A STUDY OF THE CONFORMATIONAL STATES OF ARGININE-CONTAINING POLYPEPTIDES OF REGULAR STRUCTURE

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In recent years, the attention of workers has been attracted by nuclear proteins, protamines and histones, which enter together with the nucleic acids into the composition of the chromosomes of the cell and taking part in the regulation of the activity of the genetic apparatus [1, 2]. In spite of the fact that the investigation of the primary structure of protamines and histones has been advanced to a fairly considerable degree, a study of synthetic models, which may throw light on the establishment of the nature of the interaction between mechanical structure and biological functions of natural compounds, may be important.

We have previously [3, 4] synthesized arginine-containing polypeptides modelling individual sections of protamines and histones. In the present paper we give the results of structural investigations of (Ala—Ala—Arg) and (Arg—Arg—Ala) polypeptides with molecular weights of 7000 and 6000, respectively.

Conformational investigations of the polypeptides synthesized were performed by IR and NMR spectroscopy and optical rotatory dispersion (ORD).

Analysis of IR spectra permits an answer to the question of the presence of an α helix, a chaotic coil, or a β conformation of the protein or polypeptide molecule, and here a well-established correlation between the positions of the amide bands and the conformations of the polypeptide chains is used. For the β form, the position of the amide-I band must correspond to that at 1630 cm^{-1}, and the amide-II band to that at 1530 cm^{-1}. The coil and helix conformations give partially overlapping bands in the 1640-1660 cm^{-1} region for amide-I and 1540-1560 cm^{-1} for amide-II [5].

On the basis of the results obtained by means of IR spectroscopy it was possible to conclude that in the polypeptides that we investigated there is no β structure. This is in harmony with literature information showing the absence of this structure in all fractions of protamines and histones with the exception of the lysine-rich FI fraction.

The positions of the amide-I and amide-II bands give grounds for assigning the conformation of the polypeptides synthesized and of the natural protamine to a mixture of two conformations — coil and helix. The displacements of the amide-I bands in the low-frequency direction (by 10 cm^{-1}) observed in the spectra are connected with the replacement of hydrogen by deuterium and, as is well known [6], permit the detection of a coil—helix conformational transition. The ORD parameters for the polypeptides studied (Table 1) were obtained in 0.2 M NaCl solution at acid and alkaline pH values of the solution, while at pH > 12.3 the polypeptides precipitated, which prevented further measurements. The quantitative evaluation of the conformational changes was performed on the basis of the Moffitt equation [7]:

$$[m'] = \frac{3}{n^2+2} \cdot \frac{M_0}{100} \cdot \frac{\varphi}{l\,(\mathrm{dm})\, c\,(\mathrm{g/cm^3})},$$

where $\varphi/l \cdot c = [\alpha]$ is the specific rotation of the substance, and m' is the specific rotation of the substance referred to one residue (M_0 is the mean molecular weight of the residue) and corrected for the refractive index of the solvent.

From a graph of $-[m']/\lambda$ as a function of λ' for the (Ala—Ala—Arg) polypeptide at pH 12.3 we find $b_0 = -27°$. In view of the fact that at 100% spiralization $b_0 = -630°$, we obtain the percentage spiralization of

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TABLE 1. Optical Rotatory Dispersion Characteristics for the Ala–Ala–Arg and Arg–Arg–Ala Polypeptides

λ	λ"	φ, deg		−[m']		−[m']λ'	
		pH 4	pH 12,3	pH 4	pH 12,3	pH 4	pH 12,3
579,06	0,155	0,131 0,091	0,081 0,082	67,4 63	58,4 60	435 410,3	377 388
546,07	0,177	0,149 0,105	0,093 0,093	76,7 73	67,0 68	434 414	379 385
435,83	0,310	0,263 0,181	0,163 0,166	135,4 126	117,5 121	437 408	379 393
407,78	0.370	0,315 0,217	0,196 0,201	163,2 151	141,3 138	441 412	382 392
404,66	0,378	0,323 0,222	0,201 0,198	166,3 155	144.9 145	440 414	383 387
365,48	0,507	0,432 0,278	0,271 0,268	222,4 194	195,0 196	439 389	386 390
334,16	0,674	0,572 —	0,365 —	294,5 —	263,0 —	440 —	391 —

Note. The numerator gives the figures for the Ala–Ala–Arg polypeptide and the denominator those for the Arg–Arg–Ala polypeptide.

the polypeptide as 4.3%. For the (Arg–Arg–Ala) polypeptide, the percentage spiralization at the same pH of the solution is 2.5%. Consequently, it may be concluded that in the polypeptides in solution at pH 11–12.3 there is practically no spiralization. This is apparently connected with the high value of pK for the guanidine group of arginine. A higher pH of the solution is necessary for the neutralization of this amount of charge [8]. This agrees to some extent with literature information [8, 9] on structural investigations of synthetic poly(L-amino acid)s – polylysine, polyarginine, and polyornithine – which show that the coil–helix conformational transition is largely prevented by the magnitude of the charge on the side chain of the polypeptides. For polyarginine, for example, the conformational transition is observed only at high pH values (12–12.6) when the neutralization of the charges on the side chain takes place.

The NMR spectra of the polypeptides synthesized were taken in D_2O and in a mixture of D_2O with CD_3OD in equal proprotions. It is known from the literature that with the gradual addition of a series of organic solvents to aqueous solutions of proteins and polypeptides the latter are capable of undergoing the coil–helix conformational transition [9]. Consequently, in the interpretation of the NMR spectra attention was attracted in the first place by the behavior of the α-CH proton resonance, since its position depends on the conformation of the polypeptide chain.

As is known, in a study of such systems as polylysine, polyalanine, and poly(glutamic acid) and a number of others, a single α-CH proton peak is observed which corresponds to a rapid coil–helix interconversion. For other systems – in particular, for polyarginine – two α-CH proton peaks are observed corresponding to a slow conformational interchange: at 4.0 ppm, which characterizes the helical region of a polypeptide, and at 4.4–4.3 ppm, which characterizes the region of a random coil. This is also characteristic for polypeptides with a relatively high degree of polymerization (n = 20–30) [10].

In the NMR spectrum of the conformational transitions for the (Ala–Ala–Arg) polypeptide taken in D_2O, two signals of the α-CH proton resonance can be seen: a signal in the weak field, at 4.65 ppm, which is usually assigned to the coil form, and a signal at 4.15 ppm assigned to the helical form. These signals differ considerably in intensity and show that in pure water the predominating conformation is the coil.

With an increase in the proportion of methanol, together with a broadening of the coil region it is possible to observe a simultaneous decrease in its intensity, while the intensity of the region of the helix rises. On this basis it can be stated that at a ratio of D_2O to CD_3OD of 1 : 1 and above structurization of the polypeptide chain takes place with a predominance of the helical conformation.

For the (Arg–Arg–Ala) polypeptide taken in D_2O a single broad signal is observed in the region of the α-CH peak at 4.63 ppm. On the addition of CD_3OD, the signal splits into two at 4.7 and 4.56 ppm, which shows the presence of two conformations. Consequently, the intermediate values of the signal of the α-CH proton in D_2O (4.63 ppm) can be ascribed to a slow interchange between conformational states. A further increase in the proportion of methanol leads to a predominance of the helical conformation.

EXPERIMENTAL METHOD

The IR spectra were taken on a UR-20 instrument with NaCl prisms. To obtain the spectra, films were cast from deuterated water (D_2O) and deuterated methanol (CD_3OD), a 0.2 M solution of NaCl, and trifluoroacetic acid (CF_3COOH) on a CaF_2 support. The film thickness was 3-4 μ.

The ORD parameters were obtained on a Perkin-Elmer 141 M spectropolarimeter with 0.2% solutions at 18-20°C with a cell thickness of 1 dm.

The NMR spectra were taken on a JNM 4H-100 instrument with a working frequency of 100 MHz. Tetramethylsilane was used as internal standard.

SUMMARY

On the basis of the results obtained it may be concluded that the arginine-containing polypeptides with a relatively low degree of polymerization that have been synthesized exist in aqueous solution predominantly in the form of a coil, and on the addition of organic solvents they may exist as mixtures of two conformations – coil and helix with a predominance of the helical conformation.

LITERATURE CITED

1. V. G. Allfrey and A. E. Mirsky, Proc. Acad. Sci., 44, 981 (1958).
2. V. M. Bresler and V. I. Vorob'ev, Tsitologiya, 5, 69 (1963).
3. G. F. Kasymova, V. K. Burichenko, M. B. Berdyeva, and K. T. Poroshin, Dokl. Akad. Nauk TadzhSSR, 13, No. 10, 25 (1970).
4. V. K. Burichenko, K. T. Poroshin, G. F. Kasymova, and V. A. Shibnev, Izv. Akad. Nauk SSSR, Ser. Khim., No. 11, 2597 (1972).
5. E. M. Bradbury, C. Crane-Robinson, H. Goldman, H. W. E. Rattle, and R. M. Stephens, J. Mol. Biol., 29, 507 (1967).
6. E. M. Bradburg, and A. Elliot, Polymer, 4, 47 (1963).
7. W. Moffitt, J. Chem. Phys., 25, 427 (1956).
8. J. M. Rifkind, Biopolymers, 8, No. 5, 685 (1969).
9. E. M. Bradbury, C. Crane-Robinson, and H. W. E. Rattle, Eur. J. Biochem., 12, 258 (1970).
10. M. Boiblik, E. M. Bradbury, and C. Crane-Robinson, J. Biochem., 12, 258 (1970).