UP99476 FeBABE

Product

Part number : UP994760
Name : FeBABE , p-Bromoacetamidobenzyl-EDTA, iron (III) chelate, MW: 589.14
see product description

Review

Innovative method for the determination of contact sites for nucleic acid-to-protein or protein-to-protein interactions

- Applications of FeBABE (iron-p-bromoacetamidobenzyl EDTA) -

by Akira Ishihama

Summary

Iron(S)-1-(p-bromoacetamidobenzyl) ethylenediaminetetraacetate (**FeBABE**) can be conjugated to protein Cys residues, and introduces the contact-dependent cleavage of nucleic acids and proteins in the presence of hydrogen peroxide and ascorbate. The sequence-nonspecific cleavage of nucleic acids and proteins by FeBABE can be used for mapping of the contact sites of protein-protein and protein-nucleic acids (DNA and RNA). By using 2-iminothiolane (2-IT) as a linker, FeBABE can also be conjugated to protein Lys residues. The protein-tethered chemical probe with nuclease and protease activities has been employed for mapping of molecular interactions within transcription apparatus and translation machinery.

Key Words:
Chemical nuclease; Chemical protease; FeBABE; FeEDTA; protein-protein contact site; Protein-nucleic acid contact site

1. Introduction

The last half of the twentieth century was a magnificent era for life science research thanks to the progress in the molecule-level studies. Since the 1980's, in which the genome project was started, the focus of the research has shifted from single reaction mechanisms to composite phenomena and higher-order mechanisms of life carried out by molecular assemblies. The major research theme in the 21st century is expected to be the structure and function analyses of these molecular assemblies. The structure analysis of molecular assemblies is one of the most important factors for the advancement of the research. In recent studies, a technical improvement directly leads to the advancement of research, and a technological innovation is an important driving force for the development of research. In this article, we will introduce a newly developed method for the determination of the contact sites between constituent molecules in the molecular assemblies.

2. Study of the molecular mechanisms of genetic information transmission

In life sciences of the 20th century, the molecular biology methods have been utilized as the most powerful tools for the elucidation of mechanisms involved in the transmission of genetic information during genome replication and gene expression. These processes are typical biological reactions carried out by molecular assemblies. The replication, transcription and translation apparatus consist of a number of proteins. The specific interaction between these proteins and genomic DNA and/or RNA transcripts is the base for an accurate transmission of genetic information. So far, most of the components forming large molecular assemblies, involved in the genetic information transmission, have been identified. The focus of the research has shifted onto assembly mechanisms of the components, molecular interactions between the components, modification

mechanisms of the function of each component by the interaction with other components and the expression of new functions after the molecular assembly.

The transcription apparatus consists of the RNA polymerase with the catalytic function of RNA synthesis and a group of transcription factors that recognize the gene to be expressed and transport the RNA synthesis apparatus to the gene (Fig. 1).

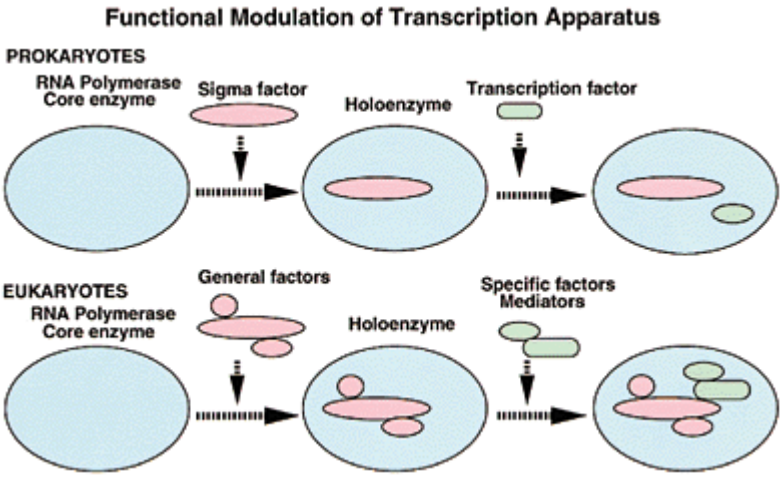


Fig. 1: Functional modulation of transcription apparatus

RNA polymerase is a molecular machine of RNA synthesis. The selection of the gene to be expressed is made by transcription factors. By the interaction between transcription factors and enzymes, RNA polymerase modulates to various transcription apparatuses that have different gene selectivity.

There are about 100 to 200 different species of transcription factors in a bacterial cell such as *E. coli*, several hundreds in yeast and over a thousand in mammalian cells. The transcription factors contact with the RNA polymerase at several different regions of the polymerase surface, and the transcription is initiated at a specific gene selected from numerous genome genes. In the case of *E. coli* RNA polymerase, for instance, the selectivity changes after two different stages of molecular interaction with transcription factors (Fig. 2). [1, 2] The gene expression pattern among 4000 species of genes in the *E. coli* genome is thus determined by these molecular interactions.

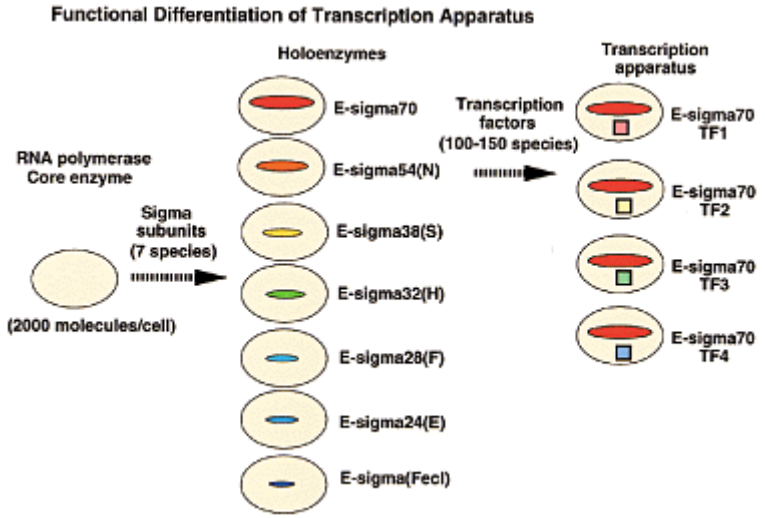


Fig. 2: Functional differentiation of transcription apparatus

E. coli RNA polymerase consists of 3 species of subunits ($Alpha_2BetaBeta'$ structure). It becomes holoenzyme by the combination with one of 7 species of sigma subunits, and highly specific selective differentiation occurs followed by the interaction with transcription factors.

3. Method for the determination of the point-of-contact site on DNA with a transcription apparatus

The transcription apparatus recognizes a promoter region and starts transcription at that site (Fig. 3). At the first stage, RNA polymerase binds to a genomic DNA without any sequence specificity, and slides on the DNA, and then finds the promoter region. Sometimes, a transcription factor binds to the RNA polymerase-promoter complex and induces opening of the promoter DNA. It is then followed by transcription of single stranded DNA. A simple method, so called "DNA footprinting method," which analyzes protected sites of DNA from the DNase digestion, is widely used for the determination of transcription factor binding sites on DNA. Instead of enzymes, chemical reagents or radicals that can cut or modify DNA are also used for the DNA footprinting method.

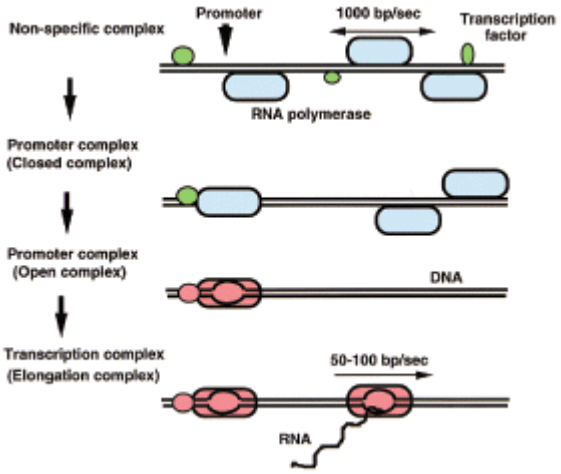


Fig. 3: Transcription mechanism

RNA polymerase selects a gene by the interaction with a transcription factor, and it synthesizes RNA. The transcription factor helps RNA polymerase to recognize the transcription starting point of the promoter, and initiates RNA synthesis.

In the case where many factor proteins combine together on a DNA, however, it is difficult to determine the binding site of each protein on the DNA. Moreover, it is extremely difficult to determine which part of a protein directly associates with the DNA. The development of a method to conjugate Fe-EDTA on a specific site of a protein now enables us to determine the binding site of each protein of multi-component assemblies on the DNA. Furthermore, this method allows us to identify the region of each protein component that makes direct contact with DNA.

4. Development of FeBABE

EDTA coupled with Fe^{3+} ion generates radicals under reducing conditions, and these radicals cut biological macromolecules. For the purpose of identifying contact sites of nucleic acid-protein and protein-protein interactions, BABE (*p*-bromoacetamidobenzyl-EDTA), which can be cross-linked to specific residues of proteins, has been developed and widely used in the life science research. In the beginning, BABE was developed as a cross-linking reagent to prepare metal-conjugated biomaterials. It was utilized for pharmaceutical and diagnostic experiments such as the identification of tumors in mice by radioactive In^{3+}-chelated EDTA conjugated at a terminus of breomycin A2, an anti tumor antibiotic.[3] Recently, the activity of Fe^{3+}-chelated BABE, FeBABE, which can cut both peptide bonds of proteins and phosphodiester bonds of polynucleotides in a sequence independent manner, has attracted researchers' attention [4,5], and its value has been recognized. Thus, an efficient method for FeBABE synthesis has been established. [6]

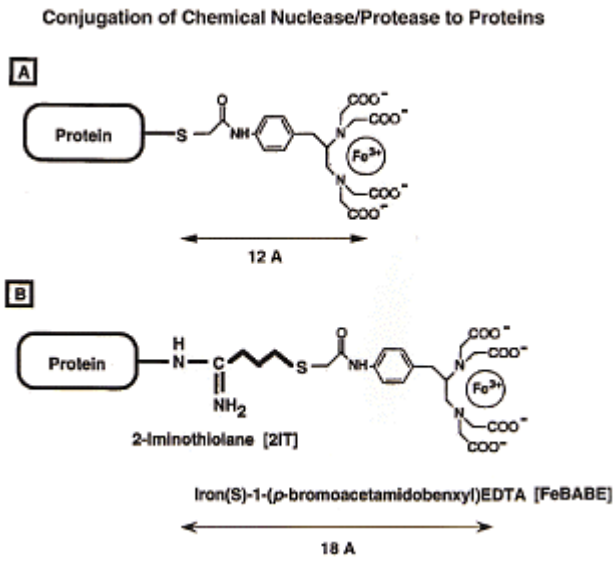


Fig. 4: Conjugation of chemical nuclease/protease to proteins

FeBABE was developed for protein labeling with EDTA. It is conjugated with cystein residues (A). Since 2-IT modifies amine to SH, it enables FeBABE to be conjugated with lysine residues through 2-IT, widening the application range of FeBABE.

EDTA compounds that have a protein binding capability are called "Meares Reagents." BABE reacts with the SH group of a cystein residue in a protein through its bromoacetamide linker under a mild condition (Fig. 4).

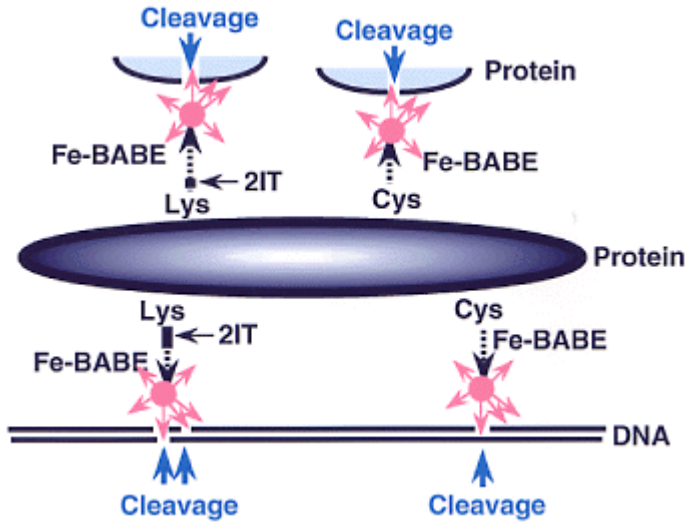


Fig. 5: Cleavage of nucleotides and proteins by FeBABE

In the presence of hydrogen peroxides and ascorbic acids, radicals are generated, which cut the DNA or protein around FeBABE. By the analysis of the cleavage sites, the point-of-contact sites can be estimated.

In the presence of ascorbic acids and hydrogen peroxides, reactive radicals are generated by Fe^{3+}-chelated BABE (FeBABE), which is conjugated with a native cystein residue of a protein or an introduced cystein residue by genetic manipulations. These radicals can cut a nucleotide chain or a peptide chain, which are located within the range of the reactive radicals' reach (Fig. 5). Since this reaction takes only several seconds, the cleavage process completes in a very short time such as 10 seconds to 10 minutes. It is estimated from the chemical structure of FeBABE that the cleavage site is at 12 angstroms distance from the cystein residue because Fe^{3+} of FeBABE is positioned in that area. By nucleotide sequencing and amino acid sequencing of the cleavage sites, a factor, with which FeBABE was in contact, and the contact site on the factor can be determined. The advantages of FeBABE include: (i) mild conjugation reaction with a peptide or a protein; (ii) mild cleavage reaction condition; (iii) rapid reaction and high yield; and (iv) chain cleavage reaction of nucleotides or proteins with no sequence specificity.

5. Use of FeBABE for the identification of point-of-contact sites between DNA and protein.

It was expected that the protein binding site of DNA or RNA could be identified by using a FeBABE-conjugated protein to determine the cleavage site of the DNA or RNA. Ishihama and his colleagues clearly identified RNA polymerase binding sites on promoter regions [7-11], and Dr. Noller and his colleagues also identified ribosome protein binding sites on rRNAs[12, 14].

E. coli RNA polymerase consists of four different species of subunits (Fig. 6). The sigma subunit is one of these subunits, and acts as the recognition factor for the promoter DNA for transcription initiation. The sigma subunit, however, is not necessary once the RNA synthesis is initiated. It is dissociated from the core enzyme soon after the transcription initiation, and is then reused by binding to another core enzyme that is not in the transcription cycle. *E. coli* has seven different kinds of sigma subunit, and each sigma subunit is involved in transcription of a different set of genes. In the study of transcription regulation mechanisms, it is important to identify what DNA sequences are recognized by each sigma subunit and which part of the sigma protein is involved in this process. We prepared a large number of sigma subunit mutants that have a single cystein residue at various regions of the subunits, and conjugated FeBABE to the purified mutant sigma proteins. By the analyses of the DNA cleavage sites using these mutants, the contact sites of each sigma subunit along the respective promoter DNA were determined.[9-11] By this experiment, it became clear that the four commonly existing domains in sigma subunits are involved in the bindings at different sites of promoter DNA (Fig.6).

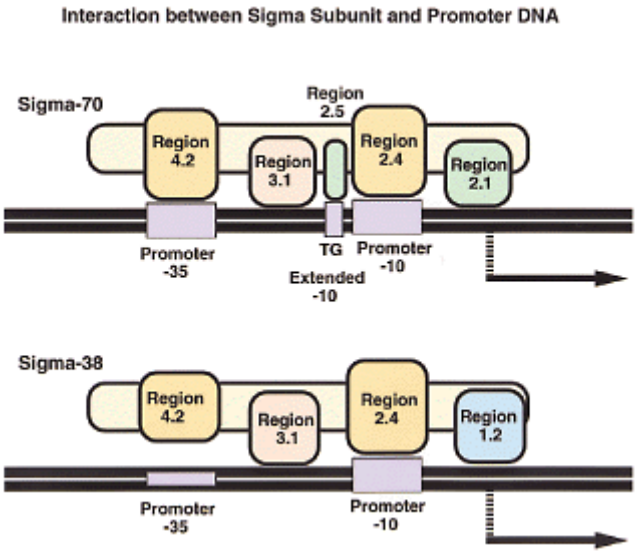


Fig. 6: Interaction between sigma subunit and promoter DNA

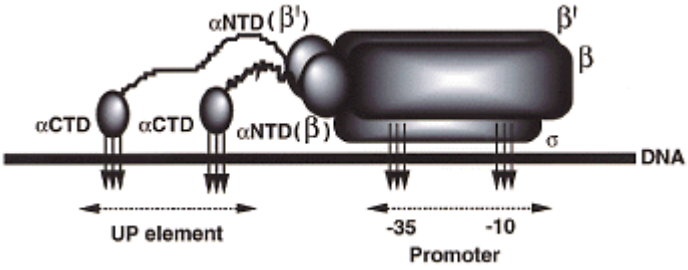
FeBABE was introduced at each region of *E. coli* RNA polymerase sigma subunit, and the FeBABE-conjugated sigma subunit was attached to the promoter site. By the analysis of the cleavage site, the point-of-contact site was estimated.

On the other hand, it has been speculated, mainly with the use of the DNA sequencing method, that the C-terminal domain (CTD) of *E. coli* RNA polymerase alpha subunit is involved in the interaction with class-I transcription factors and DNA enhancers (UP elements), and it plays an important role in transcription regulation. In order to prove the binding of alpha subunits to the UP element of ribosomal RNA genes, a FeBABE-conjugated alpha subunit at the C-terminal domain was

prepared and utilized for the analysis of DNA cleavage sites by the protein-bound FeBABE. This attempt successfully showed the alpha subunit contact with the UP element. Furthermore, it was revealed that: (i) the alpha subunit, which associates with the beta subunit, binds close to the promoter site; and (ii) another alpha subunit, which is associated with the beta-prime subunit, binds to DNA apart from the promoter site. The C-terminal domain (CTD) of the alpha subunit also interacts with class-I transcription factors that generally bind to DNA at sites upstream from the promoter. The direct protein-to-protein interaction between the alpha CTD and class-I transcription factors was indeed observed since the cleavage sites by the alpha CTD-conjugated FeBABE shifted their locations depending on the addition of the transcription factors (Fig.7).

Fig. 7: Identification of the point-of-contact site of an alpha subunit enhancer with a promoter

FeBABE was introduced at each region of *E. coli* RNA polymerase sigma subunit and the FeBABE-conjugated sigma subunit was attached to the promoter site. By the analysis of the cleavage site, a direct contact with the enhancer was shown, and the point-of-contact site was estimated.



6. Use of FeBABE for the determination of protein-to-protein interactions

The protein footprinting method, a method used to determine the parts exposed on the surface of a protein as digestion sensitive areas by free FeEDTA, is widely used in recent life science research. On the other hand, we indicated that FeEDTA, which was covalently conjugated to a protein, could cut proteins that had contact with it. Therefore, the information of three-dimensional structure of a protein should be available by the sequential analysis of intramolecular cleavage sites by FeBABE conjugated at various positions along the protein. For the DNA cleavage, it is considered that the cleavage reaction is an oxidative reaction by hydroxyl radicals. For the peptide bond cleavage, however, an attack to carbonyl carbon by peroxo-intermediate coordinated with Fe is more likely than the hydroxy radical theory.

The RNA polymerase contains two alpha subunits. In order to identify the RNA polymerase subunits that make direct contact with an alpha subunit, FeBABE was conjugated at the N-terminal assembly domain (NTD) of an alpha subunit. It was found that cleavages were observed not only within the FeBABE-conjugated alpha subunit, but also in the unconjugated pairing alpha subunit that contacts with the FeBABE-conjugated alpha subunit.

Generally, there are a lot of sigma subunits in bacterial cells, and RNA polymerase changes its selectivity for genes in transcription through changes in those subunits (Fig. 2). The specificity and affinity of sigma subunits with promoters differ among seven sigma subunits. In order to analyze the contact sites on the core enzyme with these sigma subunits, single cystein mutant sigma subunits at various positions were prepared, conjugated with FeBABE and allowed to bind to the core enzyme to determine the binding sites. From this type experiments, two cleavage sites on the beta subunit and one cleavage site on the beta-prime subunit have been identified.

7. FeBABE binding to the lysine residue of protein

In the beginning, FeBABE was utilized for direct binding to the cystein SH of proteins. However, in this case, a lot of mutants, which have only one cystein residue at various regions of the protein, had to be prepared. Recently, a simplified method was proposed. This method is to convert lysine residues or amino terminus of a protein to FeBABE-reactive residues by the use of 2-iminothiolane (2-IT) (Fig. 4). Generally, there are many lysine residues that are exposed on the surface of proteins. Therefore, it is necessary to control the molar ratio of FeBABE and protein in order to conjugate one molecule of FeBABE with one protein molecule. This method was successfully utilized to identify the contact sites

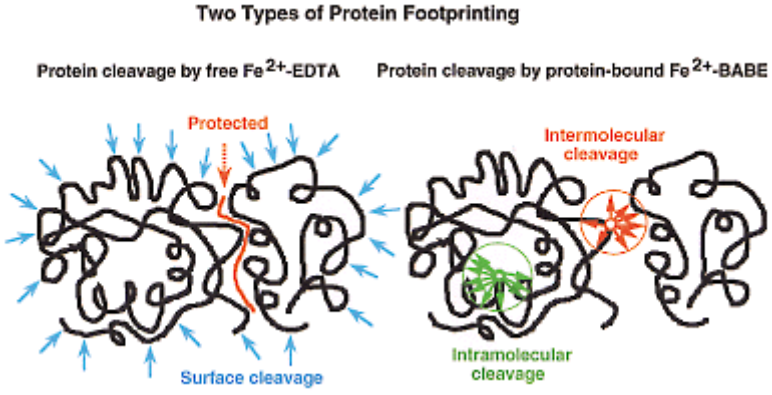


Fig. 8: Identification of protein-to-protein interaction sites by FeBABE

between many *E. coli* transcription factors and RNA polymerase [19]. Thanks to the development of this method, the applications of FeBABE have been expanded (Fig. 8).

FeEDTA has been utilized for the determination of exposed parts on the protein surface. The analysis of a cleavage site enables us to estimate the contact sites within a protein or in protein-to-protein interactions.

8. Conclusion

It was proved that FeBABE is a very powerful tool for the analyses of protein-to-nucleic acid and protein-to-protein networks of macromolecular complexes in many biological reactions. FeBABE and BABE were commercialized due to a large number of requests from researchers. We expect that these reagents will be widely used for the life science research in the future. For more detailed information about the development of FeBABE and its use, please refer to the paper, reference number 20.

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Other information

Part number :	UP994760		
Nom(s) :	p-Bromoacetamidobenzyl-EDTA, iron (III) chelate, MW: 589.14		
Packaging :	1mg	Appearance:	yellowish brown powder
Storage :	–20°C	Purity:	> 95.0% (HPLC)
Shipping :	Room Temperature	Solubility:	1 mg/ml H_2O
		Water content:	3.0-6.0 %

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