preparations with a molecular mass (Mm) of 300 to 600 kDa (the so-called low-molecular-weight form) are recommended as effective in the treatment and prevention of influenza, cardiovascular, respiratory and allergic diseases. These compounds as positive modifiers of the biological response of the body to aggressive exogenous and endogenous effects are recommended for immunotherapy and immunorehabilitation in oncology (**Lipford *et al.*, 1997; Goodman *et al.*, 1998; Van Uden and Raz, 1999; Rykova *et al.*, 2001; Besednova and Zaporozhets, 2008; Besednova *et al.*, 2018; Jiang *et al.*, 2019**).

DNA efficiency with a molecular mass higher than $4\text{-}6 \cdot 10^6$ Da was confirmed by Ukrainian researchers as a macromolecular matrix that binds cisplatin (dichlorodiaminoplatin) molecules, one of the most commonly used cytostatics (**Gómez-Ruiz *et al.*, 2012; Kulinchik, 2013**). Based on these studies, the drug "Polyplatillen" was developed and introduced into medical practice (**Volchemkova *et al.*, 2007; Shalimov *et al.*, 2007; https://platos.uaprom.net/p288127 onkologicheskij-himiopreparat-poliplatillen.html**). This drug represents a conjugate of cisplatin and a macromolecular carrier - DNA from the cattle spleen. This drug has a high selectivity of action on tumors of different localization, while its chemotherapeutic index is three times higher compared with cisplatin, and the overall toxicity is almost an order of magnitude lower.

There are published data on the use of DNA of various origins for immunotherapy of some malignant neoplasms, both as an independent drug and also in combination with some known chemotherapeutic agents (**Rybalko *et al.*, 2006; Nikolin *et al.*, 2006; Alyamkina *et al.*, 2012**). It is assumed that the origin and Mm can significantly affect the half-life period of DNA, its biodegradation, vascular permeability, uniform distribution, accumulation and localization directly in the tumor. The efficiency of the molecular transport and the intracellular delivery of DNA can change 250-1000 folds and depends on the structure of the molecule (**Rybalko *et al.*, 2006**).

The limited raw material base that is currently used and a low yield of the target product made it necessary to search for new DNA sources; we offer for this purpose male gonads of hydrobionts (fish and invertebrates). Unlike farm animals, aquatic organisms of Pacific Ocean are significantly more environmentally and toxicologically safe; they are provided by large fishing resources and, moreover, we can obtain from hydrobionts a high yield and purity of the target component. Classical methods for isolation of nucleic acids from complex initial samples include a number of stages that use organic solvents such as phenol and/or chloroform, sodium perchlorate, lithium chloride, and guanidine derivatives (**Bowtell, 1987; Tan and Yiap, 2009; Antonova *et al.*, 2010**). The use of such reagents greatly complicates the procedure for isolation of DNA preparations and requires a thorough and complete removal of the applied reagents.

Endogenous enzymes (proteases and nucleases) originally found in fish sperm cells have a great influence on the degree of DNA polymerization: this determines the need to study endogenous enzymatic systems and methods for regulating their activity in the first stages of purification.

The aim of this work was to study the biochemical composition of male gonads of hydrobionts, including the activity of endogenous enzymes, to develop on this basis effective methods for producing high polymer DNA and to study its molecular characteristics.

MATERIAL AND METHODS

Reagents, chemicals and objects

The following reagents were used in the work: substrates for determination of the activity of enzymes - casein according to Hammersten (Calbiochem, USA), bovine hemoglobin; high-molecular-weight DNA ("ISN", USA); chymopsinum (Samson Med Ltd., Russia); phage λ DNA (Sibenzym, Russia)

The objects of our study were male gonads (milts) with the maturity stages 4-5 of the chum salmon *Oncorhynchus keta*, Amur sturgeon hybrid *Acipencer schrenckii* and kaluga *Huso dauricus*, the Pacific herring *Clupea pallasi pallasi*, the pollock *Theragra chalcogramma*, and the Japanese scallop *Patinopecten yessoensis*, the fish specimens were harvested and delivered to the laboratory frozen. For analysis, we used specimens that had been stored frozen for 3 months at temperature minus 18 °C until the study.

Determination of protein, lipids and water

Determination of protein included a Kjeldahl assay (Nx6.25) on a Kjeltec Auto 10 SO Analyzer (Sweden) according to the Official Methods of Analysis of AOAC International - 20th Edition 2016. Lipids were extracted using ethanol-chloroform 1:2 in an amount of 8–10 mL per gram of wet tissue, according to the method of Folch. Amount of water was measured on a Kett F-1A infrared moisture balance (Kett Electric Laboratory, Japan).

Determination of DNA

The DNA content was determined by the Dische method (**Patterson *and* Mura, 2013**) in raw materials and by the method of Spirin in preparations (**Karklinya *et al.*, 1989**).

Enzyme Assay

Total proteolytic activity was determined by an increase in the optical density in the solution containing a substrate (casein) and an enzyme. A 2-mL portion of 2% solutions of the appropriated substrate was mixed with 2 g milt homogenate and incubated at 37 °C for 60 min. The reaction was terminated by addition of an equal volume of 5% TCA. After filtering through paper filters for coarse precipitation (yellow tape), optical density of supernatants was measured at 280 nm against a control solution. A unit of activity was assumed to be the amount of the preparation (g) which causes the absorption to increase by 1 optical unit per 1 min (**Gokcek *et al.*, 2016**).

The activities of deoxyribonucleases were determined by the number of acid-soluble oligonucleotides formed during the enzymatic hydrolysis of native DNA. When determining the activity of Ca, Mg-dependent deoxyribonuclease, a reaction mixture consisting of 5 mM Tris-HCl buffer (pH 7.5), 10 mM $MgSO_4$, and 400 μg double-stranded DNA was used. When determining the activity of acid deoxyribonuclease, a reaction mixture consisting of 0.12 M sodium acetate (pH 5.0) 8 mM EDTA-Na, and 400 μg double-stranded DNA was used. Further procedures were the same. 1 mL of 3% aqueous milt extract was added to 1 mL of the reaction medium. Samples were incubated for 1 h at 37 °C. The reaction was stopped by adding 3 mL of 0.5 M perchloric acid solution. Samples were centrifuged for 15 min at 4000 rpm. The optical density of the supernatant was measured at 260 nm against the control solution (**Guéroult *et al.*, 2010**).

Determination of DNA molecular weight

The molecular weight of DNA was estimated by gel electrophoresis in 1% agarose gel. The volume of the applied sample was 5 μL. Electrophoresis conditions: 0.1 M Tris-borate buffer, pH 8.3; the electric field strength of 1 V/cm; duration of 40-60 min. Staining was performed with ethidium bromide at a concentration of 70 μg/mL. To determine the molecular weight (Mw) of DNA, we used a pUC/Msp I marker set, containing markers from 34 to 501 bp (base pairs) (Sibenzim Ltd., Russia).

Statistical analysis

All experiments were carried out least in triplicate, and data were expressed as means ± standard deviation (σ). Statistical processing of experimental data was performed using the Statistica 6.0 and Microsoft Excel, software package. One-way analysis of variance (ANOVA) was applied, arithmetic mean (M), standard deviation (σ), confidence interval (p) performed wherever applicable. Differences were considered significant at $p < 0.05$. The confidence level was 95%.

RESULTS AND DISCUSSION

The chemical compositions of milts of hydrobionts

To develop methods for isolating DNA preparations, we used fishery objects of the Far Eastern region with their significant reserves and large volumes of fishery harvest. Important prerequisites for their choice were a high weight fraction of gonads (at least 2% of the body weight) with at least 3% DNA content. Table 1 lists these species, the general chemical composition of gonads and – for comparison – the data for the currently used source for industrial production of DNA – the spleen of cattle (*Bovinae*). An insignificant amount of RNA (up to 0.3% of the initial tissue weight) contained in sperm cells of hydrobionts allows excluding an additional separation stage for this component (**Kasyanenko *et al.*, 1997**).

Table 1 Comparative characteristics of the chemical compositions of milts of hydrobionts and the bovine spleen, % of the initial weight

Object	DNA	Water	Protein	Lipids
Chum salmon	8.0 ±0.35^a	78.5±3.14^a	12.5±0.51^a	1.0±0.04^a
Sturgeon	9.5±0.45^b	60.0±1.8^b	5.5±0.22^b	25.0 ±1.1^b
Pollock	3.2±0.15^c	84.3±4.6^c	11.2±0.43^a	1.9±0.09^c
Herring	4.5±0.23^d	75.9±3.02^d	15.8±0.62^c	3.8±0.15^d
Japanese scallop	3.3±0.13^c	77.9±3.11^d	16.8±0.66^c	2.9±0.11^e
Bovine spleen	0.9±0.03^e	77.9±3.09^d	16.9±0.67^c	2.8±0.12^e

Note: Different superscript letters in the same column represent significant difference between the all species with same treatment. $n = 6$, $p < 0.05$

The DNA content in gonads of fish and scallop is subject to changes in accordance with the degree of the development of the reproductive tissues. The highest DNA concentration was recorded at the pre-spawning and spawning stages as shown in Table 1.

The highest content of DNA was found in the gonads of sturgeon (9.5%) and chum salmon (8.0%). This amount was significantly higher than that in the bovine spleen (0.9%). The amount of protein ranged from 11.2 to 16.8%, with the exception of the value for sturgeon gonads (5.5%). The lipid content was not high – from 1.0 to 3.8%, with the exception again for sturgeon gonads (25.0%). Proteins and lipids are ballast components in DNA preparations; their contents are strictly regulated for pharmaceutical drugs.

Enzymatic activities of milts of hydrobionts

The enzymatic activities of the reproductive tissue of aquatic organisms are directly related to the maturation of the reproduction cells. Activity of deoxyribonucleolytic enzymes (DNAse) is of great importance for regulation of these processes. In gonads of various fish species, enzymes have been identified that hydrolyze single- and double-stranded DNA molecules: alkaline metal-dependent DNAse I (EC 3.1.4.5), the reaction products of which are mono-, oligo- and polynucleotides with terminal 5'-phosphoryl groups and acid DNA bases II (EC 3.1.4.6), forming oligonucleotides with 3'-terminal phosphoryl groups (**Moore, 1981**).

The same enzymes have a significant effect on the degree of DNA polymerization during isolation (**Epstein *et al.*, 1998**). In addition, fish sperm cells contain proteolytic enzymes that hydrolyze histones or protamines (in salmon fish). By destroying proteins, proteases are able to create conditions for direct contact of DNA with endogenous nucleases. It is also known that endonucleases are formed from a high-molecular-weight precursor and are activated by proteases (**Lebedeva *et al.*, 1995; Kitazumi *and* Tsukahara, 2011**).

To develop methods for monitoring the enzymatic degradation of DNA in the primary stages of isolation, we determined the activity of endogenous deoxyribonucleases and proteases in the sperm cells of fish and scallop (Table 2).

Table 2 The activity of endogenous enzymes in the gonads of hydrobionts, U/g tissue (n = 3)

Object	Proteases			DNAses	
	Acid pH 3.0	Neutral pH 6.0	Alkaline pH 8.0	Acid pH 5.0	Alkaline Ca, Mg-dependent pH 7.5
Chum salmon	0	0.07±0.003	0.33±0.010	381.0±17.1	105.0±4.3
Surgeon	0	0.06±0.002	0.14±0.005	184.4±7.7	59.7±2.4
Pollock	0.14±0.005	0.05±0.002	0.05±0.006	Traces	Traces
Herring	0	0	0.45±0.023	19.4±0.7	160.0±7.25
Scallop	0.16±0.006	0	0.16±0.007	565.0±22.6	89.8±4.48

The obtained results show the predominance of acidic DNAase, similar to DNAase II. The highest activity of such enzymes occurred in the scallop gonads, further – in descending order: in the gonads of chum salmon, sturgeon, and herring. In pollock gonads, the activity of nucleolytic enzymes has only trace values.

Protease activity in the gonads of the studied species in a slightly alkaline zone was relatively high in comparison to the values for the digestive organs in the same species. Thus, the activity of proteases in pyloric caeca (an analogue of the pancreas of higher vertebrates) was 5.3 U/g for chum salmon and 1.9 U/g for herring at pH 8.0 (**Pivnenko *et al.*, 1997**). The activity of acidic and neutral proteases was not found in herring gonads, no activity of acidic proteases – in gonads of chum salmon and sturgeon, and no neutral proteases – in scallop. Pollock was the only one among the studied species with proteases in operated in a wide pH range, but, unlike proteases in other species, they are the least active in an alkaline medium. The activity of alkaline proteases, presumably chymotrypsin type ([**Pivnenko *et al.*, 1997; Li *et al.*, 2009**) was predominant in all species, but not in pollock.

The obtained results suggest that the high activity of DNAases in fish gonads in combination with the effect of proteases can lead to rapid degradation of DNA during the process of isolation. Thus, the previously used and rapidly reproducible method for DNA isolation involved homogenization of salmon gonads in the maturity stage IV, salt extraction, followed by precipitation of the target product with organic solvents, and the final air drying (**Albulov *et al.*, 2013**). As a result, the obtained preparation had the following basic characteristics: DNA content was 70.5 ± 3.5%, protein content was 15.5 ± 0.7%, Mw of DNA was about 300 kDa. Such properties did not allow using this product as a macromolecular matrix for cytostatics.

High-polymer DNA purification

The technology proposed by us for the production of high-polymer DNA from gonads of hydrobionts was based on the method of nucleoprotein precipitation with ethanol after extraction with 2 M NaCl solution. Changes made to this method provided the most complete removal of ballast proteins while maintaining a high degree of DNA polymerization.

Defrosted milts were washed twice with distilled water, homogenized for 10 min at a speed of 8000 rpm at a temperature of 0 ± 2 °C with addition of a solution containing 0.9% sodium chloride and 0.15 M sodium citrate, in the ratio milts : solution of 1: 1. To precipitate the nucleoprotein complex, sodium chloride was added to the homogenate to a final concentration of 2 M. The resulting DNA strands were washed in an ethanol gradient with an increasing concentration from 50 to 96%. Next, the precipitate was dissolved in distilled water, in a ratio of 1: 100 (V: V). After that, 3% chymopsinum was added to solution and incubated for 3 hours. In this case, it is important to use highly purified enzyme preparations without nuclease impurities. After limited fermentolysis, the enzymes were thermally inactivated at a temperature of 70 °C for 20 min. After quick cooling, DNA was subjected to reprecipitation with an equal volume of ethanol and washing with ethanol. For effective drying of the preparation (up to 12% water), exposure to air is sufficient.

A gradual change in the composition of preparations can be observed on the example of chum salmon and sturgeon gonads. As Table 3 shows, the precipitation stage with the use of 2 M NaCl gives a nucleoprotein complex with almost equal amounts of DNA and protein; after fermentolysis, the protein content sharply decreases.

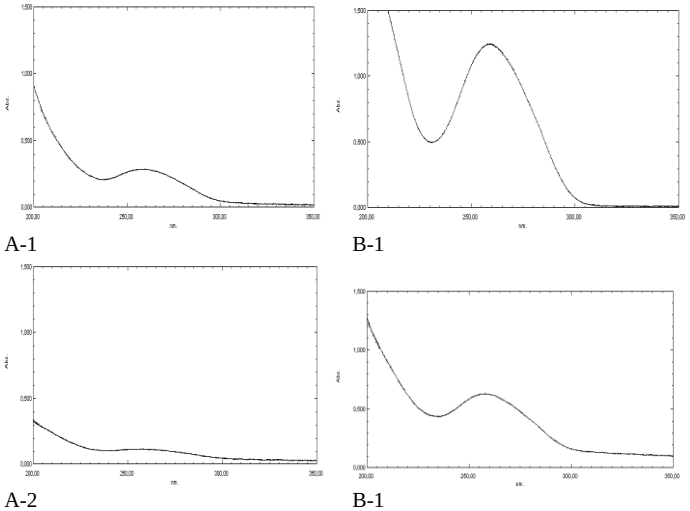
However, the preparation of sturgeon sperm cells contains a significant amount of lipid admixture, which was not completely eliminated even after the introduction of the stage of diethyl ether treatment. To assess the purity of the DNA preparation (without RNA), the optical density of the solution is measured at wavelengths of 260 and 280 nm, the absorption maximums of DNA and protein solutions. The value of the ratio $A_{260/280}$ for pure DNA should be at least 1.8 (**Tan and Yiap, 2009; Antonova *et al*., 2010; Shin, 2013**). Among the samples obtained in our study, this value was measured only in DNA preparation from chum salmon gonads. Figure 1 shows the absorption spectra of aqueous solutions of the obtained preparations at a concentration of 0.05 mg/ml in the range from 200 to 350 nm at the same stages of purification as indicated also in Table 3.

Table 3 Changes in the composition of preparations from fish gonads at different stages of DNA extraction

Stage	Content, %				$A_{260/280}$	Yield, % of the initial weight
	DNA	protein	water	lipids		
Chum salmon						
Before fermentolysis	46.6 ± 2.7^a	45.4 ± 3.3^a	5.2 ± 0.3^a	-	1.5	5.7 ± 0.8^a
After fermentolysis	91.8 ± 5.9^a	4.9 ± 0.4^a	4.9 ± 0.2^a	-	1.9	4.6 ± 0.2^a
Surgeon						
Before fermentolysis	47.2 ± 2.8^b	42.0 ± 4.1^a	4.7 ± 0.5^a	5.5 ± 0.2^b	1.6	5.6 ± 0.3^a
After fermentolysis	83.8 ± 4.4^b	4.3 ± 0.7^a	5.5 ± 0.4^a	4.9 ± 0.2^b	1.7	3.7 ± 0.2^b

Note: Different superscript letters in the same column represent significant difference between the two species with same treatment. *n = 4, p < 0.05*

As a result of the effect of the enzyme preparation chymopsinum on the complex of DNA and protein from chum sperm cells, the protein admixture was removed after precipitation with 2 M NaCl. This change is indicated by spectral characteristics: the optical density at 260 nm increased 5 folds, the peak became more distinct (Fig. 1). The same effect was observed when DNA was purified from sturgeon gonads; after treatment with enzymes, a 4-fold increase of the optical density was observed at 260 nm.



A-1 B-1

A-2 B-1

Figure 1 Absorption spectra of DNA preparations in the process of their isolation.

Legend: A – after the first precipitation; B – the final preparation; 1– chum salmon; 2 – sturgeon

Composition of high-polymer DNA preparations

Using the developed technology, five DNA preparations were obtained from gonads of various hydrobiont species. Composition of these preparations is given in Table 4.

Table 4 Comparative characteristics of high-molecular-weight DNA preparations

Object	Contents, %				$A_{260/280}$
	DNA	protein	water	lipids	
Chum salmon	93.0±4.5^a	4.8±0.2^a	4.1±0.2^a	-	1.87^a
Surgeon	85.9±3.4^b	3.9±0.1^b	5.0±0.2^b	4.7±0.10^a	1.72b
Pollock	73.2±3.6^c	18.3±0.7^c	8.3±0.4^c	0.5±0.02b	1.35^c
Herring	73.5±3.6^c	16.0±0.6^c	7.1±0.3^c	3.5±0.17^c	1.63d
Scallop	80.3±3.2^d	11.7±0.5^d	8.0±0.3^c	-	1.49^e
Bovine spleen	96±3.8^e	2.7±0.1^e	4.3±0.1a	-	1.83^a

Note: Different superscript letters in the same column represent significant difference between the all species with same treatment. *n = 5, p < 0.05*

It is known that nucleic acids absorb UV radiation in the range of 240-290 nm with a maximum value at 260 nm. Pyrimidine nitrogenous bases of DNA serve in this case as chromophores, they absorb UV light in about 10-20 folds more intensively in comparison to the chromophores of protein molecules - tryptophan, tyrosine and phenylalanine, their absorption maximum is 280 nm. The maximum absorption of polysaccharides is 235 nm (**Tan and Yiap, 2009; Antonova *et al*., 2010; Shin, 2013**). In aqueous solutions of the obtained preparations, we measured the absorption spectra from 220 to 320 nm (Fig. 2). Phage λ DNA was considered as an identity criterion.

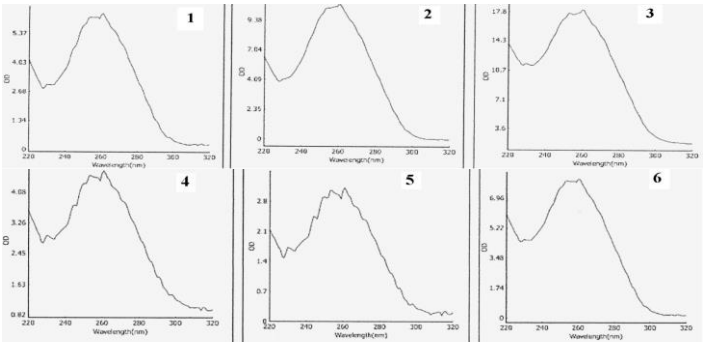


Figure 2 Spectrophotometric analysis of DNA (10-fold dilution).
Legend: abscissa: wavelength; ordinate: optical density; species: 1 - scallop, 2 - chum, 3 - pollock, 4 - sturgeon, 5 - herring, 6 - phage λ DNA

In all the samples, the absorption maximum was 260 nm, which corresponds to this value for phage λ DNA. The profiles of the DNA spectra of chum salmon, scallop and pollock gonads were the closest to the reference sample. The presence of additional small peaks in the spectrum profiles of sturgeon and herring samples is probably due to the presence of impurity components.
According to the requirements of the pharmacopeia article (**The State Pharmacopoeia of Ukraine, 2001**), the DNA content in the DNA preparation should be at least 90%, the protein content – no more than 5%, and no lipids at all. This article considers the ratio of the values of optical density in the solution of the preparation $A_{260/280}$ as a criterion of purity, the value of which should be at least 1.8. The Mw of the target product must be at least $4 \cdot 10^6$ Da.
Our studies have shown that only a preparation made from chum salmon gonads fully met all the above requirements. Other preparations had increased contents of either proteins (pollock, herring, and scallop) or lipids (sturgeon, herring, and pollock) and thus were unacceptable for the final product. The ratio $A_{260/280}$ also corresponded to the declared standards only for chum DNA. With the introduction of additional purification steps, undesirable changes appeared in the value of $A_{260/280}$ and the degree of polymerization of DNA.
The method of electrophoresis in agarose gel was applied to determine the DNA Mw in the obtained preparations (Fig. 3).

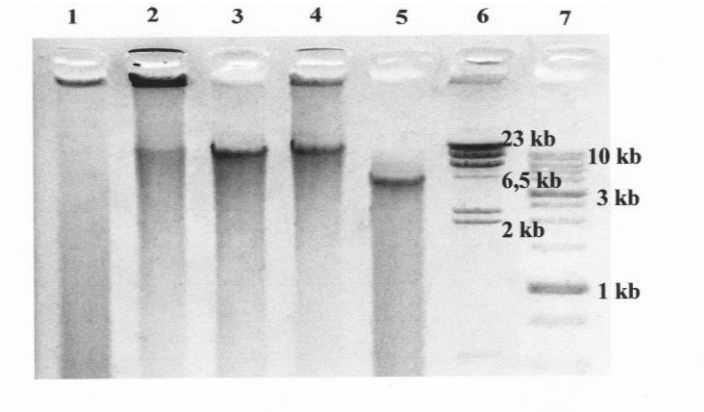


Figure 3 Electrophoregrams of DNA preparations from gonads of hydrobionts in 1% agarose gel.
Legend: 1– sturgeon; 2– chum; 3 – herring; 4 – pollock; 5 – scallop; 6-7 – markers; kb – kilobase (1000 nucleotide pairs), 1 kb = 690 kDa.

This value for herring and pollock DNAs was $1.5 \cdot 10^7$ Da, this corresponds to 23 thousand base pairs (kb). The same fraction was present in the preparation from chum salmon and sturgeon gonads, but the predominant components had Mw over $1.5 \cdot 10^7$ Da (more than 23 kb), and so this value corresponded to the requirements. The obtained scallop DNA preparations contained nucleic acid fractions with Mw from $1.3 \cdot 10^6$ Da (2 kb) and lower, this is not enough to consider this sample as the basis of the pharmaceutical preparation.

Thermal denaturation of DNA solutions

In the study of thermal denaturation of DNA solutions in the preparation from chum salmon gonads, we observed a helix-coil transition. This transition indicated a sharp increase in absorption at 260 nm in the melting temperature zone and was accompanied by a decrease in solution viscosity. When the melting point is exceeded by approximately 5°C, the DNA double helix strands diverge because of Brownian motion. For native, double-stranded DNA, the absorbance at 260 nm was significantly weaker than it would be expected if we summarize the absorption of all DNA bases. An increase in absorption at transition from polynucleotides to monomers or from double-stranded structures to single-stranded is called the hyperchromic effect. Hyperchromism increases with an increase of the chain length and reaches the limit value in oligonucleotides consisting of 5-6 residues ([**Paston *et al*., 2007**). With increasing temperature of solutions containing DNA from chum salmon gonads in 0.15 M NaCl, we observed an increase in the optical density at 260 nm. The hyperchromic effect was 56% (Fig. 4).

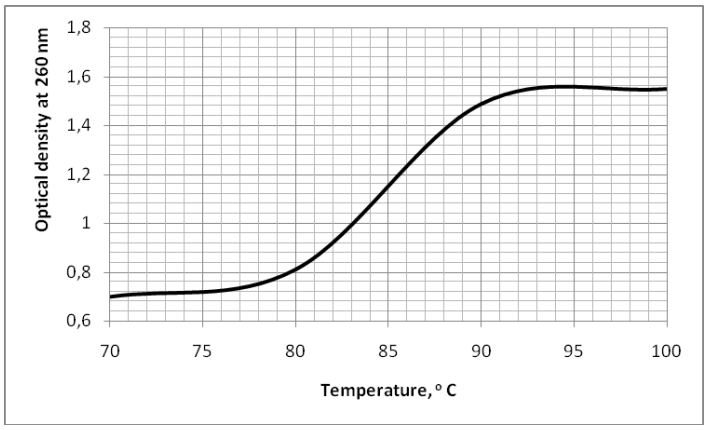


Figure 4 Temperature dependence of the optical density of DNA solutions in the preparation from chum salmon gonads at 260 nm

For comparison, we can cite data on the preparation of high-polymer DNA obtained from salmon sperm cells used as a chemical reagent isolated by the phenolic method: the N / P ratio is there 1.7-1.8, the protein content — 0.8-1.0%, the RNA content — 0.5%, the hyperchromic effect — 30%, and the melting point was 86.5°C (**MedicalPlanet. https://medicalplanet.su/genetica/541.html**).
It is known that any DNA and RNA molecule in solution is a nucleic acid salt, the cation in its composition corresponds to the salt, in the presence of which this molecule was precipitated. In this form, the DNA molecule is more stable. The DNA molecule has several sites of binding metal ions. The DNA that we obtained from salmon gonads is the sodium salt. It was previously shown that the sodium salt of DNA from chum salmon with an Mm of about 300 kDa can form fairly stable complexes with Ca^{2+}, Zn^{2+}, Fe^{2+} through an interaction of these cations with phosphate groups and heterocyclic bases of DNA; these complexes might be easily assimilated in human body (**Kasyanenko *et al*., 1997; Kasyanenko *et al*., 2017; Paston *et al*., 2017**). The type of such binding depends on the degree of denaturation and polymerization, the double-stranded structure has a higher negative charge on the helix groups, while the nitrogenous bases are hidden inside the helix. The type and the charge of the ion also determine the nature of the interaction. So, ions of alkali (Na, Li, K) and alkaline-earth (Mg, Ca, Ba) metals interact mainly with phosphate groups, while transition metal ions (Mn, Zn, Ni, Cu) bind to DNA bases also. The characteristics obtained in our study allow us to assume that DNA from hydrobiont gonads has a high complex-forming ability and ensures strong binding to the currently accepted or potential drugs and can reduce their toxic effects on the human body.

CONCLUSION

Thus, the developed method is capable to fulfill the basic condition for the quality requirements to high-molecular-weight DNA used in production of drugs with a cytotoxic effect – namely, a high DNA content in the final preparation (at least 95%) and a low protein content (not above 5%). Among the studied raw sources, salmon gonads are the most promising material. The yield and purity of the obtained preparations depend not only on the chemical composition of sperm cells, but also on the activity of endogenous nucleases and proteases. The revealed high activity of acid DNAases and alkaline proteases can cause DNA degradation in the process of isolation from the studied raw material. These characteristics differ significantly among hydrobiont species. The sufficient suppression of the activity of these enzymes at low temperatures at addition of sodium citrate can be achieved only for certain species (chum salmon and sturgeon). However, DNA preparations from sturgeon gonads contain an excessive amount of lipids, and the spectral characteristics do not correspond to the quality of a pure preparation. The high hyperchromic effect of DNA obtained from chum salmon gonads also proves the purity of the preparation obtained by us.
As a result of these investigations, we have obtained a high-polymer DNA from gonads of the chum salmon. These DNA preparations correspond in its quality to a high-molecular-weight matrix for sorption of drugs, including antitumor, antimetastatic, and immunostimulating remedies. In order to obtain a similar preparations from gonads of other fish species and from scallop gonads, technological methods require further refinement.

Acknowledgement: The authors are grateful to Dr. Nikolay N. Kovalev from Chair of biochemistry, microbiology and biotechnology of School of Natural Sciences of Far Eastern Federal University for his help in the analysis the DNA Mw by method of electrophoresis in agarose gel.

REFERENCES

Albulov, A.I., Frolova, M.A., Samuilenko, A.Ya., Vasilchenko, O.M., Shinkarev, S.M., Grin A.V., Rogov R.V. & Nemtsev, D. C. (2013). Method for producing DNA from salmon milt. Patent Russian Federation no. 2488634. (In Russian)

Alyamkina, E.A., Leplina, O.Y., Ostanin, A.A., Chernykh, E.R., Nikolin, V.P., Popova, N.A., Proskurina, A.S., Gvozdeva, T.S., Dolgova, E.V., Orishchenko, K.E., Rogachev, V.A., Sidorov, S.V., Varaksin, N.A. Ryabicheva T.G., Bogachev S.S. & Shurdov M.A. (2012). Effects of human exogenous DNA on production of perforin-containing CD8+ cytotoxic lymphocytes in laboratory setting and clinical practice. *Cellular Immunology.* 276(1-2), 59-66. http://dx.doi.10.1016/j.cellimm.2012.04.004

Antonova, O.S., Korneva, N.A., Belov, Yu.V. & Kurochkin, V. E. (2010). Effective nucleic acid isolation methods for analysis in molecular biology (review). *Nauchnoe Priborostroenie (Scientific Instrumentation).* 20(1), 3-99. (In Russian)

Attarwala, H., Han, M., Kim, J. & Amiji M. (2018). Oral nucleic acid therapy using multicompartmental delivery systems. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*. 10(2):10. http://dx.doi.10.1002/wnan.1478

Besednova, N.N., Kovalev, N.N., Fedyanina, L.N. Pozdnyakova, Yu.M., Kryzhanovsky, S.P. & Zaporozhets, T.S. (2018). Deoxyribonucleic acid of pro- and eukaryotes: medical aspects of use. Vladivostok : Dalnauka. (In Russian)

Besednova, N.N. & Zaporozhets, T.S. (2008). Fundamental and applied aspects of the study of biopolymers from hydrobionts of the Pacific Ocean. *Bulletin SB RAMS*, 4(132), 16-21. (In Russian)

Bowtell, D.D. (1987) Rapid isolation of eukaryotic DNA. *Anal Biochem.* 162(2). 463-465. http://dx.doi.10.1016/0003-2697(87)90421-0

Chiesa G., Busnelli M., Manzini S. & Parolini C. (2016). Nutraceuticals and Bioactive Components from Fish for Dyslipidemia and Cardiovascular Risk Reduction. *Mar. Drugs*.14(6). 113-128. http://dx.doi:10.3390/md14060113

Epstein, L.M., Kasyanenko, Yu.I. & Artyukov A.A. (1998). A method for producing a nucleoprotein complex. Patent Russian Federation no. 2122856. (In Russian)

Gómez-Ruiz, S., Maksimović-Ivanić, D., Mijatović, S. & Kaluđerović, G.N. (2012) On the Discovery, Biological Effects, and Use of Cisplatin and Metallocenes in Anticancer Chemotherapy. *Bioinorg Chem Appl*. 2012:140284. http://dx.doi.10.1155/2012/140284

Goodman, J.S., Van Uden, J.H., Kobayashi, H., Broide, D. & Raz, E. (1998). DNA immunotherapeutics: new potential treatment modalities for allergic disease. *Int Arch Allergy Immunol*. 116(3). 177-187 http://dx.doi.org/10.1159/000023943

Gokcek, K., Szabo, T., Alptekin, C., Tore, Y. & Urbanyi, B. (2016). A preliminary study on protease activity of Russian sturgeon, *Acipencer gueldenstaedtii Brandt and Ratzenburg, 1833*, at early life stages. *Turkish Journal of Fisheries and Aquatic Sciences*. 16: 1025–1029. http://dx.doi.10.4194/1303-2712-v16_4_29

Guéroult, M., Picot, D., Abi-Ghanem, J., Hartmann, B. & Baaden, M. (2010). How cations can assist DNAse I in DNA binding and hydrolysis. *PLoS Comput Biol*. 6(11): e1001000. http://dx.doi: 10.1371/journal.pcbi.1001000

Jiang, J., Kong, X., Xie, Y. Zou, H., Tang, Q., Ma, D., Zhao, X., He, X., Xia, A., & Liu P. (2019). Potent anti-tumor immunostimulatory biocompatible nanohydrogel made from DNA. *Nanoscale Res Lett*. 14, 217 http://dx.doi.org/10.1186/s11671-019-3032-9

Karklinya, V.A., Birska, I.A. & Limarenko, Y.A. (1989). Quantitative determination of nucleic acids in salmonidae milt by various methods. *Chem Nat Compd.* 25. 109–112. https://dx.doi.org/10.1007/BF00596713

Kasyanenko, N. A., Qiushi, Z., Varshavskii, M. S., Bakulev, V. M., & Demidov, V. N. (2017). DNA metal complexes and metallization of a macromolecule in solution. *Journal of Structural Chemistry*, *58*(2), 406-412. https://dx.doi:10.1134/S0022476617020275

Kasyanenko, Yu.I., Kovaleva, Yu.V., Epshtein, L.M. & Artyukov A.A. (1997). Preparation and properties of DNA derivatives from salmon milt. *Izvestiya TINRO (Transactions of the Pacific Research Institute of Fisheries and Oceanography)*. 120. 37–43. (In Russian)

Kim, S.K. (2014). Marine cosmeceuticals. *J. Cosmet. Dermatol*. 13(1). 56–67.https://dx.doi.org/10.1111/jocd.12057

Kitazumi, I. & Tsukahara M. (2011). Regulation of DNA fragmentation: the role of caspases and phosphorylation. *FEBS Journal.* 278 (3). 427–441. https://dx.doi.10.1111/j.1742-4658.2010.07975.x

Kulinchik, T.V. (2013). Cisplatin: a history of the discovery of antitumor activity. *Medical technology. Evaluation and Selection.* 1(11), 87-90. (In Russian)

Lebedeva, L.G., Aleksandrova, S.S., Banaskyan, A.G. & Votrin, I.I. (1995). Characterization of nuclear endo-DNases of rat liver in molecular weight and cationic dependence. *Biochemistry.* 60 (5). 798-804. (In Russian)

Li, P., Hulak, M. & Linhart, O. (2009). Sperm proteins in teleostean and chondrostean (sturgeon) fishes. *Fish Physiol Biochem*. 35(4). 567-581. https://dx.doi.org/10.1007/s10695-008-9261-y

Lipford, G.B., Sparwasser, T., Bauer, M., Zimmermann, S., Koch, E.S., Heeg, K. & Wagner, H. (1997). Immunostimulatory DNA: sequence-dependent production of potentially harmful or useful cytokines. *Eur J Immunol*. 27, 3420-3426, https://dx.doi:10.1002/eji.1830271242

Moore, S. (1981). Pancreatic DNase. *In* The Enzymes, Vol. 14, pp. 281–296. Edited by P.D. Boyer. Academic Press, New York. https://dx.doi.org/10.1016/S1874-6047(08)60341-8

Nikolin, V.P., Popova, N.A. & Sebeleva, T.E. (2006). Influence of exogenous DNA the growth of transplantable tumors. *Voprosy onkologii* . 52(1), 66-69. (In Russian)

Paston, S.V., Zyryanova, I.M., Zaichikova, Y.V. & Zamotin, V.V. (2007). Conformation changes in the DNA molecule caused by γ-irradiation of its water ethanol solutions of great ionic strength. *Journal of Structural Chemistry*, *48*(4), 734-739. https://dx.doi.org/10.1007/s10947-007-0112-y

Patterson, J. & Mura, C. (2013). Rapid Colorimetric Assays to Qualitatively Distinguish RNA and DNA in Biomolecular Samples. *Journal of Visualized Experiments.* 72 (e50225). https://dx.doi:10.3791/50225

Pivnenko, T.N., Epstein, L.M., & Okladnikova, S.V.(1997) Comparative Analysis of Substrate Specificity of Pancreatic Serine Proteinases of Different Origin. *Journal of Evolutionary Biochemistry and Physiology*. 33(6). 540-544 (In Russian)

Rybalko, S.L., Shlyakhovenko, V.A., Kozak, V.V., Olishevsky, S.V. & Yanish, Y.V. (2006). Immunomodulating CPG DNA: prospects for clinical use in oncology. *Oncology*. 8(2). 209-217. (In Russian)

Rykova, E.Yu., Laktionov, P.P. & Vlasov, V.V. (2001). The activating effect of DNA on the immune system. *Usp Sovrem Biol*, 121(2), 160-171. (In Russian)

Shalimov, S.A., Volchenskova, I.I., Yatsenko, L.D. Maydanevich, N.N., Korchevaya, L.M., Nezhina, M.V., Maydanevich, N.N. & Lenok, E.V. (2007). Phase-specific action of polyplatillen. *News of the Ukrainian Medical Dental Academy*. 7, 88-90. (In Russian)

Shin J.H. (2013) Nucleic Acid Extraction Techniques. In: Tang YW., Stratton C. (eds) Advanced Techniques in Diagnostic Microbiology. Springer, Boston, MA. https://dx.doi.org/10.1007/978-1-4614-3970-7_11

The State Pharmacopoeia of Ukraine. (2001). Kharkov: Publisher: RIREG. 572 p.

Tan S.C. and Yiap B.C. (2009). DNA, RNA, and protein extraction: the past and the present. *J Biomed Biotechnol*. 57, 43-98. https://dx.doi.org/10.1155/2009/574398

Van Uden, J., Raz, E. (1999). Immunostimulatory DNA and applications to allergic disease. *Journal of Allergy and Clinical Immunology*. 104(5). 902-910.). https://dx.doi.org/10.1016/S0091-6749(99)70066-7

Volchemkova, I.I., Maidanevich, N.N. & Budarin, L.I. (1990). Derivatives of platinum (P) with polysmon of deoxyribonuclcic acid. Method for obtaining them and pharmaceutical antitumor preparation based on them. Patent WO 1099011851

Yamamoto, S., Wang, M.-F., Adjei, A.A. & Ameho C.K. (1997). Role of nucleosides and nucleotides in the immune system, gut reparation after injury, and brain function. *Nutrition.* 13(4), 372-374.). https://dx.doi.org/10.1016/S0899-9007(96)00376-0