

SPECTRAL RESEARCHES OF AMITOZYN-NUCLEIC ACIDS INTERACTION

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Antitumor and immunomodulating activities are inherent to amitozyn antitumor preparation. But the mechanisms of its influence on the cell are not completely elucidated. Thus, studying of the mechanisms of amitozyn and its alkaloids action is of current importance, as it gives new possibilities of amitozyn wide use in clinics.

In the present paper, to elucidate molecular mechanisms of preparations interaction with DNA and RNA, luminescent and spectrophotometric analyses, that are the most informative in biopolymers and ligands interaction, were used.

Material and methods of researches.

Amitozyn, berberin chloride (Bch), thymus DNA, transfer RNA, ethidium bromide (EB) and actimicine D analog – ActH were used for studying of competitive connection of two ligands and DNA.

Fluorescence and phosphorescence spectra were registered using spectrometer, constructed in laboratory (of Kyiv Taras Shevchenko National University), and "Cary Eclipse" and "Hitachi" (Model 850) spectrofluorimeters. Absorption spectra were recorded using Specord UV VIS spectrophotometer.

Results and discussion

The research of lymphocytes blastogenic response revealed that amitozyn, in therapeutic doses, significantly stimulates DNA synthesis (1). This fact suggests that amitozyn interacts with DNA. Researches of fluorescence and phosphorescence of amitozyn water solutions and one of the preparation alkaloids – berberin, at DNA presence, showed that amitozyn fluorescence spectrum depends on the wave length of exciting radiation (that reveals the preparation as a multi-component one) (2). Nevertheless, berberin fluorescence spectrum, as it was expected, does not depend on the exciting wave length. Furthermore, it was determined that berberin fluorescence intensity grows at DNA presence approximately 60 times more comparing to berberin water solution without DNA.

In our opinion, this fact may be connected to DNA binding with berberin molecule (probably by intercalation). This effect, as is well known, related to decreasing of radiationless relaxation exciting probability and corresponding increasing of quantum yield of fluorescence. Similar effect is observed also for the amitozyn water solutions, however, the obtained results analysis indicates that not only berberin among all amitozyn alkaloids interacts with DNA. Amitozyn fluorescence spectrum at DNA presence differs from bound with DNA berberin fluorescence spectrum, although, fluorescence intensity of the preparation significantly increases. To identify amitozyn components, binding with DNA, further researches is necessary.

Investigation of phosphorescence spectra showed that triplet excitation is transferred through DNA macromolecule and localizes on berberin molecules (berberin phosphorescence spectrum has insignificant difference comparing to phosphorescence spectrum of berberin with DNA). Investigation of phosphorescence intensity depending on berberin concentration ratio and pairs of DNA bases revealed that minimal distance of the excitation effect is 20 pairs of DNA bases or 7 nanometers (3).

Thus, the data described above prove amitozyn and berberin interconnection with DNA. Therefore, the purpose of this work was to clarify the way of amitozyn and berberin binding to DNA. One of the approaches was spectrophotometric analyses of visible and ultraviolet spectral regions. Different DNA concentrations and permanent concentrations of the preparations were used. As the spectrophotometric measurements showed, absorption band displacement took place in the visible and ultraviolet spectral regions, together with its intensity decreasing and appearance of isobathic points. This fact indicates the connection of these substances with DNA matrix and presence of the only one connection type in the field of P/D small values. Such spectrophotometric manifestations are typical for ethidium bromide (EB) binding with thymus DNA, and forming of the complex of intercalation type is typical for the last one. And it is significant, at the high DNA concentrations, Bch spectra of compounds absorption do not pass through isobathic points, that indicates forming of the other type of complexing connection.

The exact constants and values of preparations connection, using different methods of calculating programs, were determined. It is essential to notice that constant of EB connection with thymus DNA was 2 times higher comparing to alkaloids we had studied.

For obtaining fundamental understanding of mechanism of amitozyn alkaloids and DNA connection, we have conducted research of competitive connection of its Bch alkaloid and DNA at EB intercalator presence. The research results showed that EB influences on Bch and DNA connection and is collateral for the place of connection.

We showed (using spectrophotometric and fluorescence methods) that at binding amitozyn preparation with DNA, other type of connection is carried out – furrow binding.

Study of berberin alkaloid and tRNA spectral interaction showed that spectrum shifts from 480 nm up to 535 nm and at the same time fluorescence

intensity increased. Calculations of association constant and stoichiometry of binding, conducted on one of the category of Bch binding site on tRNA molecule, showed that at alkaloid and tRNA interaction, specific Bch complex with tRNA ordered space structure has formed. And it is significant to notice that stoichiometry indicates the interaction of at least 3 ligand molecules with nucleic acid. Nature of the spectral changes indicates possible intercalation mechanism of planar cation Bch molecule between nitrogen bases areas of helical tRNA sections. In this case, Bch and tRNA interaction identifies with this intercalator and DNA connection, described above.

Conclusions

1. Using fluorescence and spectrophotometric research methods, berberin and DNA connection by intercalation was proved.
2. Spectrophotometric and luminescent researches showed that amitozyn components bind to DNA, forming predominantly complexes of the other type (probably, by furrow DNA binding).
3. Fluorometry of Bch and tRNA interaction testifies the forming of specific berberin complex with tRNA ordered space structure, and its stoichiometry indicates the interaction of at least 3 ligand molecules with nucleic acid. This fact proves probable connection by intercalation.