METHODS

Proton NMR Relaxation of the Nonaqueous Component of Human Blood Serum

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Proton magnetic resonance relaxation was experimentally studied in native blood serum in groups of healthy subjects and patients with malignant tumors. Although the longitudinal relaxation time T_1 of the aqueous component in these groups was found to differ reliably, the longitudinal relaxation time of nonexchanging or slowly exchanging protons in these samples within the attained range of accuracy was virtually the same. Moreover, it was revealed that the amplitude of the "nonaqueous component" of blood serum correlates with the protein concentration in the samples.

Key Words: *NMR relaxation; blood serum; proton exchange; malignant growth*

The experimentally established phenomenon of increased relaxation times of T_1 and T_2 of human blood serum and tissue protons during some diseases, including malignancies [5-7], is still not clear in terms of its molecular dynamic mechanisms. There have been investigations assessing the contribution of certain factors to the duration of relaxation, namely, the concentrations of paramagnetic centers of endogenous and exogenous origin [4], the role of low-molecular compounds, and the effects of variations in protein fractions on the final values of T_1 and T_2 [2].

The purpose of this study was to develop a method for assessing the effective time of longitudinal ($T_{1\rho}$) relaxation of "nonaqueous" component protons in native human serum and to compare this value with the routinely measured proton relaxation time (T_1) of the serum in two groups: healthy subjects and patients with malignancies.

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MATERIALS AND METHODS

Whole serum obtained from venous blood by 20-min centrifugation at 500 g in a laboratory medical centrifuge was studied. The measurements were carried out 2 to 5 h after the serum was prepared. The volume of a test sample was 0.6 ml. Individual control measurements were carried out after sufficient accumulation of the signal, that is, after 6-12 h of operation of the device.

Sera were collected from 22 healthy subjects, including 13 blood donors, and from 33 patients with stage II-IV cancer, including those with involvement of the gastrointestinal tract (13 patients), lungs (7 patients), and urogenital system (13 patients).

All measurements were carried out using a Minispec pc-20 relaxometer (Bruker, working frequency of MHz) at a sample temperature of 40±0.1°C. Before measurement the samples were kept at the working temperature for at least 20 min. The pulse sequence 180 - t_i - 90, where t_i is the delay between the probing pulses, was used [1]. Solutions of chemically pure (99.8%) glycerol in distilled water

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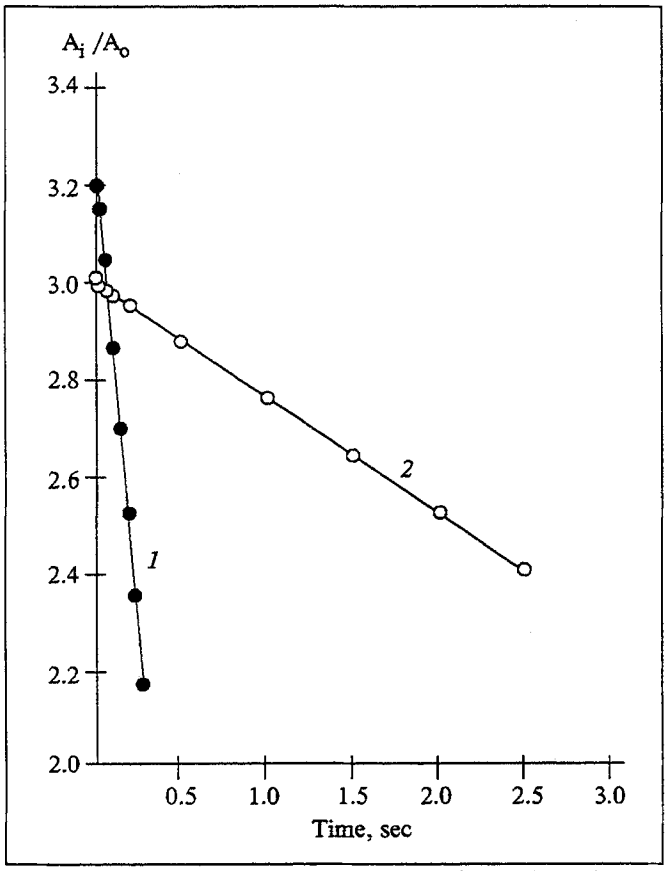

Fig. 1. Dependence of amplitude of NMR signal in glycerol (*1*) and normal saline (*2*) on delay between probing pulses.

and normal Ringer-Locke saline were taken as control samples to verify the linearity of the receiving-amplifying channel of the device. The apparatus was carefully adjusted before each new sample measured.

Minispec pc-20 relaxometers come with standard software for automated T_1 measurement, but we had to alter the algorithm and used two ranges of t_i delays between the probing 90 and 180° pulses. The signal amplitudes after 5 delays in the interval of 1 to 256 msec and 5 delays in the interval 0.5 to 12 sec were determined for each sample, in order to find the relaxation parameters. Analysis of the relaxation curves showed that an error of measurement of the T_1 relaxation time of the serum aqueous component (the usually measured T_1 time for serum or plasma [5,6]) of less than 1% may be attained for a certain number of accumulations (>16); however, excessive accumulation of the signal over several hours may introduce a systematic error in plotting the relaxation curve, making it impossible to single out the component with a short T_{10} relaxation time.

The results were processed by standard regression analysis.

RESULTS

Regression analysis of the relaxation curves for all serum samples at t_i>0.5 sec yielded a paired correlation coefficient "amplitude logarithm - delay time" R>0.999997. This permits us to consider these curves at t_i>0.5 sec as single-component curves within a ratio of component amplitudes of at least 1:100, and the error in determination of the longitudinal relaxation time of the aqueous component T_1 is less than 1%.

The mean values of this parameter in the "healthy-diseased" groups and the mean square error for T_1 in the groups were T_1=1.7±0.09 sec for healthy subjects and T_1=1.9±0.12 sec for patients, which coincides with published results obtained under comparable conditions [2,6].

Measurements in glycerol and normal saline yielded R values of more than 0.999999 for the relaxation curves, for consideration of all delays between pulses in the range from 1 msec to 20 sec (the measured values were T_1=0.29±0.001 sec for the glycerol solution, T_1=4.12±0.009 sec for normal saline). This indicates that the relaxation curves are single-component curves and that at short delays between pulses in the millisecond range of values there is no systematic instrumental error associated with the nonlinearity of the receiving channel of the device or with transient processes after exposure to fairly powerful probing pulses. Figure 1 illustrates these data. The relaxation curves for glycerol and normal saline are plotted in a semilogarithmic scale.

In the case of measurements carried out in serum (or model solutions of serum albumin), where relaxation curve points for delays of less than 150 msec are included in the regression analysis, R progressively decreases as shorter and shorter delays are considered. These data suggest the presence of a second, "rapid" component of the relaxation curve, whose protons do not participate in the exchange with water protons. The entire curve may thus presumably be described by an expression for the

TABLE 1. Content of Total Protein, Albumin, and Globulins in the Groups Examined

Group	*n*	Content, g/liter		
		total protein	albumin	globulins
Healthy subjects	18	74±6	42±4	32±4
Patients	28	78±10	39±6	40±5

measured signal amplitude A_i of nuclear magnetic resonance (NMR) as a function of the delay between t_i pulses:

$$A_i=A_{10}[1-2\exp(-t_i/T_{10})]+A_{20}[1-2\exp(-t_i/T_1)], \quad (1)$$

where A_{10} is the amplitude of the "nonaqueous" serum component, T_{10} is the time of its longitudinal relaxation, and A_{20} and T_1 are the analogous characteristics for the aqueous component. The value of T_{10} proves to be much shorter than the usually measured T_1 relaxation time ascribed to a protein solution [2,5,6]. Computer processing of the curves using equation (1) makes it possible to identify the "rapid" component (paired correlation coefficient "amplitude logarithm - delay time" of at least 0.99) and to determine parameters T_{10} and $k=A_{10}/(A_{10}+A_{20})$. In the groups examined these data are as follows: $T_{10}=35\pm6$ msec, $k=4\pm1\%$ for healthy subjects and $T_{10}=32\pm6$ msec, $k=4\pm1.2\%$ for patients.

For all measurements ($n=46$) the value of k correlates with the total protein content in the samples (correlation coefficient $R=0.31$, $p>0.05$). Figure 2 shows two typical relaxation curves characterizing the two groups examined. We see that at low t_i values the linearity of the functions is destroyed.

During a comparative analysis of NMR data in the groups of patients and healthy subjects the serum in a zeroth approximation may be regarded as a protein solution of known concentration. Since the groups do not reliably differ in terms of this parameter, or in terms of the albumin/globulins ratio (Table 1), it is possible at this stage to regard the sera simply as media with a known concentration of a certain effective biopolymer, on the surface of which the so-called "rapid" exchange of water molecules between the arbitrary "fractions" of "free" and "bound" water takes place, this resulting in the T_1 value of longitudinal relaxation usually measured in a solution [6,8]. The effect of a biopolymer on T_1 depends on the protein concentration and on the characteristic frequency relation [3,9].

Nonexchangeable (or very slowly exchangeable in comparison with the NMR-relaxation processes) protons of water and the organic component, primarily proteins, determine the rapidly recovering component of the relaxation curve, which is characterized in equation (1) by the following parameters: effective time of longitudinal relaxation T_{10} and amplitude A_{10}. Evidently, the k ratio should correlate with the independently measured protein concentration in the solution. However, in our case the assessment of k, based on the percent weight content of hydrogen in proteins, proves to be somewhat lower than the measured value (theoretical k is approximately 6-8%, measured k is approximately 4%), although these values reliably correlate. It is possible that the "lost" protons belong to rather mobile segments of macromolecules or to mobile molecular fractions, which fact prolongs their relaxation and does not permit their detection by this method within the range of accuracy attained.

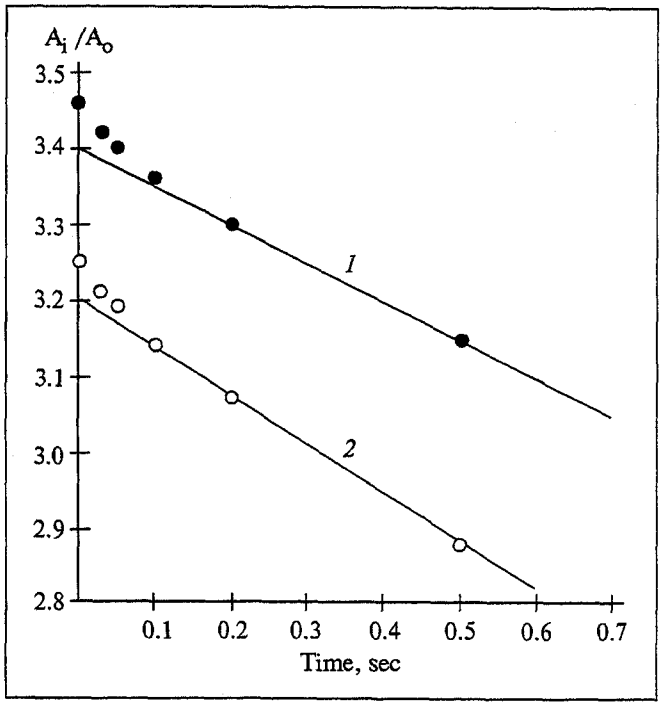

Fig. 2. Dependence of amplitude of NMR signal in serum of a healthy subject (*1*, $T_1=1.6$ sec) and a patient with stomach cancer (*2*, $T_1=2.01$ sec) on delay between probing pulses.

The absence of a reliable difference in T_{10} values between the two groups for about a 20% difference in T_1 (after taking account of the contribution of water to the total relaxation rate) means either that the measurements of T_{10} in our experiments were insufficiently accurate or that there are no differences in the time course of the macromolecules in the serum in health and disease. If the former assumption is true, it is possible to improve the accuracy of determining the parameters of the "rapid" component by partly substituting deuterons for protons (provided the protein fractions remain native). If the second assumption is true, this mechanism has to be excluded from analysis of the effects of disease on proton NMR-relaxation in the serum.

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