SPIN-EXCHANGE INTERACTION IN POLYURIDILYC ACID MODIFIED WITH A SPIN-LABELLED CARBODIIMIDE

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1. Introduction

A spin-labelled water-soluble carbodiimide, *N*-oxyl-2,2,6,6,-tetramethylpiperidyl-*N*′-β-(4-methylmorpholinium)-ethyl-carbodiimide (*S*-carbodiimide), has been synthesized recently as a reagent for modification of nucleic acids [1]. The compound reacts with nucleosides and nucleic acids in aqueous solution under extremely mild conditions affording a preparation modified to a great extent [2]. In particular, completely modified polyuridylic acid with stable radical residing on each nucleoside moiety became available.

If radical collisions are possible due to their motion, the spin-exchange interactions should arise owing to overlapping of unpaired electron orbitals during collisions. The frequency of these collisions will be determined by mobility and local concentration of spin labels.

The present communication is concerned with studies of the spin-exchange interactions in the dinucleotide UpU and in polyuridylic acid extensively modified with *S*-carbodiimide.

2. Materials and methods

The EPR-spectra were taken with a Varian E-3 EPR-spectrometer. UpU, sodium salt, homogeneous in paper chromatography, was kindly supplied by Dr. V.F. Zarytova (of Novosibirsk Institute of Organic Chemistry); polyU, sodium salt, was a preparation from Reanal (Hungary) purified as described earlier [2]; *S*-carbodiimide *p*-toluenesulfonate was obtained as described earlier [1].

2.1. *Synthesis of modified UpU*

UpU (20 mg) was dissolved in water (5 ml), *S*-carbodiimide (50 mg) added and the mixture incubated for 2 hr in a pH-stat at pH 7.5. Completely modified UpU was isolated from the reaction mixture by thin-layer chromatography on cellulose in n-butanol saturated with 0.05 N CH_3COOH. The lower yellow band was eluted with water, the effluent evaporated to dryness, the residue dissolved in water (1 ml) and applied to a column of Dowex-1 Cl^- (5 ml) to separate the product from the admixture of *p*-toluenesulfonate and UpU. The column was eluted with water, the coloured zone collected and evaporated to dryness at 20°, the residue dried by evaporation of dry ethanol and finally precipitated with ether from dry ethanol. Completely modified UpU thus obtained was homogeneous in paper chromatography in the above solvent ($R_f = 0.2$) and in 80% ethanol ($R_f = 0.3$).

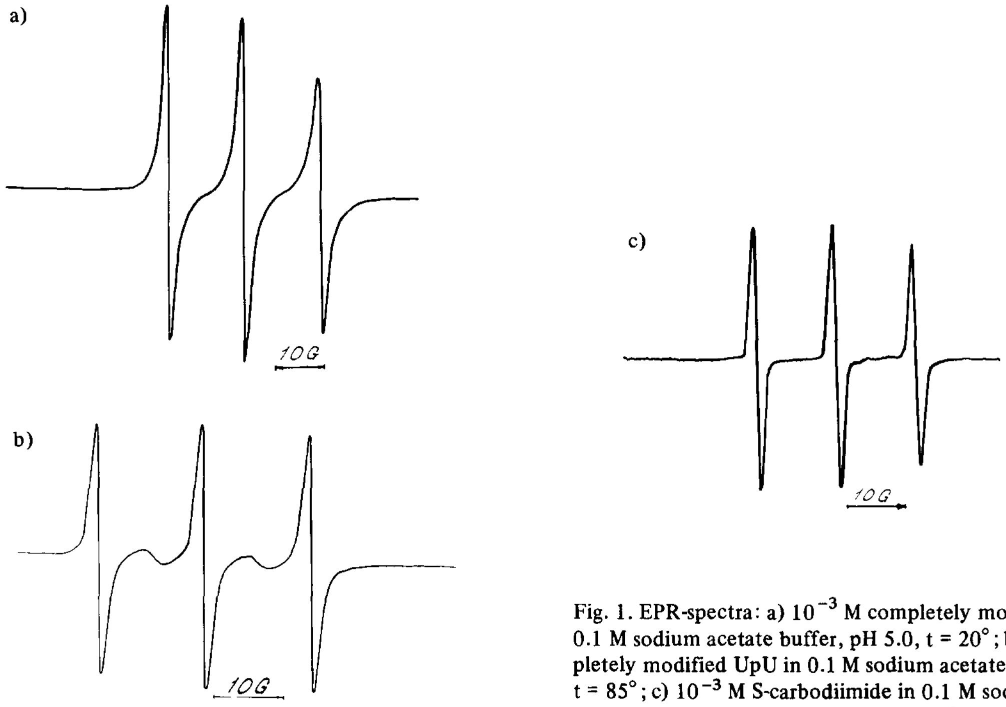

Fig. 1. EPR-spectra: a) 10^{-3} M completely modified UpU in 0.1 M sodium acetate buffer, pH 5.0, t = 20°; b) 10^{-3} M completely modified UpU in 0.1 M sodium acetate buffer, pH 5.0, t = 85°; c) 10^{-3} M S-carbodiimide in 0.1 M sodium acetate buffer, pH 5.0, t = 20°.

2.2. *Synthesis of completely modified poly U*

A reaction mixture containing 10^{-2} M polyU, 0.1 M *S*-carbodiimide and 0.1 M *N*-methylmorpholinium chloride pH 7.5, was incubated for 4 hr at 25°. Completely modified polyU was isolated by gel-filtration on a Sephadex G-25 column in 0.1 M CH_3COONa, pH 5.0. The preparation contained no free radicals in monomer form as revealed by the EPR-spectrum. To determine the extent of modification, an aliquot of completely modified polyU solution was treated with 0.1 M carbonate-bicarbonate buffer, pH 10.5, and the content of poly U determined by gel-filtration followed by spectrophotometry. Another aliquot was hydrolyzed with alkali and the concentration of spin labels was determined from the intensity of EPR-signal compared with that of a standard solution of *S*-carbodiimide. The extent of modification found was 92%.

3. Results and discussion

The molecule of UPU modified with *S*-carbodiimide contains two radical centers: see fig. 3.

It is known that spin-exchange interactions in a biradical system in the fast exchange region ($\omega_e > a$, where ω_e is the frequency of spin exchange, and a is the nitrogen hyperfine coupling constant) lead to emergence of two additional components in the EPR-spectra [3]. Fig. 1 presents the EPR-spectra of completely modified UPU at 20° and at 85° compared with that of *S*-carbodiimide. It is seen that the spectrum of the former compound at 20° is quite similar to that of *S*-carbodiimide, whereas at 85° it is a typical spectrum of a biradical system with $\omega_e > a$. The ω_e value calculated from the linewidths of the additional components according to [4] appeared equal to 8×10^8 sec^{-1}. Thus the possibility has been demonstrated of spin-exchange interaction between spin labels residing on adjacent nucleoside residues.

The EPR-spectrum of completely modified polyU at 20° is a triplet with strongly broadened components (fig. 2a). Dilution of the sample as well as variation of pH from 3 to 7 do not affect the appearence of the spectrum. The majority of the EPR-spectra have been measured at pH 5.0 since at more alkaline pH-values hydrolysis takes place affording *S*-urea [2]. Increase

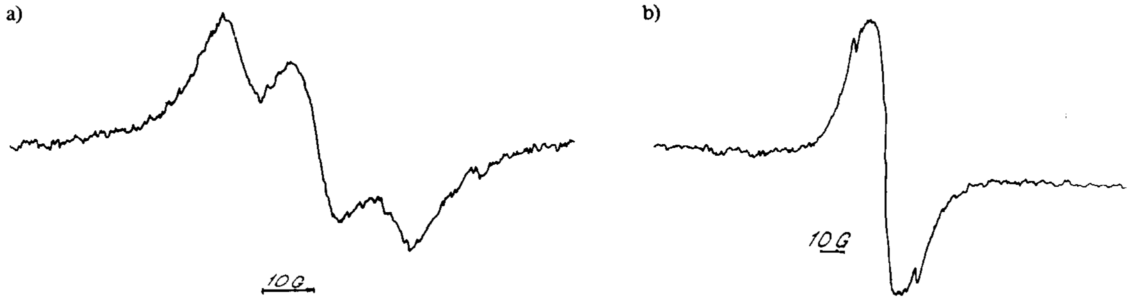

Fig. 2. EPR-spectra of 2×10^{-3} M completely modified polyU in 0.1 M sodium acetate buffer, pH 5.0. a) t = 20°, b) t = 57°. The slight distortion of the latter spectrum is due to presence of some S-urea (see above). The hydrolysis above takes place even at pH 5.0 at high temperatures (under heating).

of temperature results in broadening of the spectrum until at temperatures above 50° it becomes a singlet (fig. 2b), whose linewidth decreases with further increase of temperature. This behavior of completely modified polyU is not surprising. At lower temperatures, both anisotropy of g-value and dipolar interactions on one hand, and the spin-exchange interactions of a great number of radicals due to their collisions at $\omega_e > a$, on the other hand, contribute to the form of the spectrum. Broadening due to former types of interaction decreases with increasing temperature due to their more effective averaging while the exchange linewidth increases due to growth of ω_e, until at $\omega_e \approx a$ individual hyperfine lines collapse to a singlet whose width decreases with further increase of ω_e (for theory see [3]).

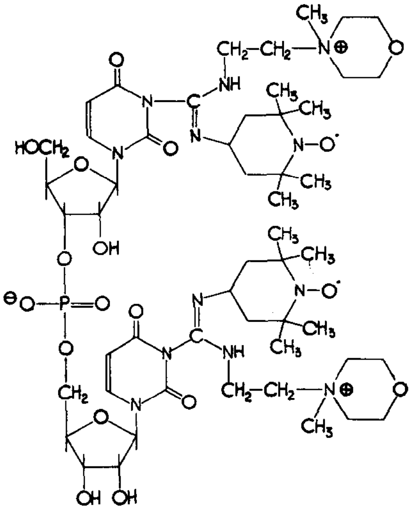

Fig. 3.

It was found according to [3] that at 85° the frequency of spin exchange in completely modified polyU in terms of this theory is 7×10^8 sec^{-1}, a value close to that found for modified UpU (see above) and suggesting that spin exchange in polymer occurs between nearest neighbouring monomer residues.

In order to calculate the value of spin-exchange constant $K = \omega_e/[R]$, the rate constant of the "reaction" of radical collisions, it was necessary to evaluate the effective local concentration of free radicals [R] in completely modified polyU. For this purpose we studied the EPR-spectra of S-carbodiimide solutions and those of modified polyU at 77°K. It appeared that a frozen 0.21 M water–glycerol solution of S-carbodiimide at 77°K exhibited the same EPR-spectrum as a 10^{-3}–10^{-4} M water–glycerol solution of completely modified polyU. It was concluded that in completely modified polyU [R] = 0.21 M and hence at 85° $K = 3.3 \times 10^9$ M^{-1} sec^{-1}.

The K value found for S-carbodiimide itself (narrowing region) in aqueous solution at 85° was approx. 3 times greater. It appears that such a distinction results from the different manner of radical motion in solution and in modified polyU.

It follows from above, that valuable information on localisation and mobility of spin labels can be obtained by means of the spin-exchange technique. The authors hope this technique will be of use in studies of spin-labelled natural nucleic acids.

References

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