Molecular Motion of Drugs in Hydrocolloids Measured by Electron Paramagnetic Resonance

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The effects of a polymer, the Li-salt copolymer of methylmethacrylic acid, and its methyl ester on the motion of drug molecules in hydrocolloids were studied. The investigation was carried out by means of electron paramagnetic resonance (EPR) using the model nitroxide tempol, and the spin-labeled drugs lidocaine (sl-lid) and dexamethasone (sl-dex). Synthesis of sl-dex was performed. Spin-labeled molecules dissolved in hydrocolloids undergo a fast reorientation motion. The decreasing order of rotational correlation times (τ) —sl-dex > sl-lid > tempol—suggests that the size and the shape of the molecules strongly affect their motion. The inhibition of motion of larger molecules depends also on their flexibility. The τ values indicate proportionality of the microviscosity of hydrocolloids to the polymer concentration. Rotational motion is dependent on the local environment conditioned by the free spaces between polymer molecules.

KEY WORDS: hydrocolloid; electron paramagnetic resonance; rotational correlation time; spin-labeled dexamethasone; viscosity.

INTRODUCTION

Molecular motion is an important factor in characterizing the behavior of drugs in hydrocolloid preparations (1,2). The mobility of a molecule in hydrocolloids is sensitive to the steric hindrance imposed by its environment. Therefore the state of a medium can be indirectly explored by introducing a nitroxide radical. Spin labeling facilitates the study of the mobility of drug molecules (3). Electron paramagnetic resonance (EPR) with a magnetic field gradient has been used to study the translational diffusion of spin probes in such systems (4), while the rotational motion can be evaluated from the line shape of the EPR spectra (5,6).

The aim of the present work was to investigate the rotational motion of spin-labeled molecules in hydrocolloids, measuring the rotational correlation time (τ), and to stress the difference between the local mobility of dissolved molecules and the long-range translational motion as a function of the polymer concentration. Qualitatively we are comparing the effect of micro- and macroviscosities.

MATERIALS AND METHODS

Preparation of Hydrocolloids

Polymethylmethacrylate-Li salt (PMMA-Li) was prepared by mixing the polymer (copolymer of methylmethacrylic acid and its methylester—Eudispert, nv, with average molecular weight of 500,000, Roehm Pharma, Darmstadt, FRG) with LiOH solution (7). The gels were prepared with a weighed amount of the polymer, which was swollen in water. After the addition of LiOH solution (7.5 mmol/g polymer), the final appropriate gel polymer concentration was adjusted by the addition of deionized water.

Spin-Labeled Molecules

The chemical structures of the spin-labeled molecules are shown in Figs. 1 and 2. In this study we used the model compound tempol and the spin-labeled drug molecules lidocaine (sl-lid) and dexamethasone (sl-dex), which were synthesized especially for this study. The preparation of sl-lid has been described previously (4). A 10^{-5} *M* concentration of the spin probes in the gel preparation was used.

Synthesis of Spin-Labeled 22-Nitroxyl Ester of Dexamethasone

Seven hundred milligrams (1.78 mmol) of dexamethasone (I) (kindly donated by the Krka Pharmaceutical Factory, Novo mesto, Yugoslavia), 700 mg (2.73 mmol) of nitroxyl mixed anhydride (II) (8), 217 mg (1.78 mmol) of 4-dimethylaminopyridine, and 200 ml of dry tetrahydrofurane were mixed and heated at 60°C for 2 hr (Fig. 2). After cooling to room temperature, the solid [salt (I) and 42 mg 11-nitroxyl ester of dexamethasone] was filtered off. The filtrate was concentrated *in vacuo*. The semisolid residue, containing I, II, III, and 1-oxyl-2,2,5,5-tetramethyl-3-carboxylic acid, was dissolved in chloroform (40 ml) and washed with a saturated solution of $NaHCO_3$ (5 × 5 ml) and then with brine (2 × 5 ml). After drying, the mixture of I, II, and III was separated on preparative TLC plates (Merck Art. 5717) using ether as

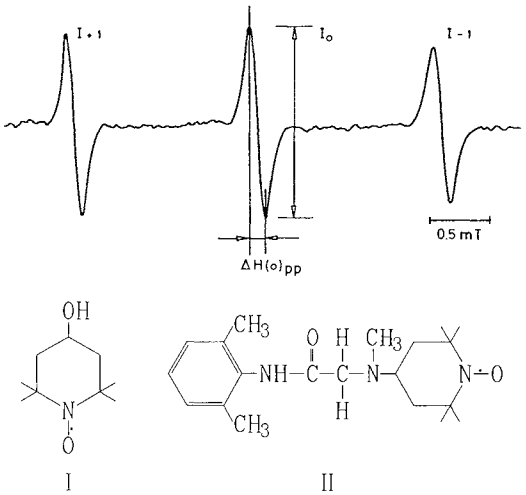

Fig. 1. A representative EPR spectrum and the chemical structure of the nitroxides. The experimental parameters used to evaluate the rotational correlation time τ for the fast motion approximation (9): $\tau = 6.02 \times 10^{-10}\ \Delta H(o)_{pp}(\sqrt{I_o/I_{-1}} - 1)$. I, tempol (1-oxyl-2,2,6,6-tetramethyl-4-hydroxypiperidine); II, sl-lid{α-[*N*-methyl-(1′-oxyl-2′,2′,6′,6′-tetramethyl-4′-piperidinyl)]-amino-*N*′-2,6-dimethylphenylacetamide}.

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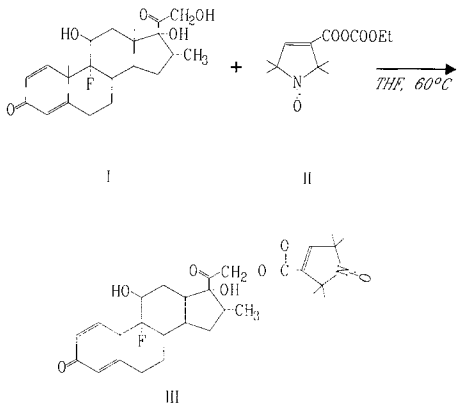

Fig. 2. Synthesis of spin-labeled 22-nitroxyl ester of dexamethasone. I, dexamethasone; II, nitroxyl mixed anhydride; III, 22-nitroxyl ester of dexamethasone—sl-dex.

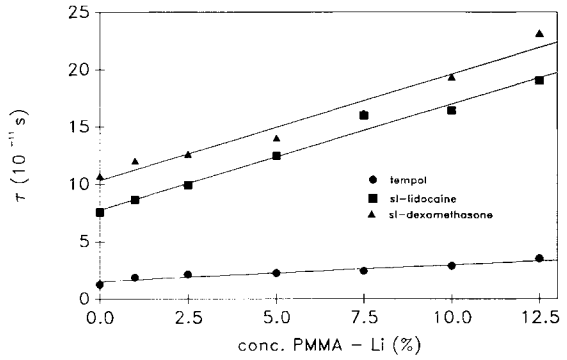

Fig. 3. The rotational correlation times τ of the spin-labeled drugs and the model nitroxide, tempol, dissolved in the PMMA–Li hydrocolloids, at various polymer concentrations (T = 20°C). The functional dependence is shown by straight lines with the equation $\tau = kc + n$, where c is the concentration of the polymer. The line parameters k and n are $k = (0.142 \pm 0.019) \cdot 10^{-11}$, $n = (1.517 \pm 0.335) \cdot 10^{-11}$, for tempol, $k = (0.920 \pm 0.057) \cdot 10^{-11}$, $n = (7.773 \pm 0.400) \cdot 10^{-11}$, for sl-lid, and $k = (0.929 \pm 0.081) \cdot 10^{-11}$, $n = (10.321 \pm 0.570) \cdot 10^{-11}$, for sl-dex. Each point is an average of six measurements. The coefficients k and n with corresponding uncertainties are calculated by the least-squares approximation.

the mobile phase and ethyl acetate as eluent. The yield was 190 mg (32%), mp = 172–175°C, R_f (ether) = 0.40, m/e = 558, IR (KBr) = 1730 cm^{-1} (ester).

The presence of the nitroxide group on the dexamethasone molecule alters the physicochemical properties, especially its lipophilicity, with an increased value of R_f (ether) from 0.19 (for the nonlabeled dexamethasone) to 0.40.

Measurement of Viscosity

Viscosity measurements were performed with a rotational viscometer (Haake, SV-1 system) with a shear rate of 3.75 sec^{-1} at 21°C.

EPR Experiments

EPR spectra were collected with a computer interfaced with an E-9 X-band Varian EPR spectrometer. Data acquisition and analysis were performed using an EPR software package. From the line shapes of the spectra the rotational correlation time τ (sec) was evaluated by the fast motion approximation as indicated in Fig. 1.

RESULTS AND DISCUSSION

The EPR line shapes show that the spin-labeled compounds dissolved in hydrocolloids undergo a fast reorientational motion, which allows the determination of τ values using the established theories (6). The rotational mobility of different spin-labeled compounds in PMMA-Li hydrocolloids is presented as a relationship between the rotational correlation time (τ) of the spin probes and the polymer concentration (c) (Fig. 3). The results reflect the properties of the microenvironments in which the molecules move. The τ values, which means the average time for which the molecule persists in any given orientation in space, are higher at higher polymer concentrations. A linear relationship was observed for each of the three solutes in the range of studied polymer concentrations. The slopes of the straight lines increase with solute size. The linear relation indicates that the microviscosity of the hydrocolloids is proportional to the polymer concentration.

In a qualitative view we could expect τ to be proportional to the cube of the effective radii of the labeled molecules. The rate of rotational motion would be restricted primarily by motions perpendicular to the longer molecular axis. From the EPR line shapes the restriction on rotational motion imposed by the polymer did not show up in the line distortion and broadening typical for slow motion. There-

Table I. Concentration Dependence of Rotational Correlation Time τ Evaluated by Labeled Molecules of Different Structure and Size

Spin-labeled molecules	d(nm)[a]	$(d_i/d_t)^3$[b]	$\tau_i \times 10^{11}$, sec, in water[c]	$(\tau_i/\tau_t)_{H_2O}$[d]	k(sec/%)[e]	k_i/k_t[f]
Tempol	0.84	1.00	1.20	1.00	$(0.142 \pm 0.019) \cdot 10^{-11}$	1.00
sl-lid	1.54	6.16	7.50	6.25	$(0.920 \pm 0.057) \cdot 10^{-11}$	6.48
sl-dex	2.16	17.00	10.70	8.92	$(0.929 \pm 0.081) \cdot 10^{-11}$	6.54

[a] d is the maximal size of the long axis of the stretched molecule, evaluated from the CPK model.
[b] The cube of the normalized molecular size d_i to that of tempol d_t.
[c] τ_i is the experimental rotational correlation time for the molecule i in water.
[d] τ_i values normalized to the corresponding value for tempol.
[e] The slopes (k) of the plots in Fig. 3.
[f] The slopes k_i normalized against the corresponding value for tempol k_t.

fore, the measured rotational mobility could be approximated by an axially symmetric prolate molecule. The values in Table I show that the values of τ in water are qualitatively in agreement with the cube of the large axis dimension.

When the concentration of the polymer in the gel increases, we can expect that the inhibition of motion will be more effective for larger molecules as observed in the larger slopes of $\tau(c)$ in Fig. 3. For the largest molecule sl-dex, the measured slope is smaller than expected from the molecular size (Table I). Therefore we assume that the flexibility of the molecule diminishes the effective size, which helps to alleviate the steric hindrances.

We suppose that the shape of the spin probe molecules might increase the probability of local-segmental motion of the nitroxide. This is especially obvious for sl-dex, where the value of τ is diminished more in hydrocolloid than in water, in comparison to the expected value for rotational motion of the whole molecule. With respect to the structure of spin-labeled drugs, the probability of a bent or folded conformation of sl-dex molecules is larger than that of sl-lid. Qualitatively we can assume that such conformations are even more probable in the hydrocolloid.

On the other hand, the lateral diffusion experiment with tempol shows that the diffusion coefficient is inversely proportional to the macroscopic viscosity (4). Constant linear magnetic field gradient EPR furnishes information on the long-range mobility of the labeled molecules. Due to the nonlinear dependence of the macroviscosity on the polymer concentration (7), we can conclude that the rate of translational motion is stronger affected in the low polymer concentration range.

With regard to the free space between polymer molecules, the rapid decrease in this free space with increasing polymer concentration is detected by the fast drop of the rotational rate of motion ($1/\tau$) and an even faster drop for the translational rate of motion. We conclude that the long-range motion of the diffusing molecule reflects the sum of many barriers, while the local mobility is relatively less perturbed. On the other hand, the rotational motion is restricted to the local environment, where we observe a linear increase in τ with polymer concentration.

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