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THE THERMAL DENATURATION OF DNA COMPLEXES WITH PROTAMINE PEPTIDES

It is shown that in a 10% solution of NaCl the melting temperature of DNA obtained from herring sperm (T_m = 94° C) is shifted to 85° C upon attachment of protamine to DNA (Zimmermann, 1965). The opposite is reported for DNA complexes with protamine in solutions of low ionic strength (Paукас, 1965b). In this experiment, DNA from sperm of *Acipenser güldenstädti* was used with the midpoint of the DNA thermal transition in 0.01M NaCl equal to 69.6° C. Upon adding protamine or basic polypeptides to DNA solution, an especial shape of transition curve was observed, consisting of two independent regions, with the second step between 89 and 97° C (see fig.). The height of the second step is proportional to the quantity oi polypeptide added and therefore obviously represents the melting of the DNA-polypeptide complex. The shift of the first step towards higher temperatures was interpreted as preferential complexing of protamine and basic polypeptides with AT-regions of the DNA molecule by means of H-bonds. Similar two-step thermal transition curve is also observed by other investigators in case of polylysine-DNA complexes (Tsuboi et al., 1966).

The unique shape of DNA-protamine ane DNA-basic polypeptide complexes thermal transition curves must be considered as an indication of the unique structure of these complexes if compared with complexes of DNA with other substances (including basic substances). For the variety of complexes studied so far, no strict stoichiometry was noted in most cases between the quantity of DNA and substances added, although the saturation was observed usually to be revealed in the constancy of T_m. It is supposed that the increase in the melting temperature is caused by reducing a negative charge of the DNA macromolecule and/or connecting the opposite chains of DNA with each other.

In the case of protamine, on the contrary, there is a precise correspondence between the size of the second step and the protamine/DNA ratio. These observations, together with the X-ray diffraction evidence (Paукас, 1965a), enabled us to assume that all basic polypeptides investigated accomodate a conformation earlier proposed for complexes of DNA with protamine (Feughelman et al., 1955). The dimensions of DNA double helix are in good conformity with those of the polypeptide chain (Paукас, 1965b), and therefore it may be supposed that protamine or polypeptide molecules lie in the small groove of the DNA macromolecule in a specific trans-configuration, the positively charged groups of side chains of arginine, lysine or ornithine fixed to DNA phosphates.

The purpose of this investigation was to establish the minimal length of the basic polypeptide chain, capable to form a triple-helical type complex with DNA, as judged by thermal transition curves.

The peptides were derived from stelline (the protamine of the sperm of *Acipenser stellatus*), which belongs to the group of triprotamines and contains all three basic amino acids: arginine 67.4%, lysine 12.9%, and histidine 8.9%.

After a short digestion of stelline with chymotrypsin, the undigested part of protamine and enzyme were precipitated from the hydrolysate, the free amino acids separated by chromatography and the obtained mixture of peptides fractionated on carboxymethylcellulose (Na^+). The resulting fractions differed in their amino acid composition and the peptide size (see the table).

The mean peptide length of each fraction was deduced from the content of free amino groups before and after a complete acid hydrolysis by the method of van Slyke. The amino acid composition was determined on an automatic recording apparatus after D. Spackman, W. Stein and St. Moore.

The first fraction consists almost entirely of ammonia salts and contains only traces of amino acids. The fraction II is a mixture of tri- and tetrapeptides with the ratio of basic and nonbasic amino acids considerably lower than that of the stelline. Peptides III and IV are fairly close in their amino acid composition, the latter containing relatively more lysine and less arginine than peptide III. The ratio of basic and nonbasic amino acids of peptide III is the same as in the case of initial stelline; the peptide fraction IV reveals somewhat higher basic amino acid content. The mean length of the polypeptide chains of the peptide fractions III and IV are 8—10 and 10—12 amino acid residues, respectively. The small content of various nonbasic amino acids obviously indicates that all the three fractions are peptide mixtures.

Amino acid composition of *Acipenser stellatus* sperm protamine (stelline) and protamine peptide fractions in mMoles per 1 g of free peptide*

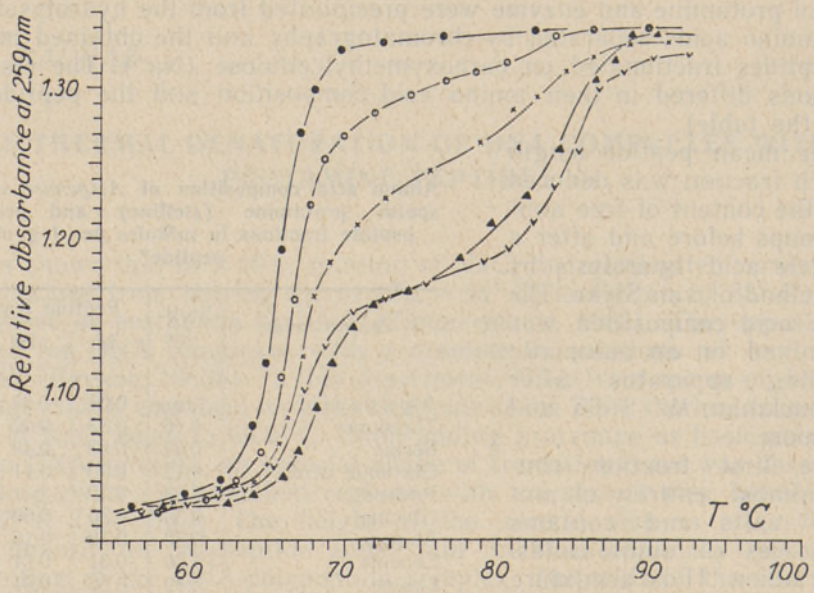
Amino acid	Stelline	Peptide fraction		
		II	III	IV
Aspartic acid	Trace	0.03	0.04	0.01
Threonine	0.16	0.33	0.07	0.02
Serine	0.37	0.93	0.49	0.51
Glutamic acid	0.12	0.30	0.11	0.03
Proline	0.08	0.20	—	—
Glycine	0.30	0.30	0.50	0.67
Alanine	0.32	0.78	0.16	0.11
Leucine	0.20	0.31	0.07	—
Lysine	0.89	0.56	0.91	1.54
Histidine	0.58	0.41	0.77	0.81
Arginine	3.87	2.95	3.31	3.02
Ratio of basic and nonbasic amino acids	3.45	1.23	3.47	3.98
Average number of amino acid residues per peptide	—	3—4	8—10	10—12

* The data are provided by Dr. E. Kaverzneva.

The melting of DNA complexes with various protamine peptide fractions were followed by optical absorbancy changes at 259 nm, making use of an SF-4 type spectrophotometer with a special cell. For experiments, phenol-extracted DNA from phage T2 was used. Stock solution of DNA in 0.24M NaCl was diluted 24 times with distilled water and the peptide solution, immediately before each experiment, to a DNA concentration of 5 μg/ml. Distilled water was added to 0.125 ml of DNA stock solution with such a consideration that when the peptide solution (up to 0.3 ml) is

added, the total volume of the sample would be 3.00 ml, and the salt concentration 0.01M NaCl.

To prepare peptide solutions, small quantities of peptides were weighed and dissolved in distilled water in a concentration of 50 μg/ml (500 μg/ml for peptide II). Since the peptide fractions contain large quantities of salt, this concentration is not equal to a concentration of free base in solution.



Effect of stelline and stelline peptide IV on the thermal denaturation of T2 DNA (5.0 μg/ml) in 0.01M NaCl. ● — DNA; o, ×, ▲ — DNA complexes with the peptide IV, peptide/DNA ratio 1 : 6, 1 : 3 and 1 : 2, respectively; v — DNA-stelline complex, stelline/DNA ratio 1 : 3.

The melting of DNA complexes with peptide fraction IV closely resembles the meltig of DNA-protamine complexes (see fig.). However, the second step on the melting curve of the DNA complex with peptide IV is somewhat smaller and less pronounced at the same peptide/DNA ratio (1:3). The obvious explanation for these observations seems to be the greater salt content of peptide fraction and the lesser regularity of the complex as compared with the DNA-protamine complex.

A melting curve of quite different kind is observed for DNA complexes with peptide fractions II and III. In these cases the midpoint of the DNA thermal transition increases proportionally to the quantity of peptides added, no changes in the shape of the transition curve were observed. Thus, the mode of interaction of these peptides with DNA is very similar to the other polyanions, such as spermine (Tabor, 1962) or diamines (Mahler, Mehrotra, 1963) in small concentrations.

As seen from the table, the peptide fractions III and IV do not drastically differ from each other, peptide IV being a little longer and containing relatively more lysine residues than peptide III. Nevertheless, the shape of the melting curve of the DNA-peptide IV complex differs essentially from that of the DNA-peptide III complex and has a shape very similar to the shapes of DNA-protamine and DNA-basic polypeptide

melting curves. Possibly, this effect is due to the greater peptide length, although the increased lysine content may also influence the melting of the complex. This view is supported by the fact that the thermal transition midpoint of the DNA-polylysine complex is higher than that of the DNA-polyarginine complex (Раукас, 1965b). Similarly, the effect of the lysine-rich fraction of calf thymus histone upon melting of DNA is far more pronounced than the effect of arginine-rich histone (Huang et al., 1964). However, the similarity of the melting curves in both cases (polylysine-DNA and polyarginine-DNA, lysine-rich histone-DNA and arginine-rich histone-DNA) enables us to assume that the change in the shape of the melting curve is mainly due to the length of peptide IV as compared with peptide III.

It may be concluded therefore that the critical length of the polypeptide chains having a characteristic triple helix configuration in a complex with DNA (Feughelman et al., 1955) and melting at its own, characteristic temperature must contain no less than 10—12 amino acid residues (including 8—9 basic amino acid residues).

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DNH JA PROTAMIINPEPTIIDIDE KOMPLEKSIDE TERMILINE DENATURATSIOON

Resümee

Ultravioletse absorptsiooni meetodil uuriti DNH ja protamiinpeptiidide komplekse. Protamiinpeptiidid olid saadud stelliini (protamiin *Acipenser stellatus*'e spermast) hüdrolüüsil. Näidatakse, et DNH ja protamiini kompleksile iseloomulik kaheastmeline «sulamiskõver» avaldub alates peptiidist, mille pikkus ei ole alla 10—12 aminohappe jäägi.

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ЭРГО РАУКАС

ТЕПЛОВАЯ ДЕНАТУРАЦИЯ КОМПЛЕКСОВ ДНК С ПЕПТИДАМИ ПРОТАМИНА

Резюме

Методом ультрафиолетового поглощения исследовалась температура плавления комплексов ДНК с основными пептидами, полученными гидролизом стеллина (протамин из спермы *Acipenser stellatus*).

Показано, что характерная для комплексов ДНК-протамин двухступенчатая кривая плавления появляется при длине пептидной цепи не менее 10—12 аминокислотных остатков.

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