On the Interaction between Basic Telopeptides and DNA

The peptide fraction (telopeptides) which is released from the collagen molecule by the action of proteolytic enzymes like pepsin, trypsin or pronase is no doubt of considerable interest for both structural and biological studies. DRAKE et al.[1] and DEYL and ROSMUS[2] have shown the importance of telopeptides in the maturation of the collagen molecule. The increase in the cross-linking density is apparently due to the covalent-bond formations in the peripheric regions of the collagen molecule – telopeptides – and involves presumably the lysine residue[2,3].

The sequences of basic peptides released by pronase action from the collagen molecule resemble very much those isolated by PHILLIPS and SIMSON[4] and SATAKE et al.[5] from the arginine-rich calf thymus histones. As calf thymus histones are known to exhibit a typical DNA-protein interaction, presumably with some biological consequences, it seemed to be favourable to test the liberated peptides for possible T_m increase.

Materials and methods. In our experiments calf-skin insoluble collagen purified by the procedure of DRAKE et al.[1] was used as starting material. Pronase digestion was carried out in the Ca-acetate media and the pepsine digestion in 0.05% acetic acid, both as described by DRAKE et al.[1].

The fractionation of telopeptides (ROSMUS et al.[6]) involved a continuous high-electrophoresis run in a pyridine-acetate buffer (pH 6.3, ionic strength 0.1) at a voltage of 33 V/cm. The basic fraction of this preliminary procedure was submitted to 2 paper-chromatographic separations: *n*-butanol-acetic acid-pyridine-water in a ratio of 30:20:6:24 and a propanol-water system (70:30) were applied stepwise. In the isolated peptides the sequence was determined by Edmann's degradation using a slight modification for the N-terminal determinations (GRAY and HARTLEY[7]). The N-terminal amino acid in each step was converted into the 1-dimethylamino-5-naphthalene-sulphochloride derivative and identified by thin-layer chromatography on Eastman-Kodak silica gel sheets as described previously by DEYL and ROSMUS[8].

The DNA used in our experiments was prepared from calf thymus using the technique of SAVITSKY and STAND[9].

50 μg of calf thymus DNA and a roughly equal amount of the peptide tested (or tenfold of the peptide mixture) were heated in 3 ml of 2.8 m*M* NaCl and 0.2 m*M* citrate buffer, pH 7.2. The variation of absorbance at 260 nm as a function of temperature was studied.

Results and discussion. The results are briefly summarized in the Figures. The effect of the whole peptide mixture is shown in Figure 1, whereas Figure 2 illustrates the effect of the following peptides on the T_m of the calf-thymus DNA:

Peptide No. 12: arg.glu.lys.gly.asp.gly.lys.
No. 28: gly.arg.gly.arg.ser.
No. 37: arg.glu.lys.gly.
No. 41: gly.ala.arg.gly.arg.
No. 44: lys.gly.asp.gly.lys.

In addition to these peptides another peptide released by ozonolysis from the collagen molecule[10], namely trp.leu.lys.arg, was tested.

As far as the structure of the telopeptides is concerned, the following conclusions can be made from the results presented: (1) For the stabilizing effect of a telopeptide, the presence of 2 basic amino acids at least 1 amino acid residue apart is necessary. (2) The peptides with basic

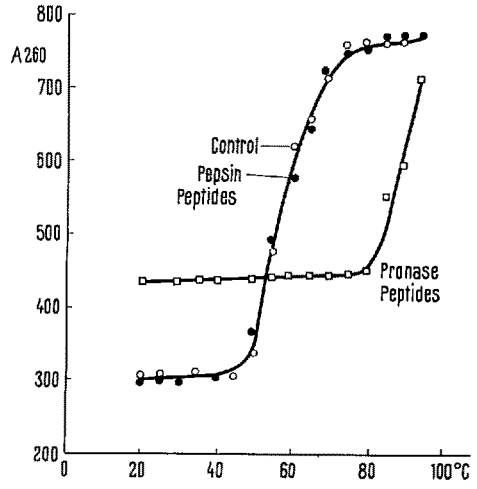


Fig. 1. The temperature profile of absorbancy (A) of the calf thymus DNA stabilized by the mixture of telopeptides. Peptides were released by pronase from the collagen molecule. Peptides split off by pepsin do not exhibit any stabilizing effect on DNA. For experimental details see text.

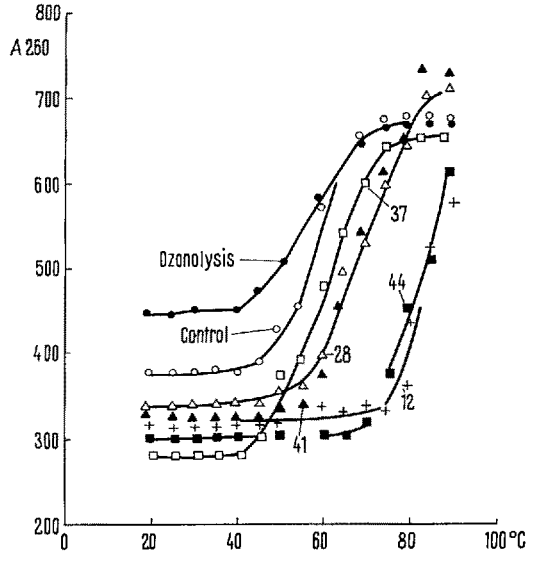


Fig. 2. Stabilizing effect of some peptides from the pronase digest (absorbancy vs temperature). Sequences of peptides: No. 12 arg.glu.lys.gly.asp.gly.lys; No. 44 lys.gly.asp.gly.lys; No. 37 arg.glu.lys.gly; No. 28 gly.arg.gly.arg.ser; No. 41 gly.ala.arg.gly.arg. Peptide liberated by ozonolysis: trp.leu.lys.arg. For experimental details see text.

[1] M. P. DRAKE, P. F. DAVISON, S. BUMP and F. O. SCHMITT, Biochemistry *5*, 303 (1966).
[2] Z. DEYL and J. ROSMUS, J. exp. Gerontol *2*, 97 (1967).
[3] P. BORNSTEIN, A. H. KANG and K. A. PIEZ, Proc. natn. Acad. Sci. U.S.A. *55*, 417 (1966).
[4] D. M. P. PHILLIPS and P. SIMSOW, Biochem. J. *82*, 236 (1962).
[5] K. SATAKE, P. S. RASMUSSEN and J. M. LUCK, J. biol. Chem. *235*, 2801 (1960).
[6] J. ROSMUS, M. P. DRAKE and Z. DEYL, Biochim. biophys. Acta, in press.
[7] J. GRAY and B. S. HARTLEY, Biochem. J. *89*, 380 (1963).
[8] Z. DEYL and J. ROSMUS, J. Chromat. *20*, 514 (1965).
[9] J. P. SAVITSKY and F. STAND, Biochim. biophys. Acta *114*, 419 (1966).
[10] Z. DEYL and J. ROSMUS, Experientia *23*, 610 (1967).

residues 3 amino acids apart exhibit the maximal effect. (3) The neighbourhood of the stabilizing sequence does not influence the increase in the T_m value.

Compared with the results published by PHILLIPS and SIMPSON[4], the pronase-liberated telopeptides are very close to the tryptic digestion of arginine-rich histones, from which sequences like gly(2glu,arg).arg or lys.(pro,his).arg were isolated. For this reason, the stabilizing effect of telopeptides upon DNA is to be expected. Speculation on the possibility of an inhibitory effect of these peptides upon survival of diploid fibroblasts in a tissue culture seems to form an attractive basis for further investigation.

Zusammenfassung. Es wird festgestellt, dass die von dem Tropokollagenmolekül abspaltbare Telopeptidmischung das T_m der Thymusdesoxyribonukleidsäure erhöht. Für die Erhöhung erweisen sich die folgenden Peptide verantwortlich:

arg.glu.lys.gly.asp.gly.lys.
gly.arg.gly.arg.ser.
arg.glu.lys.gly.
gly.ala.arg.gly.arg.
lys.gly.asp.gly.lys.

Z. DEYL, H. MÁLKOVÁ and J. ROSMUS

Laboratory for Gerontology, Physiological Institute, Czechoslovak Academy of Sciences, Prague-Krč, and Central Research Institute of Food Industry, Prague-Smichov (Czechoslovakia), 7th March 1967.

Tryptophan Production by Mutant Strains of *Escherichia coli* K 12

The purpose of this research is to investigate the development of a general method of obtaining strains able to accumulate amino acids in the medium.

It is well known that strains of *Escherichia coli* resistant to 5-methyl-tryptophan (5-MT) excrete small amounts of tryptophan and indole into the medium[1,2]. 5-MT is an analogue of tryptophan which is not incorporated into proteins but which represses the formation of the enzymes involved in tryptophan synthesis. Tryptophan and indole production has generally been attributed to the fact that strains resistant to 5-MT are non-sensitive to repression by either 5-MT or tryptophan[1,2].

Materials and methods. An Hfr strain of *E. coli* has been chosen as a starting strain. Throughout the work a minimal medium (MM) and a complete medium (CM) described by JACOB and WOLLMAN[3] have been used.

5-MT resistant mutants were selected by seeding cells of *E. coli* on solid MM supplemented with 5-MT to a final concentration of $4.10^{-4}M$.

UV- and X-rays were used as mutagenic agents at doses giving about 10% survival. The X-ray treatment was carried out in liquid medium with a Gilardoni-Neoderma Be apparatus (V = 3.3 70 Kvp, 5 uA). The depth of the medium was 1.6 mm and the dose was approximately 10^5r.

Some resistant colonies produce a small turbid halo of non-resistant bacteria. These are colonies that excrete small amounts of indole or tryptophan into the medium which permits the non-resistant cells to grow. Such colonies were picked up and streaked on MM + 5-MT; finally they were plated on the same medium to purify the mutants.

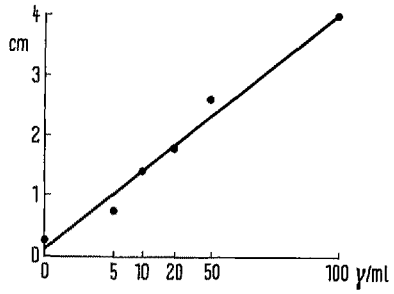
Tryptophan production has been measured microbiologically by the 'fish-spine-bead' technique by using a general method described in [4]. This technique utilizes the growth-stimulating action of tryptophan on 7-aza-tryptophan inhibited cells. The toxic action of 7-aza-tryptophan is overcome by tryptophan but not by indole (Figure). Therefore indole production was measured by the Kovacs colorimetric method[5]. For the assays the mutant strains were cultured in bottles on MM that was neither aereated nor stirred. A preliminary test has shown that either aereation or stirring do not improve production of tryptophan or indole.

The medium with the bacterial suspension was heated 1 h at 70 °C in order to kill cells and then the production titrated. Heating for 1 h at 70 °C does not destroy tryptophan; destruction of indole is approximately 10%.

Results. 60 spontaneous, 220 X-ray induced and 35 UV-induced mutants were cultured in liquid MM and the production of tryptophan and indole assayed.

Among the 315 mutants examined production varied from traces non-measurable to a maximum of 4 γ/ml and that of indole from 0–0.2 γ/ml.

Cells from 5 mutants resistant to 5-MT (code numbers D, E, Z, 3, 63) were plated on MM supplemented with 5-fluoro-tryptophan (5-FT) at the final concentration of $7.10^{-4}M$. 5-FT is an analogue of tryptophan which is probably incorporated into the proteins[6]. The 5 mutants, sensitive to 5-FT, spontaneously and after mutagenic



The dose-response curve of L-tryptophan on MM supplemented with 7-aza-tryptophan ($1.65 \cdot 10^{-3}$). On the abscissa scale the log doses of the amino acid, on the ordinate scale the diameter of growth zones in cm. The bacterium used was *E. coli* K 12. Indole is not titrated with such a system and does not disturb tryptophan titration.

[1] G. N. COHEN and F. JACOB, C. r. hebd. Séanc. Acad. Sci., Paris *248*, 3490 (1959).
[2] A. MATSUSHIRO, J. molec. Biol. *11*, 54 (1965).
[3] F. JACOB and E. L. WOLLMAN, in *Sexuality and Genetics of Bacteria* (Academic Press Inc., London 1962), p. 62.
[4] M. F. MASTROPIETRO CANCELLIERI and G. MORPURGO, Sci. Rep. Ist. Super. Sanità *2*, 336 (1962).
[5] P. R. EDWARDS and H. EDWING, in *Identification of Enterobacteriaceae* (Burgess Publ. Comp., Minneapolis 1962), p. 248.
[6] N. SHARON and F. LIPMANN, Archs Biochem Biophys. *69*, 219 (1957).