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Interactions of tannic acid and its derivatives (ellagic and gallic acid) with calf thymus DNA and bovine serum albumin using spectroscopic method

Magdalena Labieniec, Teresa Gabryelak *

Department of General Biophysics, Institute of Biophysics, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland

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Abstract

In the present investigation, an attempt has been made to study the interaction of chosen polyphenols (tannic, ellagic and gallic acids) with calf thymus DNA and bovine serum albumin (BSA) employing spectrofluorimetric technique. The fluorescence quenching of DNA-bound ethidium bromide (EB) and BSA-bound 1-anilinonaphthalene-8-sulfonic acid (ANS) by phenolic acids has been examined. As BSA contains two tryptophan residues, the polyphenols influence on protein by measuring the changes in the fluorescence of BSA in the presence of phenolic acids was also evaluated.

Our experiments prove that there is a direct interaction between phenols and DNA or BSA. The obtained data suggest that used acids can intercalate to DNA and interact strongly with BSA. The strongest interactions were observed between DNA and ellagic acid and between BSA and tannic acid. The conformational changes were revealed in DNA and BSA after incubation with tested phenolic acids and the extent depended on the phenol structure and the used concentration.

Keywords: BSA; ANS; Polyphenols; Calf thymus DNA; Stern–Volmer plot; Tryptophan fluorescence

1. Introduction

Higher plants synthesize various secondary metabolites including tannic acid and its derivatives such as ellagic or gallic acid [1]. As revealed in structural studies [2,3], these natural phenolic acids have a variety of biochemical and pharmacological properties, which are, at least in part, due to interactions between them and biomolecules such as DNA or proteins.

In our previous papers [4–7] we evaluated the influence of chosen acids on DNA and proteins in in vitro studies using digestive gland cells of freshwater mussel *Unio tumidus* and Chinese hamster cell line B14. Based on the experimental data we concluded that the used polyphenols contributed to the formation of DNA-strand breaks and caused oxidative changes in proteins by the formation of carbonyl groups. The next step in our work was to use the calf thymus (ct) DNA and bovine serum albumin (BSA) as simple molecular models to check the ability of phenolic acids to express the strong affinity to ct DNA and BSA which are the main targets of interactions with phenols. The results of the experiments which are described below let us complete and explain some of the observations obtained in our earlier studies.

Nucleic acid bound to some molecules generally exhibits marked changes in fluorescence properties and this phenomenon is used in the studies with DNA [8]. Ethidium bromide (EB), a polycyclic aromatic dye, is the most widely used fluorescence probe for DNA structure. It binds to DNA by intercalation, and shows little base pair specificity to bind to 5′-C-G-3′ dinucleotide step [9]. It is reported that the enhanced fluorescence of the ethidium bromide–DNA complex can be quenched at least partially by the addition

* Corresponding author. Tel.: +48 42 6354478; fax: +48 42 6354474.
E-mail address: tgabryl@biol.uni.lodz.pl (T. Gabryelak).

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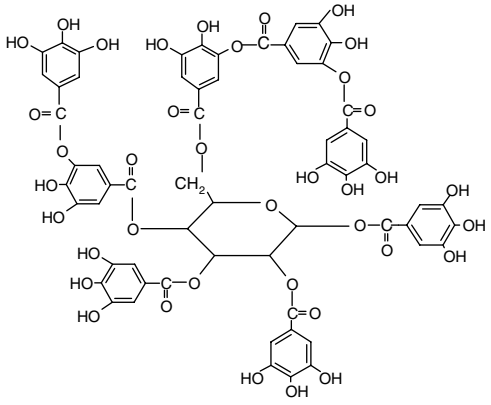
of a second molecule. Two mechanisms have been proposed to account for the quenching: the replacement of molecular fluorophores and/or electron transfer [10]. An experimental strategy for determining stability constants for ligand molecules based on quenching of ethidium bromide fluorescence via a competition for binding sites in DNA has become a standard method in nucleic acid studies. The binding of chosen polyphenols to DNA has been examined by some independent laboratories [11,12]. Their results revealed that several different mechanisms have been suggested for DNA damage caused by these acids, also including their binding to DNA.

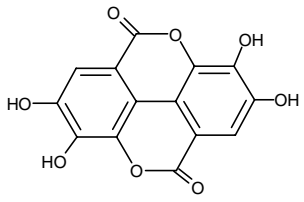
Bovine serum albumin (BSA) has often been used as a model protein to measure the reactivity with phenols and form soluble complexes with them. As the major soluble protein constituent of circulatory system, it has many physiological functions and plays a key role in the transport of many endogenous and exogenous ligands [13].

Because the used acids have numerous food and pharmacological applications, their influence on DNA and protein molecules is important for the investigation. Polyphenols also characteristically possess a significant binding affinity to proteins, which can lead to the formation of soluble and insoluble protein–phenol complexes [14]. Questions remain concerning whether and to what extent the protein–polyphenol interaction influences functionality.

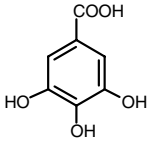
In the present work, we studied the DNA intercalation properties of tannic acid and its derivatives: ellagic and gallic acids (Fig. 1). We describe the fluorescence quenching of the DNA-bound ethidium ion by chosen phenolic acids.



tannic acid



ellagic acid



gallic acid

Fig. 1. Chemical structures of used phenolic acids.

These quenching processes probably occur by the replacement of the fluorophore and, therefore, we suggest that polyphenols can intercalate to DNA. Additionally, the experiments with BSA and with a fluorescent probe 1-anilinonaphthalene-8-sulfonic acid (ANS) indicate that the same phenolic acids interact with bovine serum albumin and change its conformation.

Although, quite a lot is known about biological properties of the used compounds, more information about their influence on biomolecules (DNA, proteins) is crucial for further investigation of their different applications.

2. Materials and methods

2.1. Apparatus

All fluorescence measurements were made with a Perkin–Elmer LS-50 B spectrofluorimeter. Absorption spectra were recorded on a Pharmacia LKB Biochrom 4060 ultraviolet–visible spectrophotometer.

2.2. Reagents

The calf thymus (ct) DNA, essentially-fatty-acid-free (fraction V) BSA, 1-anilinonaphthalene-8-sulfonic acid (ANS), ellagic acid and ethidium bromide (EB) were purchased from Sigma (St. Louis, MO, USA). Tannic acid and gallic acid were obtained from Aldrich–Sigma (London,UK). The distilled deionized water was used to prepare the solutions, all other chemicals were analytical reagent grade.

2.3. Procedures

2.3.1. The fluorescence studies of ethidium bromide bound to DNA in the presence of phenolic acids

The calf thymus DNA was used as received. Solutions of ct DNA gave a ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280} of 1.8–1.9, indicating that the DNA was sufficiently free of protein. DNA concentrations were determined spectrophotometrically with an extinction coefficient of 6600 $M^{-1}\,cm^{-1}$ at 260 nm [10]. All experiments were carried out at pH 7.0 in the buffer containing 50 mM NaCl and 5 mM Tris–HCl. DNA and EB were dissolved in buffer at the concentrations of 3 and 1 μg/ml, respectively. Phenolic acid's concentration was 10 μM.

EB displays very weak fluorescence in the aqueous solution. However, in the presence of DNA it exhibits intense fluorescence because of the intercalation to base pairs in DNA. Polyphenols were added to EB bound with ct DNA and the intensity of fluorescence of EB was measured. Fluorescence spectra were using excitation wavelength 478 nm and the emission range set between 480 and 850 nm.

Before examining the fluorescent properties of EB, it was checked if the used phenolic acids did not quench the EB fluorescence.