NUCLEAR MAGNETIC RELAXATION IN BIOCHEMICAL ANALYSIS OF PLASMA AND SERUM

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Measurable parameters of NMR-relaxation (spin-lattice relaxation time T_1 and spin–spin relaxation time T_2 of water protons) are known to depend on the nature, concentration, hydration, and conformation of proteins in solution [1, 2]. Because of this state of affairs it is possible to use the NMR method for biochemical analysis of blood plasma and serum. The writers previously suggested a new approach to the description of NMR-relaxation of the blood and presented a theoretical model, according to which relaxation rates are proportional to the concentrations of its components [2, 5, 4].

The aim of this investigation was a detailed study of the relaxation parameters T_1 and T_2 of blood plasma and serum.

EXPERIMENTAL METHOD

Original samples of blood serum were prepared by centrifugation of blood at 1000*g* for 10 min; plasma was obtained by stabilizing the blood with heparin. Tests were carried out during 1 h after taking the blood sample. Relaxation measurements were made on the "Minispinecho" instrument at 25°C and an NMR-frequency of 5 MHz [4]. Altogether 32 samples of plasma and serum were tested. Isotonic NaCl solution (0.9%), the relaxation rate $(T_{1,2})^{-1}$ of which was 0.42 sec^{-1}, was used as the original comparison solution.

EXPERIMENTAL RESULTS

Analysis of the results (Table 1) shows that $(T_1)^{-1}$ and $(T_2)^{-1}$ are linear functions of the total protein concentration, just as was found previously [2-4]. It was also observed that the scatter of the experimental data relative to the theoretical straight line depends on the albumin-globulin ratio: the less albumin is present in the serum, the greater the value of $(T_2)^{-1}$, and vice versa.

The mathematical model of the experimental data yielded the following equations:

$$(T_2)^{-1}=0{,}024\ [C_p - k \cdot C_p\ (1{,}3 - {}^{A}_{G})\ ] + 0{,}42, \quad (1)$$

$$(T_1)^{-1}=0{,}012\ [C_p - k \cdot C_p\ (1{,}3 - {}^{A}_{G})\ ] + 0{,}42, \quad (2)$$

where C_p denotes the protein concentration in the serum (in g/liter); A and G the relative percentages of the albumin and globulin fractions; k the effective coefficient of the contribution of A and G, equal to 1.3; 1.3 reflects the accepted normal A/G ratio; 0.42 is the relaxation velocity of physiological saline (in sec^{-1}).

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TABLE 1. Biochemical and Relaxation Parameters of Serum and Plasma

№	Physicochemical properties				NMR parameters, sec			
	C_p, g/liter	albumin, %	globulin, %	fibrinogen, g/liter	of serum		of plasma	
					T_1	T_2	T_1	T_2
1	70,06	0,55	0,45	—	0,924	0,540	—	—
2	72,8	0,47	0,53	—	0,82	0,7	—	—
3	64,2	0,6	0,4	—	1,01	0,544	—	—
4	66,0	0,56	0,44	—	1,166	0,546	—	—
5	66,3	0,43	0,57	—	0,999	0,539	—	—
6	81,4	0,48	0,52	—	0,932	0,523	—	—
7	64,2	0,48	0,52	—	1,098	0,56	—	—
8	70,6	0,55	0,45	—	1,048	0,665	—	—
9	70,6	0,6	0,4	—	0,944	0,543	—	—
10	64,2	0,61	0,39	—	1,073	0,69	—	—
11	68,5	0,61	0,39	—	0,987	0,605	—	—
12	70,6	0,61	0,39	—	0,999	0,589	—	—
13	70,6	0,72	0,28	—	0,959	0,629	—	—
14	77,0	0,68	0,32	—	0,94	0,548	—	—
15	77,0	0,5	0,5	—	0,877	0,509	—	—
16	75,0	0,52	0,48	—	0,966	0,525	—	—
17	68,5	0,6	0,4	—	0,49	0,616	—	—
18	65,0	0,58	0,42	—	1,025	0,588	—	—
19	80,0	0,35	0,65	—	0,914	0,482	—	—
20	70,06	0,68	0,32	—	1,034	0,574	—	—
21	81,2	0,61	0,39	—	0,982	0,615	—	—
22	75,0	0,58	0,42	—	0,938	0,544	—	—
23	65,0	—	—	0,27	1,11	0,511	0,986	0,326
24	60,0	0,67	0,33	0,62	1,054	0,612	1,04	0,419
25	70,0	0,41	0,59	0,36	1,005	0,448	0,913	0,364
26	72,0	0,31	0,69	0,44	0,955	0,358	0,894	0,351
27	65,0	0,44	0,56	0,4	0,753	0,400	0,745	0,350
28	65,0	0,31	0,69	0,58	0,891	0,462	0,814	0,375
29	65,0	0,35	0,65	—	—	—	0,913	0,445
30	65,0	0,38	0,62	0,53	1,147	0,555	1,038	0,494
31	68,5	0,4	0,6	0,44	0,597	0,393	1,144	0,497
32	65,0	0,35	0,65	0,49	1,045	0,520	1,030	0,457

It will be clear from these equations that linear correlation between the test parameters is satisfied by the effective protein concentration (between square brackets), as a result of its hydration and conformational properties, which depend on the A/G ratio and on other factors. The expression $k \cdot C_p$ (1.3 − A/G) reflects a correction for the total protein concentration, which amounts to 25 ± 5% of the total protein concentration (C_p). For that reason, by measuring T_1 and T_2, it is possible to obtain C_p, A, and G immediately, and this has many advantages in biochemical analysis.

Comparison of the relaxation parameters of serum and plasma, as might be expected [3, 4], will also be a linear function of the fibrinogen concentration (C_f).

The equation of this function:

$$(T_2)^{-1}_{\text{serum}} - (T_2)^{-1}_{\text{plasma}} = 0{,}2 \cdot C_f - 0{,}8 \qquad (3)$$

thus obtained can be used to determine the plasma fibrinogen concentration in cases of "above normal" fibrinogen concentrations. The results undoubtedly have applied importance in the biochemical analysis of blood.

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