DOI 10.1007/s10517-017-3794-y
Bulletin of Experimental Biology and Medicine, Vol. 163, No. 3, July, 2017

BIOPHYSICS AND BIOCHEMISTRY

Effects of Fullerene Derivatives on Activity of Ca^{2+}-ATPase of the Sarcoplasmic Reticulum and cGMP Phosphodiesterase

L. V. Tat'yanenko, E. A. Khakina, A. V. Zhilenkov, P. A. Troshin, O. V. Dobrokhotova, I. Yu. Pikhteleva, and A. I. Kotel'nikov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 163, No. 3, pp. 298-303, March, 2017
Original article submitted March 18, 2016

We studied the effects of new water-soluble polysubstituted fullerene C60 (PFD) derivatives on activity of Ca^{2+}-Mg^{2+} ATPase of the sarcoplasmic reticulum and cGMP phosphodiesterase. All examined fullerene derivatives inhibited activity of both enzymes. For instance, PFD-I, PFD-II, PFD-III, PFD-V, PFD-IX, PFD-X, and PFD-XI in a concentration of 5×10^{-5} M completely inhibited hydrolytic and transport functions of Ca^{2+}-ATPase. These compounds in a concentration of 5×10^{-6} suppressed active transport of calcium ions by 51±5, 77±8, 52±5, 52±5, 100±10, 80±8, and 100±10%, respectively, and inhibited ATP hydrolysis by 31±3, 78±8, 18±2, 29±3, 78±8, 63±7, and 73±9%, respectively, uncoupling the hydrolytic and transport functions of the enzyme. PFD-I noncompetitive and reversibly reduced activity of Ca^{2+}-ATPase ($K_i=2.3\times10^{-6}$ M). All the studied fullerene derivatives (except for PFD-VII) inhibited cGMP phosphodiesterase by more than 80% in concentration of 10^{-4} M and higher and by more than 50% in concentration of 10^{-5} M. PFD-I is a non-competitive reversible inhibitor of cGMP phosphodiesterase ($K_i=7\times10^{-6}$ M). These results allow us to expect antimetastatic, antiaggregatory, antihypertensive and vasodilative activity of the studied compounds.

Key Words: *Ca^{2+}-Mg^{2+}-ATPase of the sarcoplasmic reticulum; cGMP phosphodiesterase; inhibition; fullerene derivatives*

Ca^{2+}-ATPase of the sarcoplasmic reticulum catalyzes active transport of calcium ions across the biomembrane using hydrolysis energy of the substrate of ATP enzyme. The structure of sarcoplasmic reticulum Ca^{2+}-ATPase is well studied by modern physical methods including X-ray structural analysis with the resolution of 2.6 Å [14].

Inhibition of active Ca^{2+} transport across the membrane of the sarcoplasmic reticulum disturbs the balance of calcium concentrations inside and outside the cells [13], which plays an important role in the processes of thrombus formation and adhesion of metastatic cells to the capillary endothelium [8,9]. Pervious experiments [4,5] showed a strong correlation between inhibition of the growth of experimental B16 melanoma metastasis and inhibition of active transport of calcium ions across the biological membranes.

cGMP phosphodiesterase (cGMP PDE) belongs to the family of Zn^{2+}-dependent metal phosphohydrolase that specifically hydrolyze cGMP and\or cAMP to 5'-monophosphate nucleotide and participate in the regulation of intracellular cGMP/cAMP ratio [7]. cGMP PDE hydrolyzes cGMP and cAMP, thus regulating intracellular content of these secondary messengers, which leads to activation/inhibition of some biological processes in cells. This mechanism mediates

Institute of Problems of Chemical Physics, Russian Academy of Sciences, Chernogolovka, Moscow region, Russia. ***Address for correspondence:*** kotel@icp.ac.ru. L. V. Tat'yanenko

regulation of the smooth muscle tone, thrombocyte aggregation, reduction in the synthesis of inflammation mediators, regulation of cell differentiation and apoptosis, and modulation of ion channel function, which determines antiaggregation, antihypertensive, and vasodilatory effects and mediates important physiological processes in the body [2,10].

Analysis of the effects of fullerene derivatives on activity of sarcoplasmic reticulum Ca^{2+}-ATPase and cGMP PDE is aimed at elucidation of the molecular mechanisms underlying their biological effects and selection of potential antimetastatic, antihypertensive, and vasodilatory preparations.

Here we studied the effect of new water-soluble polysubstituted derivatives of fullerene C60 (PFD) on activity of sarcoplasmic reticulum Ca^{2+}-ATPase and cGMP PDE.

MATERIALS AND METHODS

Human albumin, imidazole, cGMP, nucleosidase (cobra venom), ATP (Sigma), EDTA, trichloracetic acid (TCA), sucrose, $MgCl_2$, NaCl, KCl, $CaCl_2$, sodium oxalate, and ammonium molybdate (Reakhim) were used in the experiments after the appropriate additional purification.

Synthesis and spectral parameters of PFD (Fig. 1) were described in details previously [11,12,15].

Ca^{2+}-ATPase was isolated from the white muscles of rabbit hind limbs using as described previously [3]. Specific activity of Ca^{2+}-ATPase was 15,000 nM P_i/mg protein/min. Protein concentration was measured by the modified Lowry method.

Reaction medium contained 4 mM $MgCl_2$, 2.5 mM imidazole, 100 mM NaCl, 5 mM sodium oxalate, 0.04 mg protein, and 3 mM ATP (pH 7.2). The reaction was initiated by adding 0.1 mM $CaCl_2$.

Ca^{2+}-ATPase activity was estimated by the kinetics of pH shifts, as the ratio between protons and phosphate ions in this reaction is 1:1. Hydrolytic activity of Ca^{2+}-ATPase was calculated as the tangent of the slope of the initial part of curve describing the kinetics of accumulation of inorganic phosphate as a result of ATP hydrolysis. The rate of changes in Ca^{2+} concentration was estimated by the time of their complete uptake by vesicles of the sarcoplasmic reticulum, which stopped ATP hydrolysis. Relative activity of the enzyme was calculated using the formula: $I=100(A_0-A)/A_0$, where I is relative enzyme activity; A_0 and A are specific content of inorganic phosphate in the control and in a sample containing the test substance, respectively.

The mechanism of inhibition of Ca^{2+}-ATPase and cGMP PDE by PFD-I was evaluated by the dependence of the rate of enzyme reaction on the concentration of the substrate (ATP or cGMP) in the absence or presence of 10^{-5} M PFD-I [1].

Reversibility of Ca^{2+}-ATPase inhibition was determined by dialysis of Ca^{2+}-ATPase solution containing the test substance PFD-I in a concentration of 10^{-5} M. Dialysis was performed against 200 ml of the incuba-

TABLE 1. Effects of PFD on Hydrolytic and Transport Activities of Sarcoplasmic Reticulum Ca^{2+}-ATPase (% of Control; *M±m*)

Substance code	Substance concentration					
	5×10^{-5} M		5×10^{-6} M		5×10^{-7} M	
	active Ca^{2+} transport	ATP hydrolysis	active Ca^{2+} transport	ATP hydrolysis	active Ca^{2+} transport	ATP hydrolysis
PFD-I	100±10	100±10	51±5*	31±3	20±2*	0
PFD-II	100±10	100±10	77±8*	50±5	26±2*	11±2
PFD-III	100±10	100±10	52±5*	18±1	20±2*	0
PFD-IV	76±8	91±9	40±4	52±5	33±3	20±2
PFD-V	100±10	100±10	52±5*	29±3	11±1*	0
PFD-VI	63±6*	38±4	50±5*	29±3	20±2*	10±1
PFD-VII	60±6*	38±4	50±5*	31±3	33±3*	17±2
PFD-VIII	82±8	67±9	78±8*	44±4	61±6*	13±1
PFD-IX	100±10	100±10	100±10*	78±8	47±5*	5±0,5
PFD-X	100±10	100±10	80±8	63±7	34±3*	20±2
PFD-XI	100±10	100±10	100±10*	73±9	41±4*	21±2

Note. Results of 4-6 experiments with each substance are presented. Control was taken as 100%. *$p<0.01$ in comparison with initial activity.