PHYSICOCHEMICAL CHANGES IN DNA AND DNP INDUCED BY POTASSIUM CYANATE

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UDC 612.398.145.1.014.46:546.32'268.1

The effect of KNCO on the structural integrity of salt solutions of DNP was investigated. The use of sedimentation, viscosimetric, and circular dichroism methods showed that KNCO does not cause degradation of the polynucleotide strands of DNA but it weakens the bond between DNA and protein.

KEY WORDS: DNP; DNA; potassium cyanate; mutagens.

Chemical reactions determining the strong biological effect of N-nitroso-N-methylurea (NMU) and other nitrosoalkylureas (NAU) have not been fully explained. It is usually considered that the specificity of action of NMU, by contrast with that of the typical alkylating mutagen methylmethanesulfonate (MMS) is due to its ability to alkylate biological macromolecules by an S_N1 mechanism [10]. However, a study of the reaction of $^{14}CO_4$-NMU with DNA in vitro showed that the radioactive label is transferred from NMU to DNA, i.e., carbamylation of DNA takes place [5]. The authors cited considered that the true carbamylating agent in this reaction is isocyanic acid formed by the breakdown of NMU. Later work showed that NMU can carbamylate bases in nucleosides [13] and RNA [6], and of the four bases of RNA, cytosine is most easily carbamylated. Studies of the biological properties of isocyanic acid and its potassium salt have shown that they also possess mutagenic activity [1, 2]. Carcinolytic activity of the isocyanates has been described [12]. On the basis of these facts it has been postulated that the biological effect of NAU is due to two different chemical reactions: a methylation reaction and a carbamylation reaction [4].

This paper describes a first attempt to investigate the effect of KNCO on the physicochemical properties of DNP in vitro.

EXPERIMENTAL METHOD

Preparations of DNP and DNA were isolated from calf thymus and the solubility of the DNP was studied as described previously [7, 12]. Potassium cyanate was synthesized from urea and K_2CO_3 and recrystallized from ethanol [9].

Circular dichroism spectra were recorded on the Roussel-Jouan CD-185 dichrograph at 20°C. The number of single breaks in DNA was determined viscosimetrically in 0.1 N NaOH [3]. The dependence of the reduced viscosity of DNA in the composition of DNP on the ionic strength of the solvent was determined with a single-ball viscosimeter of the Ostwald type (diameter of capillary tube 1 mm, volume of ball 1 cm^3, H_2O gradient at 25°C 117.9 sec^{-1}).

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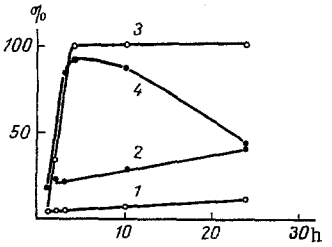

Fig. 1

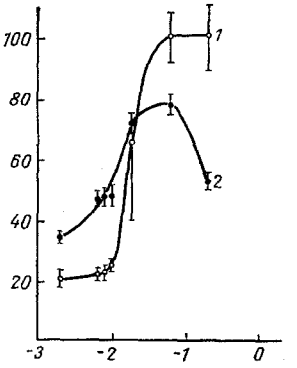

Fig. 2

Fig. 1. Kinetics of solubility of DNP in 0.11 M NaCl + 0.01 M phosphate buffer, pH 7.0, on treatment with 0.2 M KNCO at 37°C: 1, 2) DNA and protein, respectively, in control; 3, 4) DNA and protein, respectively, on treatment with mutagen. Abscissa, time (in h); ordinate, solubilization of protein and DNA (in %).

Fig. 2. Solubility of DNP (24 h, 37°C) in 0.12 M NaCl + 0.02 M phosphate buffer as a function of KNCO concentration. Abscissa, log of KNCO concentration (in M); ordinate, solubilization of protein (curve 2) and DNA (curve 1) (in %).

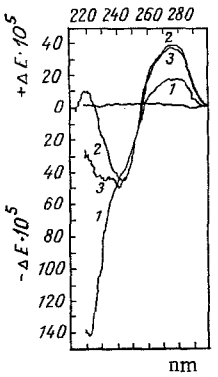

Fig. 3. Circular dichroism spectra: 1) DNP incubated for 24 h at 37°C, solvent 0.4 M NaCl + 0.05 M phosphate buffer, pH 7.0, concentration as DNA 60 μg/ml; 2) DNA, solvent 0.14 M NaCl, pH 7.0, concentration 60 μg/ml; 3) fraction of DNP soluble in 0.11 M NaCl + 0.01 M phosphate buffer, pH 7.0, after incubation with 0.2 M KNCO for 24 h at 37°C, concentration as DNA 60 μg/ml. Abscissa, wavelength (in mm); ordinate, positive (+) and negative (−) region of dichroism (in $\Delta\varepsilon \cdot 10^5$).

EXPERIMENTAL RESULTS

In the course of the reaction between DNP and KNCO a sharpchange takes place in one of the integral properties of DNP, namely its solubility in a medium with an ionic strength close to physiological (0.11 M NaCl + 0.01 M phosphate buffer, pH 7.0). Whole, uninjured DNP is insoluble under these conditions. During the process of modification of DNP by KNCO the solubility of the DNP rises (Fig. 1). The data on the change in solubility of DNP after treatment with KNCO are evidence of the general character of the effects of NMU [7] and KNCO. However, a detailed comparison of the effects of NMU and KNCO also revealed differences. Whereas on incubation with NMU no increase in solubility was observed during the first 5 h, in the case of KNCO solubility increased from the first hours of incubation, and all the DNA had gone into solution after 5 h. The character of the change in solubility of the protein component of DNP after modification of the DNP by KNCO is unique (Fig. 1). The quantity of protein in the soluble fraction of DNP rose sharply to a maximum and then fell sharply. This was evidently because the protein dissociated from DNP associated and was sedimented during centrifugation under mild conditions (8000 g, 20 min). This effect was much weaker with NMU. The DNP solubilization effect on treatment with mutagen is highly dependent on the KNCO concentration (Fig. 2). During the action of low concentrations of KNCO (one to five molecules of mutagen per nucleotide) the solubility of DNP in a solution with physiological ionic strength increased only a little, but it rose sharply with a further increase in the concentration of mutagen. This type of relationship could indicate that the critical number of DNP groups must be modified for conversion into the soluble state.

Disturbances in the structure of DNP induced by the action of KNCO could be identified from the circular dichroism spectra of the "soluble" DNP (Fig. 3). In the positive region of dichroism ΔA after incubation of DNP with KNCO in the spectral region 260-290 nm reached the values of ΔA characteristic of free DNA, evidence of transition of DNA of the soluble fraction from the C into the B form [8]. In the negative region of dichroism of DNP, because of modification of its protein, its contribution to ΔA was negligible and ΔA was due practically entirely to DNA.

An increase in the solubility of DNP in a medium with physiological ionic strength after modification by a mutagen can be produced in two ways: by degradation of polynucleotide strands of DNA and by dissociation of DNA–protein bonds.

It was shown by viscosimetry in an alkaline medium that KNCO, under all conditions of treatment tested, does not produce degradation of polynucleotide strands of DNA. This conclusion was confirmed by the study of the effect of ionic strength of the medium on the reduced viscosity of the "soluble" DNP. The reduced viscosity of "soluble" DNP in a solvent consisting of 0.11 M NaCl + 0.01 M phosphate buffer, pH 7.0, was about 90 dl/g, and on transfer into 2.6 M NaCl solution this parameter fell to 77 dl/g, i.e., it approximated to the characteristic values for control samples of DNP at the same ionic strength.

In the writers' opinion the increase in solubility of DNP after treatment with KNCO is due to dissociation of DNA–protein bonds. The validity of this conclusion is confirmed by the results of centrifugation of DNP soluble in 0.11 M NaCl + 0.01 M phosphate buffer at 180,000 g for 20 h. In this case all DNA and only 40-50% of the soluble protein were segmented. Evidence that the effect of weakening and dissociation of DNA–protein bonds is primary was given by the data presented above showing differences in the kinetics of the change in solubility of DNA and protein (Figs. 1 and 2).

The experimental data described above can be interpreted as follows. During the action of KNCO on lysine-rich histones, the ε-amino group of the latter is transformed into a urea group and can no longer interact with the phosphate group of DNA. As a result the DNP loses its stability. Since protein in DNP plays a structural and stabilizing role, the ability of KNCO to weaken the dissociate the DNA–protein bonds demonstrated by the present experiments may have biological consequences in the organization and function of chromosomal structures.

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