Chemical approaches to the study of nucleoprotein structures

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The main chemical approaches to the study of macromolecular structure and dynamics and to the elucidation of interbiopolymer contact points are considered and illustrated by particular examples. Primary attention is paid to the chemical footprinting and affinity modification methods. The use of bifunctional reagents for the study of nucleoprotein structure architecture is described. The ways of enhancing the selectivity of affinity modification available from the literature are analyzed with an emphasis on catalytically competent (superselective) labeling. The identification of proteins responsible for replication of the tick-borne encephalitis virus by this method is described to demonstrate the possibility of the application of the method to multicomponent systems such as the nucleus fraction of infected cells.

Key words: affinity modification, chemical modification, photoaffinity modification, competent labeling.

Important genetic processes in eukaryotes, namely, replication, reparation, recombination, biosynthesis of mRNA (transcription), and processing of pre-mRNA, which includes removal of noncoding sequences (splicing) and capping of the 5'-end and formation of a polyadenyl sequence at the 3'-end of the chain, occur with participation of complex supramolecular structures, which mainly consist of a set of proteins and nucleic acids. Determination of the structure of these complexes is a significant stage in the study of the mechanism of storage, multiplication, and realization (expression) of the information specified in the chromosome DNA. An important characteristic of these processes is the continuous migration of some components with respect to other components. For example, during the biosynthesis of RNA the addition of each new nucleotide residue to the growing RNA chain is inevitably accompanied by migration of the growing chain and DNA matrix relative to the active center of RNA polymerase. To understand the mechanisms of all the above-mentioned processes, one needs not only data on the structures of these complexes at a certain stage of the process but also data on the variation of these structures during the genetic process, *i.e.*, on the process dynamics. Since these structures are large, it is virtually impossible to prepare them in the crystalline state for X-ray diffraction analysis. The use of NMR spectroscopy for the structural studies is limited by the sensitivity of the method and the insufficient resolution, which precludes determination of the role and behavior of individual amino acid and nucleotide residues. Therefore, chemical methods are playing an ever increasing role in studies of the structures of nucleoproteins and the dynamics of processes involving supramolecular complexes. Photochemical methods seem especially attractive in this respect, because photochemical transformation can be performed over periods of fractions of seconds, and it is in this time scale that many dynamic events occur.

The tasks faced by chemists can be conventionally divided into three groups. The first task is to establish the general architecture of supramolecular complexes, *i.e.*, to elucidate the areas of contact between their macromolecular components (including the subunits of individual enzymes and protein factors). The second task is to solve the same problem for a series of consecutive states through which the system passes during its functioning. The third task is to identify the monomeric residues that contact within these complexes.

The overwhelming majority of chemical methods used to study biopolymers and their complexes are based on their chemical modification. There is a broad range of reagents at researchers' disposal that are able to react with definite types of functional groups of proteins and nucleic acids.

The simplest method of chemical modification is treatment of a biopolymer with some reagent, which should modify all groups of a particular type. However, in studies of biopolymer complexes, some contacting areas can appear to be protected from the access of these reagents. Elucidation of these areas provides certain information on the mutual arrangement of components in the complex. This approach underlies a widespread method referred to as footprinting. Actually, this method is based on the search for the "footprint" remaining on the biopolymer molecule after its interaction with a component. For practical reasons, this method is largely

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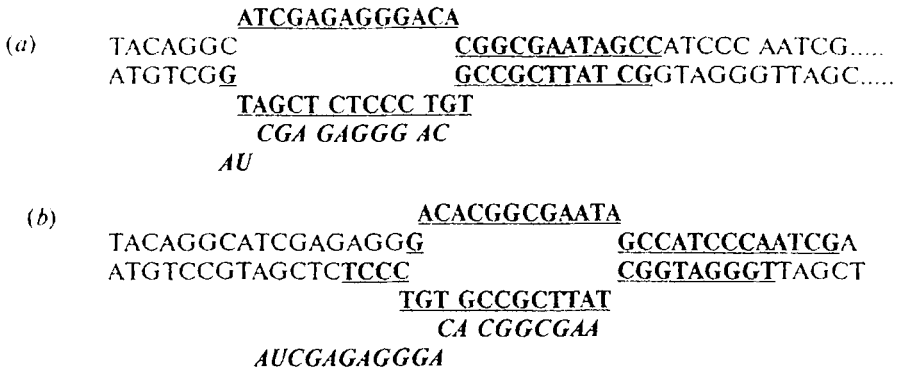

Fig. 1. Variation of the position and size of the untwisted DNA section during transcription as a function of the transcript length: (*a*) the complex for the 12-meric reaction product; (*b*) the complex for the 20-meric reaction product. The DNA sections interacting with the enzyme according to the data of OH-radical footprinting are highlighted by a bold typeface and underlined. The italic typeface designates the ribonucleotide residues.

employed for identification of the regions of nucleic acids that are cleaved on treatment with definite reagents. Nucleic acids labeled by radioactive phosphorus at one end, most often at the 5′-end, are used for this purpose. The nucleoprotein complexes being studied are treated under conditions (time, reagent concentration) that induce cleavage at one point. The points at which cleavage has occurred are determined based on the lengths of the fragments detected after separation by gel electrophoresis. The region of the nucleic acid molecule complexed with some protein under study proves to be protected from the reagent; therefore, no corresponding bands can be detected in the electrophoresis pattern. Thus, a sort of impression ("footprint") of the protein molecule bound to the nucleic acid is produced.

One of the first examples of the use of chemical footprinting is presented in the studies by V. V. Vlasov,[1] who developed a method for splitting RNA by means of nitrosoalkylureas. This method was used to study the interaction of tRNA with the corresponding aminoacyl-tRNA-synthetases. Currently cleavage of nucleic acids by OH radicals is used most widely for chemical footprinting.[2] Free radicals can detach H atoms from various positions of deoxyribose residues. In all cases, this results in the cleavage of the polynucleotide chain.

When studying the functioning of nucleoprotein complexes, it is sometimes significant to distinguish between double- and single-stranded regions. Many reagents are virtually inert toward heterocyclic bases in double-stranded sections but efficiently modify single-stranded sections of DNA.

A study[3] dealing with the arrangement and migration of the untwisted section of DNA (bulge), carried out in RNA polymerase of *Escherichia coli* (below referred to as *E. coli*), is a striking example in which information on the transcription dynamics was gained using the simplest chemical modification methods. During transcription, the DNA section contacting the enzyme is temporarily unwinded and thus becomes able to transfer the fragment of the strand being transcribed and adjacent to the enzyme active site into the one-strand state. As transcription goes on, RNA leaves the enzyme along a particular "exit passage." The strand being transcribed is combined with the nontranscribed one, which has temporarily moved away, and transcription continues downstream,* together with migration of the bulge (*i.e.*, toward the 5′-end of the DNA).

To study migration of the unwinded section relative to the enzyme, a series of transcription complexes with the transcript length ranging from 11 to 20 nucleotides were prepared in the study cited. These complexes were obtained by transcription on the DNA fragment containing a promoter and the sequence (3′)d(... TpApGpCpTpCpTpCpCpCpTpGpTpGpCpCp—GpCpTpT...)(5′) of the first 20 transcribed nucleotides. The complex with a transcript length of 11 was prepared by initiating transcription using pApUpC ribonucleotide and GTP and ATP as substrates. Elongation of the complex isolated by gel filtration with various sets of nucleoside-5′-triphosphates (NTP) gave complexes containing transcripts comprising 12, 14, 16, 18, and 20 nucleotide residues. The position of the unwinded DNA section within these complexes was determined using reagents specific toward a particular type of heterocycles not involved in the formation of double-stranded structures (OsO_4 for thymine, diethyl pyrocarbonate for adenine, and dimethyl sulfate followed by hydrazine for cytosine). These studies in combination with footprinting by OH radicals showed that up to the complex with a transcript length of 18, the double helix is unwinded ahead of RNA polymerase but the position of the contact of the enzyme with DNA in the region of the 3′-end of the DNA strand being transcribed remains unchanged. After the transcript has reached a length of 20, a saltatory change in the system occurs, and a part of the transcribed

* In the direction of transcription.

DNA returns to the double-stranded state. The scheme illustrating the structures of two such complexes, the 12-meric and 20-meric reaction products, is shown in Fig. 1.

New opportunities for the study of the architecture of nucleoprotein structures are opened up by the use of bifunctional reagents. These reagents contain two identical or two different reactive groups linked by a spacer of a definite length. Any group of a particular type in one of the numerous subunits can serve as the target for chemical modification. After the first reaction, the reagent becomes fixed at a particular component of the complex, and the ability for the second reactive group to react is restricted to reactions with those functional groups of the biopolymers that are located near the first modified group. This may give a conjugate with a neighboring biopolymer. If, after isolation of this conjugate, the components of the initial mixture from which it has been formed are determined, it can be considered with a high degree of reliability that an element of the supramolecular structure has been identified.

A study dealing with identification of the contacts between subunits from RNA polymerase II from *Schisosaccharomyces pombe* yeast can be cited as an example.[4] The enzyme contains two large subunits, Rpb1 and Rpb2, which consist in this particular case of 1752 and 1210 amino acid residues, and ten substantially smaller subunits. By using a series of bifunctional reagents (dimethyl suberimidate, 2-iminothiolane, *N,N'*-1,2-phenylenedimaleimide, dimethyl-3,3'-dithiobis(propioimidate), diepoxybutane) with different distances between the reactive groups (from 4.0 to 14.5 Å), the authors found that there exists a contact between large subunits, the contact of Rpb1 with the small subunits Rpb3, Rpb6, Rpb7, Rpb8, Rpb11, and Rpb12, and the contact of Rpb2 with Rpb3, Rpb5, Rpb6, Rpb7, Rpb10, Rpb11, and Rpb12. In addition, some contacts between small subunits were found.

The use of bifunctional reagents has also played a significant role in the determination of elements of the spatial structure of ribosome subunits.[5]

Finally, methods for affinity modification of biopolymers, which were first developed to study the interaction of biopolymers (most of all, enzymes) with monomeric ligands have found wide use in studies of nucleoprotein structures. These methods are based on the use of a derivative of a component of the system under study, in which a reactive group has been introduced, as the reagent. The chemically reactive group in the complex under study proves to be brought artificially close to the functionally significant region of the corresponding partner and the modification occurs within or, at least, near this region.[6]

A new version of affinity modification, which can be called double affinity modification, has been implemented.[7,8] The main idea of this method is that affinity modification of a particular point of one component of the system under study is carried out by a reagent, which either contains a reactive group or introduces a group that can be easily converted into a reactive one. This approach was first[7] employed to introduce a photoactive group into the G^{24} residue of $tRNA^{Phe}$ (*E. coli*). In the first step, tRNA was subjected to alkylation by $ClRCH_2NHpApApCpCpA$, where ClR is 4-(*N*-2-chloroethyl-*N*-methylaminophenyl), which occurred selectively at the G^{24} residue. After acid hydrolysis of the phosphamide bond, the resulting tRNA derivative, containing a $G^{24}RCH_2NH_2$ residue, was arylated by 5-fluoro-2,4-dinitrophenyl azide. Thus, the first affinity modification gave tRNA with a photoactive group in a single position. This photoaffinity reagent was used to study the reaction of tRNA with tRNA-(adenine-1) methyltransferase. A similar expedient was used to study the interaction between *E. coli* threonyl-tRNA synthetase and the leader region of mRNA coding this enzyme.[8] Apparently, this interaction has an important regulatory role because it terminates translation of mRNA when a sufficient amount of the enzyme has already been synthesized. In this study, several $ClRCH_2NH$ derivatives of oligonucleotides, complementary to definite sections of mRNA, were used as the first reagent. After selective alkylation of guanine residues at the position determined by the addressing oligonucleotide, a set of photoactive mRNA derivatives with a fixed position of the arylazido group were obtained by the above-described procedure. Each of them modifies threonyl-tRNA synthetase on exposure to light. At the same time, the photoactive derivatives of mRNA were not bound to nonspecific aspartyl-tRNA synthetase on irradiation.

In the direct affinity modification of larger-size enzymes such as prokaryotic and eukaryotic RNA polymerases and, especially, nucleoproteins, there is a probability that, in addition to the active site under study, the reaction would also involve some other points. For instance, photomodification of phenylalanyl-tRNA synthetase from *E. coli* by ATP γ-*p*-azidoanilide results in the addition of 20 molecules of the analog without the loss of enzymatic activity.[9] Therefore, development of methods for increasing the selectivity of affinity modification becomes a topical task.

A method of this type, applicable to reactions involving two or more substrates, has been proposed[10,11] for the photoaffinity modification of *E. coli* RNA polymerase. It is based on the use of a combination of two substrates, one of which carries a reactive group and the other of which has a radioactive label. NTP and ribonucleotide *p*-azidoanilides were used as the reactive analogs. Prior to irradiation, the enzyme was kept with the reagent (one of [α-^{32}P]NTP and, in some cases, certain combinations of nonlabeled NTP); this resulted in the synthesis of photoactive transcripts of a definite length. The scheme of the experiment is shown in Fig. 2. The reaction mixture was irradiated and subjected to gel electrophoresis. High selectivity of the approach was indicated by the facts that serum albumin, present in the mixture, was not modified and that differ-

(3′) *Up-Ap*Gp-Ap*Up-Ap*Gp-Up-Up-Gp-Up-Cp*-$NHC_6H_4N_3$ (*6*)
(3′) *Gp-Up*Up*Gp*-Up-Cp*-$NHC_6H_4N_3$ (*5*)
(3′) *Gp*Up-Cp*-$NHC_6H_4N_3$ (*4*)
(3′) *Up-Cp*-$NHC_6H_4N_3$ (*3*)
.....dGpdGpdApdTpdCpdTpdApdTpdCpdApdApdCpdApdGpdGpdApdGpdT..... (*2*)
(3′)dCpdCpdTpdApdGpdApdTpdApdGpdTpdTpdGpdTp dCpdCpdTpdCpdA... (*1*)

Fig. 2. Scheme of the experiment on the affinity modification of *E. coli* RNA polymerase: (*1*) untranscribed DNA strand; (*2*) transcribed DNA strand; (*3*) dinucleotide derivative; (*4*) the product obtained by elongation in the presence of [α-^{32}P]GTP; (*5*) the product obtained by elongation in the presence of [α-^{32}P]UTP and GTP; (*6*) the product obtained by elongation in the presence of [α-^{32}P]ATP, GTP, and UTP. The italic typeface designates the ribooligonucleotide residues.

ent sets of modified subunits of RNA polymerases are formed depending on the length of the transcript bearing the *p*-azidoaniline residue (β and σ in the case of (*4*); β, β′, and σ in the case of (*5*); and β and β′ in the case of (*6*)). Thus, the first information on the migration of the RNA product relative to the enzyme (RNA "exit package") was gained.

Among the techniques used to carry out affinity modification, the method named competent labeling deserves attention. This method is based on the recently discovered[12] fact that in some cases, the affinity analog of the substrate bound to the enzyme by a covalent bond retains the ability to undergo an enzymatic reaction with a second substrate. It was found that covalent binding of the alkylating derivative of tRNA, chloroambucilylphenylalanyl-$tRNA^{Phe}$ (chloroambucil-*p*-$(ClCH_2CH_2)_2NC_6H_4CH_2CH_2COOH$), to the ribosome peptidyl transferase site does not prevent further elongation with the formation of polyphenylalanine in the presence of the poly(U) template. Later, phenomena of this type were called "catalytic competence."[13] In this study, the enzyme estradiol-17β dehydrogenase was alkylated by bromoacetoxyestrone and it was shown that the attached steroid residue retains its capability of being reduced by NADH or NADPH to estradiol. Subsequently this method has been used most often to modify RNA polymerases[14] and later DNA polymerases.[15] In a series of studies[14] dealing with RNA polymerases this technique was defined as "superselective labeling." In these studies, a nonlabeled reagent was used (a derivative of the initiating nucleotide or oligonucleotide) and the first attached substrate was elongated by some [α-^{32}P]NTP. In the opinion of the authors,[14] the introduction of the label into the residues of the first reagent attached outside the active site of the enzyme is excluded in this case. It is assumed that the addition of the nonlabeled analog does not influence the ability of the modified enzyme to accomplish the competent labeling, which is not obvious in the general case.

It should be noted that in the large series of works devoted to the use of superselective labeling for RNA polymerases, one possible source of artifacts is not always taken into account. With some reagents, for example, phosphorylating and alkylating reagents, some unconsumed reactive reagent, *i.e.*, the reagent that has not attached to the target protein, remains in the reaction mixture at the second stage. This reagent might be able to react with the second substrate bearing the radioactive label, and the resulting labeled reagent can accomplish chemical modification of the protein. Thus, not all of the labeled proteins identified can be considered to be superselectively modified. In these cases, it is important to ensure complete inactivation of the unconsumed reactive reagent prior to the addition of a labeled substrate. It was found for modification of *E. coli* RNA polymerase by phosphorylating derivatives of the XpdCpdGprC trinucleotide, where X is the *N*-methylimidazole or *N,N*-dimethylaminopyridine residue,[16] that after the addition of [α-^{32}P]UTP along with the β-subunit, σ- and α-subunits also become modified, although to a lesser extent. However, when reactive derivatives of oligonucleotides were inactivated by hydrolysis or by addition of ethylenediamine prior to the addition of [α-^{32}P]UTP, no modification of σ- and α-subunits was observed. The researchers believe that elongation of the trinucleotide derivative results partially in the so-called abortive synthesis accompanied by ejection of a short product from the complex with the enzyme and the matrix. It is this product that modifies other subunits. Perhaps, the discrepancy between the data on the labeling of *E. coli* RNA polymerase subunits by different types of reagents[17] is due to the fact that the circumstance mentioned above was not taken into account when interpreting the results. Unlike modification by phosphorylating derivatives of short oligonucleotides and those containing a formyl group, modification by derivatives carrying a 4-(*N*-2-chloroethyl-*N*-methylaminophenyl) residue at the 5′-phosphate group resulted in the label being introduced not only in the β-subunit but also in the σ-subunit; in the case of trinucleotide, some modification of the α-subunit was also observed. Since under the conditions employed, some of the alkylating groups were retained to the instant of addition of the labeled substrate, it can be assumed that, in this case, the discrepancy between the results obtained with different reagents is due to the fact that conventional affinity labeling or even nonselective labeling occurred in parallel with the superselective labeling.

In recent years, the applicability of catalytically competent labeling was demonstrated in relation to two more enzymes. It was shown that phosphofructokinase

modified by the nonradioactive substrate analog fructose-6-phosphate *p*-formylbenzyl ester with subsequent reduction of the resulting Schiff's base by $NaBH_4$ is catalytically competent. The transfer of [^{32}P]phosphate from [γ-^{32}P]ATP to the 1-OH group of the covalently bound analog was detected.[18] Photoaddition of $tRNA^{Phe}$ containing one 4-thiouridine residue to phenylalanyl-tRNA synthetase yields a conjugate, in which enzymatic aminoacylation of tRNA is highly efficient. The experiment was carried out with preliminary isolation of the conjugate, which rules out participation of unreacted photoactive tRNA in the acylation or subsequent nonselective photomodification of the enzyme.[19]

A significant advantage of the superselective modification is that it can be used to identify functional proteins in cells and cell organelles. This possibility was used to establish the nature of proteins participating in the replication of RNA of the tick-borne encephalitis virus (TBEV).[20,21] The virus contains RNA as the genetic information carrier; RNA acts as the (+)-strand, *i.e.*, during the infection it starts working as mRNA, coding a set of viral proteins including those needed for the subsequent replication. At a later stage of the infection, it serves as a template for the generation of a (−)-strand with the formation of a double-stranded RNA, on which synthesis of a large number of (+)-RNA, required for the formation of new viruses, starts. Since viruses do not tend to exhibit RNA replicase activity, RNA replication is most likely performed by nonstructural (NS) proteins, which arise and exist only during the infection.

It can be expected that the enzyme catalyzing the synthesis of the (−)-strand on the virion RNA should appear at earlier stages of the infection than the enzyme catalyzing the synthesis of (+)-strands on the double-stranded RNA as the template. In conformity with this, modification of proteins from the nuclear fraction was studied 8 h and 45 h after the infection of cells. *p*-Formyl-*o*-hydroxymethylphenyl esters of GMP, GDP, and GTP and *p*-formylphenyl ester of ATP were used as the reagents. The first three reagents proved to be efficient for cell modification at an early stage of the infection, and the fourth one was efficient at later stages. In the former case, a GTP derivative was added to nuclear fractions and 15 min later, they were treated with $NaBH_4$ (30 min, 0 °C). After that, [α-^{32}P]ATP complementary to the second 3′-end nucleotide of the virion RNA CpU (3′) was added (15 min, 30 °C) and the proteins were fractionated. The radioactive label was found in one protein with a molecular weight of ~100 kDa, which corresponds to the NS5 nonstructural protein. Other combinations of the reagent and the radioactive NTP did not result in modifications of any protein. The whole set of obtained data allows the NS5 protein to be regarded as the first replicase. A similar experiment carried out with the nucleus fraction at a late stage of the infection showed that combination of the ATP derivative as the reagent and [α-^{32}P]GTP leads to selective labeling of another protein, with a molecular mass of ~69 kDa, which was identified as NS3. The combination of the reagent and radioactive NTP used is consistent with the 5′-end sequence of the virus RNA.[20]

To identify the proteins participating in the replication of the virus RNA at the elongation stage, CTP analogs containing a photoactive group attached to the exocyclic amino group of cytosine were introduced in the nuclear fraction.[21] After irradiation of the incubation mixture, [α-^{32}P]ATP was added to it and the proteins were analyzed by gel electrophoresis. Immunoblotting by radioactively labeled proteins with monoclonal antibodies against NS3 and NS5 virus proteins showed that affinity labeled products are also nonstructural TBEV proteins. This implies that the elongation stage involves both nonstructural proteins, apparently, bound in a complex. Thus, the use of superselective modification in the study of TBEV replication complex made it possible to identify proteins that act as replicases at various stages of the development of the viral infection.

An important aspect in the study of the structure of nucleoprotein systems is elucidation of the nucleotide residues of the nucleic acid and amino acid residues of the protein involved in the interaction of the components of nucleoprotein complexes. In the case of proteins, this is usually done by isolating peptides from the modified protein and analyzing their structure by the Edman method.

The chemical nature of the modified amino acid can be predicted in cases where the affinity reagent contains a reactive group possessing a certain specificity. For example, reagents with a formyl group selectively react with primary amino groups.

When investigating components of supramolecular structures that are difficult to prepare in amounts required for structural analysis, it is attractive to use information on the properties of the groups being modified, for example, on the chemical stability of the resulting covalent bond under various conditions.

A relatively large number of examples of such characterization of groups being modified are currently available. For example, demodification has played an important role in the identification of the amino acid residues involved in the formation of the catalytic site of template biosynthesis enzymes. Modification of RNA polymerase of *E. coli* by adenosine-5′-trimetaphosphate can be cited as an example.[22] The elongation of the added pppA residue by one unit using [α-^{32}P]UTP transformed it into pppAp*U bound by a covalent bond. At pH 4.9 and 20 °C, about 80% of the introduced label was spilt off over a period of 40 min. This rate was in good agreement with the rate of hydrolysis of the phosphoamide bond in ATP imidazolide. However, hydrolysis of the phosphoamide bond in the compound CH_3NH-pppA, which models the product of modification at the lysine residue, occurred by no more than 10% over a period of 30 min at the same pH and a tempera-

ture of 37 °C. Hence, it can be concluded that in the example considered, the enzyme modification involved the histidine residues.

It should be noted that in the case of dark reactions, the structure of the products of modification of amino acid residues in proteins is frequently known. However, little is known about the structure of photomodification products. Published data on the points of photoaffinity modification of a large group of proteins by aryl azides are summarized in a review.[23] In the majority of cases studied, modification of amino acid residues involves lysine, tyrosine, and tryptophan. Only in a few cases, did modification of aliphatic amino acid residues (Ala, Ile, Leu, Val, Pro) occur. Unfortunately, the lack of data on the structures of the products hampers interpretation of the route for their formation.

To determine the nucleotide residues contacting with partners in nucleoprotein complexes, nucleic acid derivatives containing photoreactive groups at definite positions are widely used. Covalent bonding of these derivatives to a component of the nucleoprotein complex occurring on irradiation attests unambiguously that it is the photoreactive residue that acts as the point of contact with the protein partner. Two methods used most widely for the preparation of these derivatives have been described in detail in reviews,[15,23] and here they are considered only briefly. One of them is based on the use of nucleoside-5′-triphosphate derivatives in which a reactive group has been introduced. At present, deoxyuridine-5′-triphosphate derivatives with a reactive substituent introduced in position 5 of the heterocycle, which does not participate in Watson—Crick interactions, and a deoxycytidine-5′-triphosphate derivative with such a substituent introduced at the exocyclic amino group are used most widely. Some of these analogs were found to be DNA polymerase substrates and were used for the introduction of one or several photoreactive groups into the double-stranded DNA being synthesized by elongation of the primer, complementary to a definite section of the single-stranded DNA, chosen as the investigation object. The position of the photoreactive groups is defined by the primer chosen.[24] The second approach is based on the preparation of a photoreactive DNA chain by elongation of a primer modified preliminarily at a particular heterocycle to the corresponding size using a single-stranded template. The primer is modified in a chemical synthesis using synthons bearing groups capable of adding a photoreactive residue in the subsequent stages.[25] A method of introducing photoreactive groups into definite positions of RNA based on affinity modification has also been described.[8] However, it has not yet found wide use.

In particular, photoreactive DNA derivatives were used to localize the individual points of interaction of the 5S RNA gene with transcription factors of RNA polymerase III, responsible for the transcription of short genes,[24] the points of contact of DNA with various subunits of *E. coli* DNA polymerase III,[25] the points of contact of T4 phage DNA polymerase, and some auxiliary factors of phage replication with the DNA.[26]

The information considered here clearly demonstrates that chemists have created a wide range of reagents and methods for modification of biopolymers and their complexes. The use of photoreagents in combination with the technique of fast mixing of reagents followed by pulse irradiation opens up prospects for investigation of the dynamics of functioning of supramolecular structures. However, the use of these methods is still being held up by the imperfection of structure analysis of modified proteins and peptides. At present, it seems most promising to identify modified peptides resulting from specific proteolysis (*e.g.*, by trypsine) by direct injection of the products into a mass spectrometer making use of ionization methods (ESI, MALDI) that allow recording of mass spectra of polymers. Further development of these approaches in the near future would open up prospects for establishing at the molecular level mechanisms that ensure a particular sequence of interactions of the most important biochemical components at various stages of cell functioning.

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