MITOGENETIC ANALYSIS OF THE PROTEIN STRUCTURE OF PROTOPLASM

COMMUNICATION I. EXPERIMENTS ON A GELATIN MODEL

N. S. Slavina

From the Mitogenesis Room (Director – Prof. A. A. Gurvich) of the Institute of Normal and Pathological Physiology (Director – Active Member of the AMN SSSR V. N. Chernigovskii) of the AMN SSSR, Moscow

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It may be concluded from a number of factors that in living systems the spatial relationships of the molecules are by no means entirely of the nature of stable, large complexes, so-called "structures", due to true chemical bonds.

Equal importance, however, is attached to temporary quasichemical bonds (of the hydrogen bridge or electrovalent bond type, etc.) and their continuous interchange.

In the study of these processes both in vitro and in the protoplasm of living cells, mitogenetic methods open up new prospects.

For this reason we set out to discover by means of the mitogenetic method criteria which would permit estimation of changes in the quasichemical bonds (reappearance or disappearance) in the protoplasm.

However, before embarking on this study on a living object it was necessary to define the limits of sensitivity of the method on a suitable experimental model.

EXPERIMENTAL METHOD

In selecting a test object we decided upon gelatin. According to modern ideas of colloid chemistry a gelatin sol consists of molecules in chain formation.

Interaction between the individual molecules takes place through nonpolar groups [3]. A gelatin sol, heated to 30-40°C, splits up into separate polypeptide chains which, on cooling, recombine to form a gel. Several theories of gel-formation exist. The most widely accepted is the theory of crystallization which was suggested by Levites, Lipatov, Sokolov, Pauli, Meyer, Mark and others.

This theory regards the process of gel-formation as a peculiar form of crystallization – a gradual approximation and orientation of hydrated particles.

In dilute solution gelatin exists in the form of polypeptide chains; these interact through nonpolar groups. The main forces of interaction within the chain are the valent bonds between the amino acid residues.

On cooling the gelatin, hydrogen bridges are formed between the imido (NH) and carbonyl (CO) groups, and also bonds of an electrostatic character, as a result of which the chains become aggregated into two-dimensional flat lattices, the distance between the chains being of the order of 4.5 A.

In the subsequent development of the process of gel-formation the two-dimensional flat lattices form three-dimensional complexes on account of electrovalent bonds which arise in the side chains containing ionogenic groups. The distance between the lattices is of the order of 11.3 A.

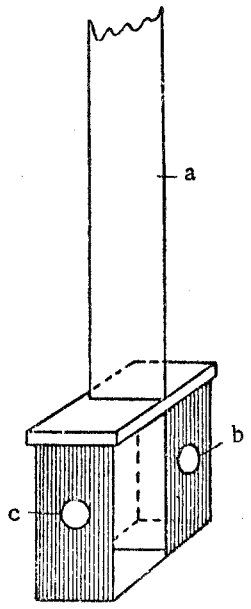

Fig. 1. Quartz chamber.
a – China partition,
b – quartz window through which the activating ultraviolet light is delivered;
c – quartz window centered in front of the collimator of the spectrograph.

In the three-dimensional complexes the bond in the direction of the chain is more closely expressed than are the bonds between the elements in the other two directions, and is due to Van der Waals forces and to hydrogen and electrostatic bonds [3]. These elements are anisometric and form a reticular structure, filled with fluid. Thus, the difference between the sol and the gel consists of the formation in the gel of new and feebly energized bonds.

It is on this condition of the formation of a gelatin gel by labile, feebly energized bonds that the use of gelatin is based as a model to enable the investigation of the possibility of determining the bonds by spectral methods. For this purpose we utilized the mitogenetic method of spectral analysis of selective dispersion, introduced several years ago [1]. As a biological indicator we used an 18-hour culture of the wine yeast Saccharomyces cerevisiae pacca chabli on solid agar.

The effect of irradiation was calculated from the formula $E = I - C / C$, where E is the effect, I the induction and C the control [2].

In order to obtain the selective spectrum of the mitogenetic radiation from the gelatin solution we used a quartz chamber, illustrated in Figure 1. Through the window b in one wall of the chamber the ultraviolet radiation is delivered.

The spectrum is obtained through the window c, in another wall of the chamber, which is divided by a china partition which extends to within 2 mm of the base. This type of construction, with the communicating vessels, permitted the continuation of chain processes underlying the selective dispersion of the mitogenetic radiation, and enabled the effects of scattered "parasitic" light to be excluded.

The investigation was made on a 3% aqueous solution of gelatin.*

The solution was prepared before the experiment in a water bath for 15 minutes, and then was transferred to the chamber, cooled to the required temperature and placed in the apparatus to secure a constant temperature. After the spectrum had been obtained from the sol, the chamber was placed in the refrigerator in order to gelate the gelatin, for 40-60 minutes. The spectrum from the gel was then obtained.

When studying the spectra of the sol and gel, we set out from the hypothesis that in the process of conversion of gelatin from the sol into the gel state, a reorganization of the intermolecular bonds takes place. It was therefore to be expected that certain changes would take place in the spectrum.

We were not concerned to obtain the complete spectrum of the gelatin. We confined ourselves to the study of the $R-C_6H_5$ group – the phenyl group of phenylalanine present in the side-chain – and the $R-C=O$ or carboxyl group, present in the main peptide chain. These functional groups were of the greatest interest to us since, as pointed out in the scheme described, a hydrogen bond appears during the formation of the two-dimensional lattice between the carbonyl and the imido groups, whereas the formation of the three-dimensional framework is due to electrovalent bonds between the functional groups of the side-chains, one of which is phenylalanine.

EXPERIMENTAL RESULTS

As seen from the Table and Figures 2 and 3, our hypothesis that changes in the intermolecular bonds of the Van der Waals and hydrogen bridge type would be sharply reflected in the spectra of selective dispersion was fully justified.

* The gelatin was obtained from Prof. V. A. Kargin's laboratory (L. Ia. Karpov Institute of Physical Chemistry).

Selective Dispersion Spectra of the Functional Group

$R-\overset{R}{\underset{|}{C}}=O$ and $C-C_6H_5$ in a Sol and Gel of Gelatin

Functional group	Wavelength in A	Av. effect (in %)		No. of expts	
		sol	gel	sol	gel
C=O	1960—1975	72,0	58,0	10	15
C=O	2100—2110	85,0	60,0	10	15
Next to c C=O	1950—1960	1,0	76,0	11	9
The same	1975—1980	1,5	1,2	8	4
» »	2090—2100	1,2	2,0	4	8
» »	2110—2120	1,1	70,0	9	10
C_6H_5	2690—2700	131,0	89,0	3	4
C_6H_5	2700—2710	78,0	94,0	5	5
C_6H_5	2720—2730	95,0	45,0	7	7
Next to c C_6H_5	2710—2720	9,5	73,0	7	8
The same	2730—2740	15,0	91,0	5	4

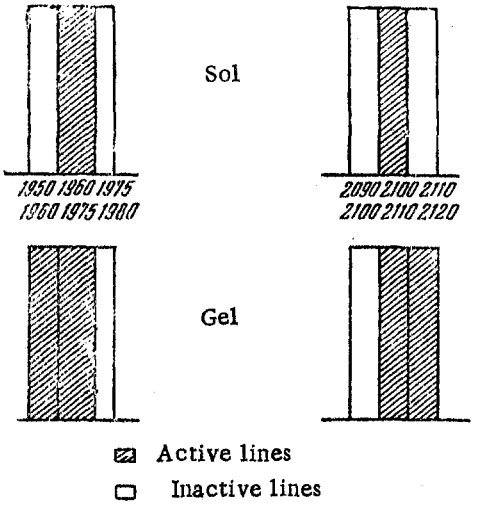

Fig. 2. Spectrum of the functional group $R-RC=O$ in gelatin.

Duration of Exposure of Activity of the Functional Groups

$\overset{R}{\underset{R}{\diagdown}}C=$ and $\overset{R}{\underset{R}{\diagdown}}C_6H_5$

Functional group	Wavelength in A	Exposure	Effectiveness of line	
			sol	gel
C=O	1960-1975	25″	+	+
C=O	2100-2110	15″	+	+
C_6H_5	2690-2700	25″	+	+
C_6H_5	2700-2710	18″	+	0
		30″	0	+
C_6H_5	2720-2730	25″	+	+

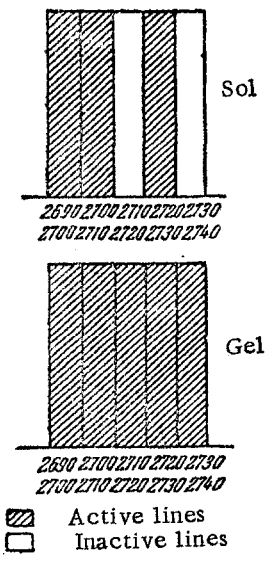

Fig. 3. Spectrum of the phenyl group $R-C_6H_5$ of phenylalanine in gelatin.

The characteristic bands of $R-C=O$ (1960 - 1975 A) and (2100 - 2110 A), studied with an accuracy of up to 5 A, were surrounded in the sol by unoccupied areas; in the gel these bands were still present and new bands appeared alongside them: in the short-wave side a band at 1950 - 1960 A, and in the long-wave side – at 2110 - 2120 A (Fig. 2).

According to the hypothesis put forward above, we regard the appearance of these new bands in the gel as an expansion of the bands of the carbonyl group.

The spectrum of this group is represented in the sol by 3 bands at 2690 - 2700 A, 2700 - 2710 A and 2720 - 2730 A, and by unoccupied areas at 2710 - 2720 A and 2730 - 2740 A. In the gel the spectrum is widened and the bands at 2710 - 2720 A and 2730 - 2740 A, inactive in the sol, begin to emit radiation, i. e. the entire area from 2690 - 2740 A becomes active.

There is a conspicuous weakening of the 2nd C_6H_5 band in the gel.

This band is effective in the sol for 18", and in the gel for 30".

We cannot give at this juncture a complete discussion of this phenomenon, but we consider it necessary to emphasize the fact itself.

The changes in the spectrum of the phenyl group bear the same character.

The filling up of the vacant interval (2710 - 2720 A) and the addition of a new band in the long-wave side (2730 - 2740 A) may, it appears to us, be interpreted with full justification as a widening of the main bands, and the phenomenon of widening may itself be considered to be a sign of the establishment of intermolecular bonds (Fig. 3).

SUMMARY

By means of mitogenetic spectral analysis it was established that lines characteristic of the functional groups RC= O (carbonyl) and R= C_6H_5 (phenyl) are of different breadth in the sol and gel of gelatin. When the gelatin sol is changed into gel the lines become broader.

Increase in the breadth of lines may be considered as the result of appearance of weak intermolecular connections, joining the peptide chains into two- and three-dimensional latticeworks.

The results of these experiments permit consideration of these data as a successful criterion in the assessment of the degree of connection of peptide chains in the substrate under different cellular conditions.

LITERATURE CITED

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