2. Conjugates of N-acetylmuramyl-ℓ-alanyl-d-isoglutamine with a synthetic peptide fragment of influenza virus hemagglutinin have been obtained.

LITERATURE CITED

1. R. Arnon and M. Sela, Ann. Inst. Pasteur Immunol., 136, No. 3, 271 (1985).
2. M. Sela, Biopolymers, 22, No. 3, 415 (1983).
3. R. Arnon, M. Sela, M. Parant, and L. Chedid, Proc. Natl. Acad. Sci. USA, 77, No. 11, 6769 (1980).
4. G. Werner, F. Floc'h, D. Migliore-Samour, and P. Jolles, Experientia, 42, No. 5, 521 (1986).
5. F. Audibert, M. Jolivet, L. Cherdid, R. Arnon, and M. Sela, Proc. Natl. Acad. Sci. USA, 79, No. 16, 5042 (1982).
6. V. Ivanov, T. Andronova, M. Berzukov, V. Rar, E. Makarov, S. Kozmin, M. Astapova, T. Barkova, and V. Nesmeyanov, Pure Appl. Chem., 59, No. 3, 317 (1987).
7. P. Lefrancier and E. Lederer, Pure Appl. Chem., 59, No. 3, 449 (1987).
8. C. Hiebert, W. Kopp, H. Richerson, and C. Barfknecht, J. Med. Chem., 26, No. 12, 1729 (1983).
9. A. Hasegawa, E. Tanahashi, and M. Kiso, Carbohydr. Res., 103, No. 2, 251 (1982).
10. D. Viley, I. Wilson, and J. Skehel, Nature (London), 289, No. 5796, 373 (1981).
11. A. F. Shval'e, V. I. Ofitserov, and V. V. Samukov, Zh. Obshch. Khim., 55, No. 9, 2152 (1985).
12. V. V. Samukov, V. V. Kalashnikov, V. I. Ofitserov, and A. F. Shval'e, Bioorg. Khim., 11, No. 8, 1037 (1985).

PRINCIPLES OF MODELING IN THE STUDY OF CONFORMATIONS OF HISTONES

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UDC 547.466.1+547.962

A study has been made by the CD method of the conformational potentialities of the polypeptides $(Gly\text{-}Lys\text{-}Gly)_n$ and $(Ala\text{-}Lys\text{-}Ala)_n$ and fragments of the terminal sections of histones H4 (the sequence 1-16), H2B (1-21), and H1 (152-184), and they have been used as models of histones in complex formation with DNA under various conditions of the medium.

It is traditional to consider that in the majority of protein molecules there are two main types of regular secondary structure — α-helix and β-structure. As exceptions have been found proteins of the collagen type in which a unique left-handed helical conformation of the type of poly-L-proline-II is realized at the positions of accumulation of imino acids and glycine (projection of a residue on the axis of the helix of the order 3 Å, 3_1 symmetry, mean values of the angles φ and ψ: 77.2 and 145.9°). However, a conformation of this type was later detected in certain synthetic peptides in aqueous solutions [1, 2], in hormones [3], and in globular proteins [4].

It has become known that the left-handed helical conformation is also realized in an aqueous medium at fairly low temperatures when hydration is most effective [1, 2]. The absence of interpeptide hydrogen bonds in the chain in the organization of this structure and the possibility of its stabilization by water ensure its lability in the conformational respect, which is important for the organization of enzyme-substrate, hormone-receptor, and other functionally important interactions.

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In a study of the complicated processes of interaction between proteins and nucleic acids, it is promising to use model systems. As model protein molecules it is possible to employ sequenal polypeptides and synthetic peptide fragments (or their analogs) of definite sections of a protein molecule the structure and functional properties of which it is proposed to study.

It is natural also to use model compounds in the study of histones, the structure of which bears a complex fibrillar-globular nature (the ends of the peptide chains are not included in the globules).

The two- or three-domain structure of the polypeptide chain of histone permits one to speak of the presence in it of sections enriched with basic amino acid residues and responsible for the interaction of the histones with DNA and of sections enriched with hydrophobic amino acids responsible for protein-protein recognition. Such a distribution of the residues in histones permits the modeling of individual elements of their primary structure.

Taking as examples the sequenal polypeptides $(Gly\text{-}Lys\text{-}Gly)_n$ and $(Ala\text{-}Lys\text{-}Ala)_n$ [5] and fragments of the terminal sections of histones H4, H2B, and H1 [6, 7] synthesized according to [6, 8-10], we have made a study of the conformational potentialities of polypeptide chains of definite sequences under various conditions of the medium and in their complexes with DNA [11, 12].

Analysis of the CD spectra of solutions of sequenal polypeptides containing basic amino acids $(Gly\text{-}Lys\text{-}Gly)_n$ and $(Ala\text{-}Lys\text{-}Ala)_n$ (with molecular masses of about 7000 Da) have shown that in water at pH 7.0 both polypeptides are characterized by a small positive band in the 215-220 nm region, a more intense negative CD band at 196 nm, and a weak negative band in the 235 nm region (Fig. 1, a, b, curves 1).

Clear empirical spectral criteria of the presence of left-handed helices in polypeptides are characterized by a maximum on the curves of the CD spectra in the region of positive values of the ellipticity at 220-230 nm and a minimum with a negative value of the ellipticity in the region of 190-206 nm [13]. The amino acid residues capable of making a contribution to the CD spectra in the region of wavelengths of 190-250 nm through electronic transitions in the chromophoric side chains [14, 15] are absent from the peptides mentioned. Therefore the very form of the CD spectrum and its transitions taking place through a change in the conditions of the medium are due only to the basic conformation of the polypeptide chain and its transformations.

CD spectra similar in the form and in the asymmetric position of the bands with respect to the zero line belong to the completely disordered (random coil) conformation of the polypeptide chain. Theoretical calculations of CD spectra for regular structures of the types of α-helices and β-forms [14, 16-18] agree well with the experimental CD curves for these conformations. However, similar calculations of the CD spectra for unordered systems do not give a satisfactory explanation of the experimental CD spectra of the peptides being studied. And only for a limited range of angles φ and ψ of the conformational map ($-180° \leq \varphi \leq -60°$; $120° \leq \psi \leq 180°$, i.e., for a region a large part of which corresponds to the angles φ and ψ for an extended left-handed helical structure [18]) does the calculated CD spectrum bring us close to the asymmetric CD spectra such as we see in Fig. 1, a, b.

Thus, the polypeptides represented in Fig. 1, a, b, curves 1, which include lysine residues, exist in the conformation of an ε-helix [5, 7]. The intensity of the CD band in the 235 nm region characterizes a defectiveness (loosening) of the ε-helix [3]. Fluctuations in the conformations of the peptide residues in solution lead to a decrease in the ellipticity maximum in the region of positive values which are the most sensitive to conformational changes.

Since an extended left-handed helical structure can be considered as a structure where the sequenal residues of the peptide chain strive to be present in similar, although not completely identical, conformations, then, apparently, a fluctuation of the angles φ and ψ for each residue takes place in solution within limits that are close to the conformation of the left-handed helix of the poly-L-proline II type [13].

According to the conformational energy map of a glycine residue [19] this may be extremely mobile and may disturb a left-handed helical conformation. Therefore, in the CD spectra of aqueous solutions of polypeptides with glycine residues, as a rule, a minimum

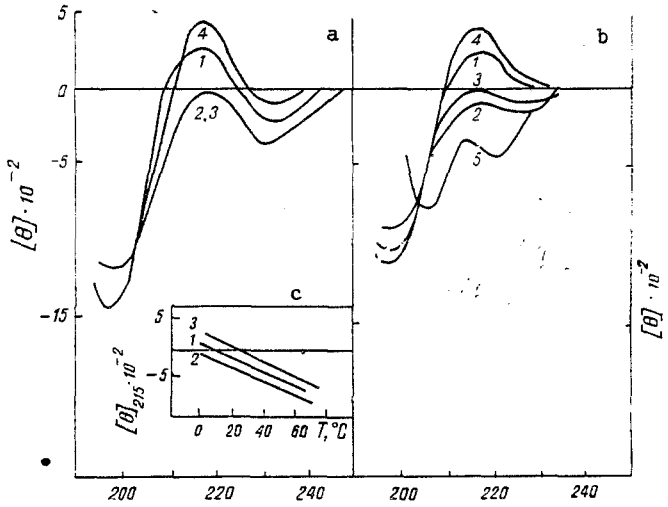

Fig. 1. CD spectra of the polypeptides $(Gly\text{-}Lys\text{-}Gly)_n$ (a) and $(Ala\text{-}Lys\text{-}Ala)_n$ (b) under various conditions (2°C): 1) H_2O, pH 7; 2) H_2O, pH 12; 3) 1 M NaF; 4) 5 M Gu·HCl; 5) 1% SDS; c) dependence of the molar ellipticity $[\theta]_{215}$ of $(Gly\text{-}Lys\text{-}Gly)_n$ on the temperature in solutions in: 1) H_2O, pH 7; 2) H_2O, pH 12; 3) 5 M Gu·HCl.

with a negative value of the molar ellipticity is observed in the 230-240 nm region characterizing defects in an ε-helix. The position of the maximum in the 220 nm region under certain conditions may shift into the region of negative values of the ellipticity (Fig. 1, a, curves 2 and 3).

In the case of the other, alanine-containing peptide there is also distortion of the left-handed helical structure. But the reason for the distortion here may be connected with a transition of part of the polypeptides investigated in the region of the angles φ and ψ that are characteristic for an α-helix (Fig. 1, b, curve 5).

When the charges of the side chains of the amino acid residues of the polypeptide studied are neutralized (in the region of alkaline pH values, Fig. 1, a, b, curves 2) or these charges are screened by ions (in 1 M NaF solution, Fig. 1, a, b, curves 3), the left-handed helical nature of the CD spectra of both polypeptides is preserved, in the main, and there is only an increase in the number of defects. The addition of solutions of urea and of guanidine hydrochloride (Fig. 1, a, b, curves 4) has little effect on the structure of these polypeptides, in contrast to their influence on globular proteins [20].

Curves of the dependence of the amplitude with the molar ellipticity at 215 nm on the temperature for the polypeptide $(Gly\text{-}Lys\text{-}Gly)_n$ in aqueous solution are given in Fig. 1c. A similar dependence was obtained for the polypeptide $(Ala\text{-}Lys\text{-}Ala)_n$. In all cases, with a rise in the temperature there was a linear fall in the amplitude of the CD band at 215 nm, which shows the noncooperative mechanism of the change in the conformation of an extended left-handed helix as a function of the temperature.

The nature of the CD spectra of the N-terminal sections of histones H4 and H2B of the sequences 1-16 and 1-21, respectively, and their analogs taken under the same conditions is similar to that of the spectra of the polypeptide $(Gly\text{-}Lys\text{-}Gly)_n$ given in Fig. 1a [7, 21]. The formation of an ε-helix has also been detected in the 152-184 fragment of histone H1 [6]. These observations indicate that even the appearance in the polypeptide chain of a certain number of hydrophobic amino acids does not prevent the formation of an ε-helix, which is fairly stable in a wide range of conditions of the medium. For some peptides, even the addition of large amounts of an alcohol to the solution (up to 80% of ethanol, methanol, or 2-chloroethanol) or of a detergent (1% solution of SDS), which substantially changes the structure of histone molecules [22], does not appreciably affect the conformation of the peptides. However, the alanine-containing polypeptide undergoes substantial changes under these conditions (Fig. 1, b, curve 5), indicating that under these conditions a considerable part of the polypeptide chain adopts the α-helical conformation, which is stabilized at low temperatures.

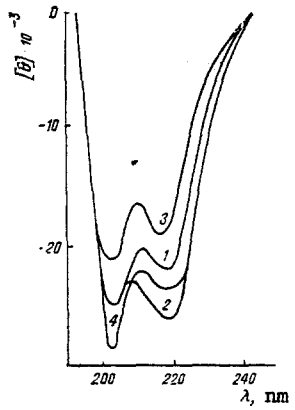

Fig. 2. CD spectrum of the polypeptide $(Ala\text{-}Lys\text{-}Ala)_n$ (molecular mass ~20,000 Da) under various conditions (2°C): 1) H_2O, pH 7; 2) H_2O, pH 12; 3) 1 M NaF; 4) 80% TFE.

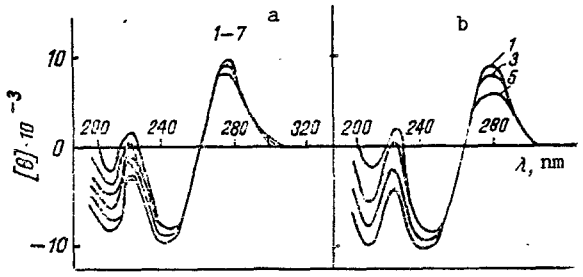

Fig. 3. CD spectra of complexes of DNA with the polypeptides $(Gly\text{-}Lys\text{-}Gly)_n$ (a) and $(Ala\text{-}Lys\text{-}Ala)_n$ (b) in 0.015 M NaCl with various amounts of polypeptide in the complex: 1) r = 0 (–); 2) r = 0.2; 3) r = 0.3; 4) r = 0.5; 5) r = 0.6; 6) r = 0.7; 7) r = 1.0.

Thus, while the features of the primary structure in any polypeptide permit the existence of several conformations, the nature of the conformational transition observed with a change in the temperature is determined by the solvent [5].

A sample of the polypeptide $(Ala\text{-}Lys\text{-}Ala)_n$ with a molecular mass of about 20,000 Da behaved completely differently (Fig. 2), exhibiting a tendency to form α-helical sections even under conditions in which a sample of a polypeptide with a molecular mass of about 7000 Da formed a left-handed helical structure.

The differences observed in the conformational potentialities of sequenal polypeptides also affect the nature of their interaction with DNA and features of the conformational behavior of the complexes obtained.

The CD spectra of complexes of DNA with both polypeptides in solution with a low ionic strength (0.015 M NaCl), presented in Fig. 3a, b, showed that the glycine-containing polypeptide in a complex at r = 1 (r being the ratio of lysine groups in the peptide to phosphate groups in the DNA) changed its structure only slightly and did not change the local parameters of the secondary structure of the DNA (Fig. 3, a, curve 1). The fragment of histone H4 with the sequence 1-16 behaves similarly [11]. The alanine-containing polypeptide within a complex under the same conditions was partially α-spiralized and considerably changed the parameters of the DNA (Fig. 3, b, curve 1).

In a solution with the physiological ionic strength (0.15 M NaCl), the complexes of DNA with the polypeptides exhibited a tendency (to a greater degree with the alanine-containing polypeptide) to undergo aggregation. And in the complexes with the low-molecular-mass DNA at r = 0.6-0.8 the transformation of the DNA into the ψ type was observed, which is characteristic for complexes of DNA with polylysine [23] and with histone H1 [24]. This phenomenon is interpreted as a highly ordered asymmetric supermolecular packing of the associates of

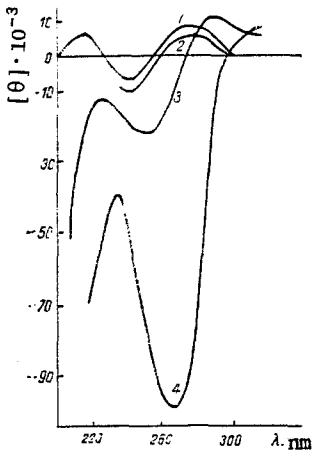

Fig. 4

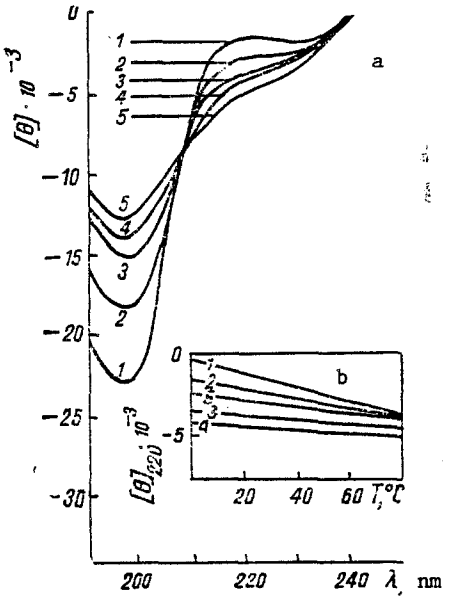

Fig. 5

Fig. 4. CD spectra in 0.015 M NaCl solutions of the complexes of low-molecular-mass DNA with the peptide 1-21 at various concentrations of the latter: 1) r = 0; 2) r = 1.0; 3) r = 1.5; 4) r = 2.0.

Fig. 5. CD spectra of histones: a) H_2O, pH 7.0 (2°C); b) dependence of the molar ellipticity $[\theta]_{220}$ of the histones on the temperature in solutions: H_2O, pH 7.0; curves 1) H1; 2) H2A; 3) H2B; 4) H3; 5) H4.

the nucleotides in the presence of the salt. We observed a similar phenomenon in the case of complexes of fragments of histone H2B with the sequence 1-21 with low-molecular-mass DNA. In solutions with a low ionic strength (0.015 M NaCl) at r ≥ 1.5 ψ-complexes of DNA are formed (Fig. 4, curves 3 and 4) [8].

The study of the structural transitions of models of histones has enabled a comparison to be made of them with the conformational potentialities of the histone molecules themselves. Together with α- and β-structured sections in the histones it is possible to single out fragments close in structure to an extended left-handed helix of the type of poly-L-proline II [20].

In the CD spectra of aqueous solutions of histone fractions (Fig. 5, a), the presence of a shoulder was observed in the 220 nm region and an intense negative band in the 190-200 nm interval confirming the assumption that in the structure of the histones there is a considerable proportion of sections with the conformation of an ε-helix but with different degree of defectiveness, depending on the amino acid sequence. When the histone solutions were heated there was the noncooperative change in their conformation that is also characteristic for polypeptide chains with the structure of an ε-helix, and this indicates the involvement in this process of sections of histones with the given conformation (Fig. 5, b). A change in the conditions of the medium led to a change in the conformation of the histone molecule (see, for example, histone H1) from a clearly expressed left-handed helix in 8 N urea and 5 M guanidine hydrochloride (Fig. 6, curve 6) as far as α-spiralization in suitable solvents (80% ethanol and 1% SDS) (Fig. 6, curves 4 and 5).

Analysis of the primary structures of histone has shown that the majority of sections capable of adopting the conformation of an extended left-handed helix are located at the ends of the histone molecules enriched with basic amino acid residues. There are grounds for considering that the structure of the polypeptide chain is determined by the global minimum of the potential energy of the peptide–solvent system. This minimum occurs at the lowest dipole-dipole (in the peptide groups), Van der Waals (the backbone and side chains), and charge-charge (side chains) interactions. According to calculations taking into consideration nonvalent and electrostatic interactions, the minimum energy is located in the region of the regular structures of α-helix, β-structure, and extended left-handed helix

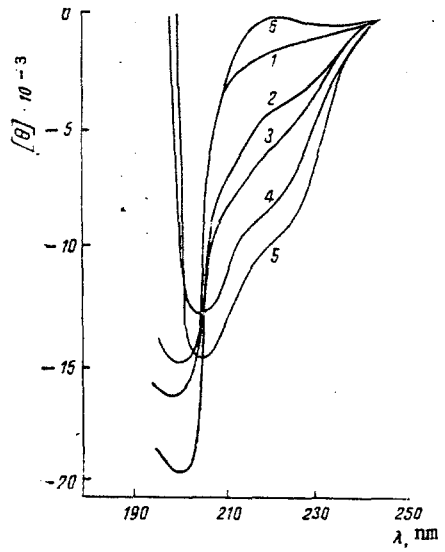

Fig. 6. CD spectra of histone H1 under various conditions: 1) H_2O, pH 2-6; 2) H_2O, pH 12; 3) 1 M NaF; 4) 1% SDS; 5) 80% ethanol; 6) 5 M Gu·HCl or 8 M urea.

[25]. The random-coil conformation, conversely, greatly increases the energy of dipole-dipole interactions.

Until recently, it was assumed that the extended left-handed helix of the type of poly-L proline II was characteristic of only a narrow class of polypeptides containing a large proportion of imino acids. As a result of calculations of the frequency of finding α-helices, β-structures, and ε-helices in globular proteins, it has been shown that these are, respectively, 33.7, 11.4, and 9.4%, i.e., the frequency of finding an ε-helix in globular proteins is close to the frequency of β-structures [4].

The side chains of certain amino acids (lysine, alanine, serine, proline) within a polypeptide chain may additionally stabilize an ε-helix and, consequently, there is a probability of their more frequent inclusion in sections with such a structure.

The ε-helix is preferred for some small segments of a polypeptide chain if no specific interactions of any kind are superposed that lead such sections of the polypeptide away from this conformation.

Thus, an extended left-handed helical conformation of the type of poly-L-proline (II) is the third main regular form of polypeptide chain. It bears a universal nature, and this includes the fact that it permits the incorporation of any amino acid residue, provides the possibility for a polypeptide chain to be incorporated in the chains of other biopolymers to effect the necessary interactions, and can be found in both fibrillar and globular proteins.

LITERATURE CITED

1. W. B. Rippon and A. G. Walton, J. Am. Chem. Soc., 94, 4319 (1972).
2. M. L. Tiffany and S. Krimm, Biopolymers, 12, 575 (1973).
3. A. A. Makarov, N. G. Esipova, Yu. A. Papkov, B. A. Grinevskii, V. M. Lobachev, and G. A. Sukhomudrenko, Mol. Biol. (Moscow), 10, 704 (1976).
4. A. A. Adzhubei, V. V. Poroikov, N. G. Esipova, V. G. Tumanyan, Conformational Type of Polyproline II in Globular Proteins [in Russian], Dep. Rukop. [deposited manuscript] No. 4372-79, VINITI [All-Union Institute of Scientific and Technical Information], Moscow (1989).
5. E. I. Ramm, R. R. Kamilova, O. L. Polozova, V. I. Vorob'ev, and V. K. Burichenko, Biofizika, 24, 815 (1979).
6. L. I. Mar'yash, V. K. Burichenko, and V. A. Shibnev, Bioorg. Khim., 10, No. 4, 467 (1984).
7. E. I. Ramm, N. I. Koryakina, A. I. Pisachenko, V. M. Lobachev, N. G. Esipova, V. K. Burichenko, V. I. Vorob'ev, and Yu. A. Lazarev, Biofizika, 22, 32 (1977).
8. V. K. Burichenko, R. R. Kamilova, and E. E. Meitus, Dokl. Akad. Nauk TadzSSR, 20, No. 6, 35 (1977).
9. N. I. Koryakina and V. K. Burichenko, Khim. Prir. Soedin., 788 (1975).
10. V. A. Shibnev, L. I. Mar'yash, and K. T. Poroshin, Dokl. Akad. Nauk SSSR, 207, No. 3, 625 (1972).

11. V. A. Moroshkin, E. V. Kartasheva, É. V. Frisman, E. I. Ramm, V. N. Nikun, V. I. Vorob'ev, R. R. Kamilova, N. I. Koryakina, and V. K. Burichenko, Mol. Biol. (Moscow), 14, No. 4, 795 (1980).
12. L. I. Mar'yash, L. V. Abaturov, and V. A. Shibnev, Stud. Biophys., 43, No. 1, 41 (1974).
13. N. G. Esipova, V. M. Lobachev, V. N. Rogulenkova, A. A. Makarov, V. A. Shibner, and M. Finogenova, Mol. Biol. (Moscow), 18, No. 3, 725 (1984).
14. A. J. Adler, N. G. Greenfield, and G. D. Fasman, Meth. Enzymol., 28, 675 (1975).
15. E. R. Blout, in: Fundamental Aspects and Recent Developments in ORD and CD (ed. E. Ciardelli), Heyden, New York (1973), p. 352.
16. V. Madison and J. Schellman, Biopolymers, 11, 1041 (1972).
17. D. Aebersold and E. S. Pysh, J. Chem. Phys., 11, 1919 (1970).
18. V. A. Zubkov, T. M. Birshtein, J. S. Milevskaya, and M. V. Volkenshtein, Biopolymers, 10, 2051 (1971).
19. P. J. Flory, The Statistical Mechanics of Chain Molecules, Interscience, New York (1969).
20. E. I. Ramm, V. I. Vorob'ev, R. R. Kamilova, V. K. Burichenko, and N. G. Esipova, in: IVth All-Union Symposium on the Chemistry of Proteins and Peptides [in Russian], Minsk (1977), p. 58.
21. E. I. Ramm, G. S. Ivanov, V. I. Vorob'ev, N. G. Esipova, M. V. Grigolava, V. S. Grechishko, and V. K. Burichenko, Biofizika, 28, No. 1, 35 (1983).
22. B. Jirgensons and L. S. Hnilica, Biochem. Biophys. Acta, 109, 241 (1965).
23. A. Shin and G. L. Eichhorn, Biopolymers, 16, 225 (1977).
24. G. D. Fasman, M. S. Valenzula, and A. J. Adler, Biochemistry, 10, No. 20, 3795 (1971).
25. G. N. Ramachandran, G. M. Venkatachalam, and S. Krimm, Biophys. J., 6, 849 (1966).

PRODUCTS OF PHOTOTRANSFORMATION OF PROVITAMIN D_4 OBTAINED FROM A MUTANT *Saccharomyces cerevisiae* YEAST.

II. IRRADIATION IN HEPTANE

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UDC 547.92

The composition of the photolytic mixture formed on the irradiation of provitamin D_4 in heptane has been studied. In addition to the main reaction products – vitamin D_4 and previtamin D_4 – a number of by-products were formed the structures of which have been determined by spectral methods. In contrast to that formed in ethanol, the photolytic mixture obtained in hexane contained only small amounts of by-products. The solvent therefore has an influence on the occurrence of phototransformation in the preparation of vitamins of the D group.

The phototransformation of the provitamins D is a complex process depending on many factors such as the wavelength of the source of irradiation, the initial concentration, stirring, the presence of dissolved oxygen, and a number of others [1]. One of the determining factors is the nature of the solvent, which affects the quantitative and qualitative composition of the photolytic mixture [2]. The photolysis of provitamins D is most frequently carried out in ethanol, which is due to its availability, cheapness, and low toxicity. However, because of its high reactivity, ethanol interacts with the excited reaction intermediates increasing the total number of by-products [3]. It is obvious that it is possible considerably to increase the selectivity of the process and the yield of final product by performing photolysis in a medium less aggressive towards the components of the reaction mixture.

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