polarized structure. Cells will lose their polarization and consequently the polarity of the structural orientation of tissue ensemble will disappear. In this case cells become independent from each other and receive the capability to get free from the tissue "prison". This may be one of reasons of the metastasizing processes.

Thus, all of epithelial cells display extensive keratin filament frameworks around which the cell shape and polarity are defined. The primary cytoskeletal components in vivo regulate and affect the interaction of epithelial cells and extracellular matrix developing the oriented structure of the whole tissue and at the same time in which flexible extracellular matrix is transformed into fibrillar matrix. Our results propose also the regulatory role of metal content in matrix assembly.

So the hair tissue has to be considered as the structural continuous organization providing the resistance to mechanical stresses externally applied to the tissue. Mutations that weaken this structural framework and any exogenous factors that change the extracellular matrix increase the risk of cell rupture and cause a variety of human disorders.

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Alvina Vazina *vazina@star.iteb.serpukhov.su*

6-85

THE X-RAY DIFFRACTION STUDY OF HAIR

A.A.Vazina[1], P.M.Sergienko[1], V.S.Gerasimov[1], N.F.Lanina[1], A.M.Aksirov[1], V.N.Korneev[2], G.N.Kulipanov[3], N.A.Mezentsev[3], V.I.Kondratyev[3], B.P.Tolochko[4]

[1]*Institute of Theoretical and Experimental Biophysics RAS, 142290 Pushchino, Moscow Region, Russia*

[2]*Institute of Cell Biophysics RAS, Pushchino, Moscow Region, Russia*

[3]*Budker Institute of Nuclear Physics SD RAS, Novosibirsk, Russia*

[4]*Institute of Solid State Chemistry SD RAS, Novosibirsk, Russia*

X-ray diffraction investigations of human hair were carried out using SR of VEPP-3 and the X-ray generator with rotating anode GX-20 (Pushchino). The stretching frame construction for hair clamping was used (fig. 1)

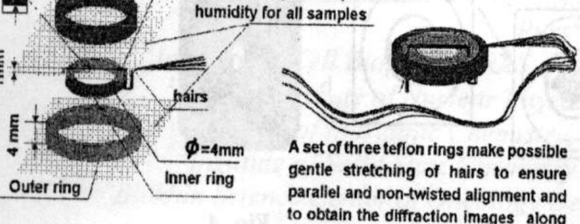

Fig. 1

The hair X-ray pattern is fibre diffraction pattern: the meridional reflections are due to supercoiling of the α-helical bundle packing in the keratin intermediate filaments (IF); the equatorial reflections, elongated parallel to the equator, give a diamond-shape appearance, such a shape may arise from helical twisting and correspond to the cylindrical Fourier-transform of an α-helical bundle. There is a slight trace of arcing across the equator that corresponds to a molecular spacing of 4,5 nm; spacing of this reflection is not variable, but angular size of this arc (texture) and arc width can increase so that the diffuse arc will be transformed into the full diffuse ring with its higher orders. The X-ray patterns with a slight arc was called as "*no ring*" one and those with rings was called as "*ring*" one.
A set of diffraction patterns from "*no ring*" to "*ring*" can be obtained from the hairs of the individual donor by scanning along the sample point by point from the root region to the tip at 5÷100 mm interval making approximately n=5÷15 images per sample depending on the lengths of hair (hair age). In this case the law of conservation of intensity for this set of diffraction patterns wasn't realized (fig. 2):

$$\int I_1(s)ds < \int I_{n-1}(s)ds < \int I_n(s)ds$$

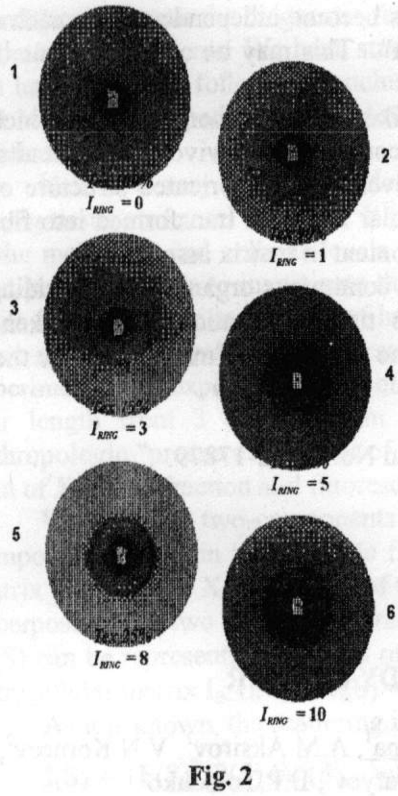

Fig. 2

It was shown that there were donors whose hairs did not give the "ring" X-ray pattern from the root region to the tip. In this case the law of conservation of intensity is realized always:

$$\int I_1(s)ds = \int I_{n-1}(s)ds = \int I_n(s)ds$$

Therefore, there are two different types of donors marked by us as "C" (constant) and "V" (variable). For "C"-type $\int I^C(s)ds$ = const along the hair length; this integral is variable for "V"-type and is a function of the hair length L: $\int I^V(s)ds = f(L)$. $f(L)$ increases from the root region (L_0) to the tip (L_n). For every i-donor $df(L)/dL$ is individual. It is necessary to note that for any donor $f_i(L_0)$ has got approximately the same values and similar to $\int I^C(s)ds$.

X-ray diffraction data allow us to suppose the two-component structural model of hair tissue, which consists of flexible component of *extracellular matrix* (ECM) in series with inflexible component of *keratin intermediate filaments*. The X-ray pattern of the two-component structural model can be considered as a result of superposition of two separate diffraction patterns of IF and ECM. In this case scattering intensity of hair $I_H(s)$ can be represented as a sum of the intensities of scattering of intermediate filament $I_{IF}(s)$ and extracellular matrix $I_{ECM}(s)$: $I_H(s) = I_{IF}(s) + I_{ECM}(s)$. Fig. 3 shows the diagrams of

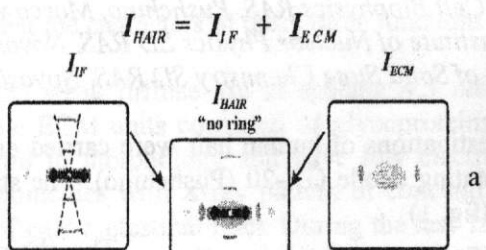

Fig. 3

Fig. 4

diffraction patterns of hair samples "V" and "C" (*"ring"* and *"no ring"*) as a superposition of two diffraction patterns due to I_{IF} and I_{ECM}.

The soaking of "no ring" type hair in solutions of heavy metal salts ($AgNO_3$) after preliminary washing in chloroform results in modified X-ray pattern and the appearance of the significant intensity ring becomes evident ("ring" type). Fig.4 (a, b) shows X-ray diffraction patterns of two Ag-derivates for "C" and "V" types of hair; X-ray patterns display the higher orders of 4,5 nm spacing: 2,33 nm; 1,56 nm and 1,24 nm.

The model diagram of ECM (fig. 3, bottom level) completely coincides with X-ray pattern of concentrated solution of glycoproteins produced by epithelial cells of gastrointestinal tract. During last 15 years we have been studying the structure of glycoproteins by X-ray small-angle diffraction [L.Zheleznaya et al., *Nanobiology* (1992) 1, 107-115], fig. 5 presents the X-ray pattern of glycoproteins. The strong higher orders of Ag-derivate arise from increasing electron density due to electrostatic interaction of heavy metal ions with multiple anion groups of polysaccharide chains. It is necessary to note that the derivates of heavy metals are not isomorphous: the positively charged metals transform the configuration of glycoproteins from fibrillar structure to random-coil one. In this case the equatorial diffuse arc will be transformed into the full ring with its higher orders.

Fig. 6 demonstrates two types of X-ray diffraction patterns of hair with their diagrams. It is evident that "*ring*" X–ray pattern is superposition of diffraction patterns of "*no ring*" type and of glycoproteins. Hair is a very suitable model for study of normal and pathologic tissues and interactions among tissue structural elements.

The X-ray diffraction pattern from concentrated solution of isolated glycoprotein and its diagram

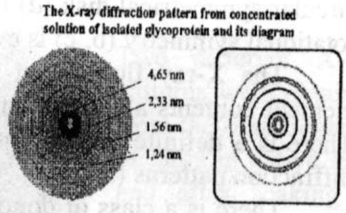

Fig. 5

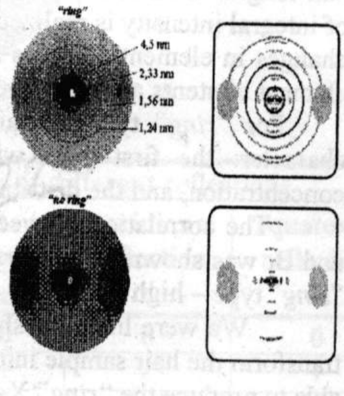

Fig. 6

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Nadezhda Fedorovna Lanina *lanina@star.iteb.serpukhov.su*

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THE SPECIFIC CHANGES OF SMALL-ANGLE X-RAY DIFFRACTION PATTERN OF HAIR AND THEIR CORRELATION WITH THE ELEMENT CONTENT

A.A.Vazina[1], P.M.Sergienko[1], V.S.Gerasimov[1], N.F.Lanina[1], A.M.Aksirov[1], V.N.Korneev[2], G.N.Kulipanov[3], N.A.Mezentsev[3], V.I.Kondratyev[3], V.A.Trounova[4], B.P.Tolochko[5], V.P.Letyagin[6]

[1]*Institute of Theoretical and Experimental Biophysics RAS, 142290, Pushchino, Moscow Region, Russia*
[2]*Institute of Cell Biophysics RAS, Pushchino, Moscow Region, Russia*
[3]*Budker Institute of Nuclear Physics SD RAS, Novosibirsk, Russia*
[4]*Institute of Inorganic Chemistry, SD RAS, Novosibirsk, Russia*
[5]*Institute of Solid State Chemistry SD RAS, Novosibirsk, Russia*
[6]*Blokhin Science Center of Oncology Department of Breast Tumor, Moscow*

X-ray diffraction and fluorescent investigations of human hair were carried out using SR of VEPP-3 and the small-angle techniques [1]. The X-ray patterns were classified into two groups according to the appearance or non-appearance of the diffuse 4,5 nm ring. X-ray patterns with the weak equatorial arc at spacing 4,5 nm were called "*no ring*" and those with rings were called "*ring*" one. We have shown that X-ray patterns obtained from the near-root segment of hair always belong to "*no ring*" type of patterns [2].

"*No ring*" patterns for most donors are transformed to "*ring*" patterns *via* intermediate ones by scanning along the sample point by point from the root region to the tip. In this case the law of conservation of integral intensity for this set of diffraction patterns wasn't realized. (fig.1a-d). Small-angle X-ray scattering (SAXS) shows the central region of diffuse scattering: equatorial streak and central diffuse disk. There is an evident correlation between the intensities of streak and disk depending on intensity of diffuse ring 4,5 nm. The transformation from the equatorial streak to the circular symmetrical disk (d) takes place via intermediate forms of intensity distribution where 6-fold rotational symmetry (b, c) is evident.

The X-ray fluorescent element analysis study has shown that there are some changes in element contents along the hair from the root region to the tip for donors with "*ring*" type of hair. There is a definite correlation between contents of Ca-Br in donor's hair and their X-ray fibrillar diffraction patterns (fig. 2).

There is a class of donors whose hair always give X-ray patterns without *ring* throughout the hair length. We call such diffraction patterns as "*ring free*" ones. In this case the law of conservation of integral intensity is realized. The X-ray fluorescent element analysis study has shown: there are no changes in element contents along hair for "*ring free*" type donors; but there are some changes in element contents along the hair from the root region to the tip for donors with "*ring*" type of hair.

For "*ring*" type of hair the distribution of element contents along the hair has bimodal character: the first pool with low element contents has got narrow distribution of element concentration, and the distribution for the second pool with higher element contents is wider.

The correlation between the character of X-ray patterns and the element contents for Ca, Sr and Br was shown: for "*no ring*" type - low Ca and Sr concentration and high Br concentration; for "*ring*" type – high Ca and Sr concentration and low Br concentration.

We were lucky to show that a prolonged soaking of hairs in 1M $CaCl_2$ at pH 10-11 could transform the hair sample initially giving a typical "*ring free*" X-ray pattern in such a way that it was able to produce the "*ring*" X-ray pattern (fig. 3).

Four different X-ray patterns of human hair

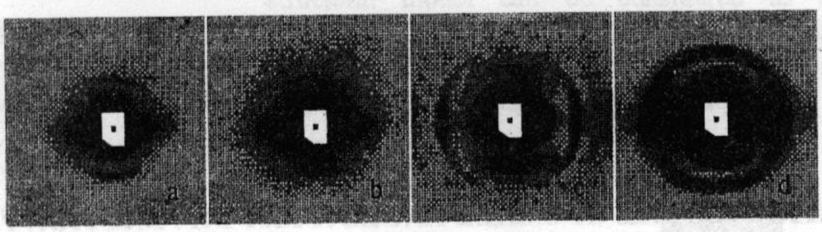


Fig. 1

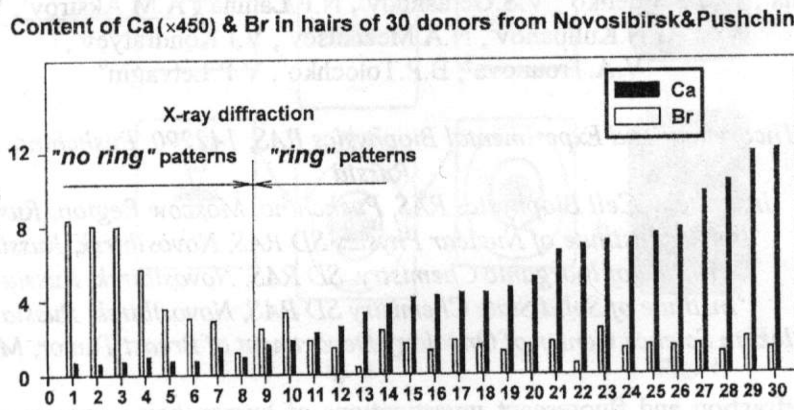
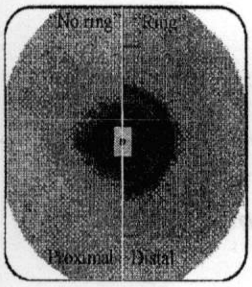
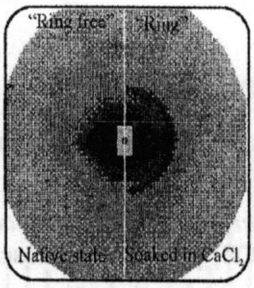


Fig. 2

The sensitivity of X-ray diffraction patterns to various reagents and heavy metals is presented in table.



Two different X-ray patterns were obtained from *proximal* and *distal* fragments of hairs of individual donor.



Two different X-ray patterns were obtained from *the same segment* of hairs in native state and after soaking in $CaCl_2$ solution.

Fig.3

Reagents		**Change of intensities of different reflections**				
		ring 4.5 nm	**diffuse small-angle scattering**		**meridional reflection**	**equatorial reflection**
			disk	**streak**		
Ethanol		0	0	0	0	0
Chloroform		0	+	+	-	+
100% Acetone		-	0	0	-	-
Hydrogen peroxide 30%H_2O_2		0	0	0	0	0
8M Urea		0	0	-	- -	-
b-mercaptoethanole		-	+	+	- -	-
0,6M $AgNO_3$	**"ring"**	++	++	++	++	++
	"no ring"	0	0	++	-	++
1,2M $AgNO_3$	**"ring"**	++	++	+++	++	+++
	"ring free"	0	0	++	-	++
0,6M $AgNO_3$ after chloroform	**"ring"**	+++	++	+++	+++	+++
	"ring free"	+	+	++	+	++
1,2M $AgNO_3$ after chloroform	**"ring"**	+++	+++	+++	+++	+++
	"ring free"	++	++	+++	++	+++
Paint shampoo	**"ring free"**	+	+	+	+	+
Tinting "Ursul" (Au, Ag, Cu)	**"ring free"**	+			+	+
Permed liquid	**"ring free"**	0	0	0	0	0
1N HCl	**"ring"**	0	0	0	0	0
NaOH pH 10-11	**"ring free"**	0	0	0	0	0
1M $CaCl_2$ pH 5	**"ring"**	0			0	0
	"no ring"	+++			+	+
	"ring free"	0			0	0
1M $CaCl_2$ pH 11	**"ring free"**	+++			+	+
5mM EDTA	**"ring"**	0	0	0	0	0

'0' - no change, **'++' - strong increase,** **'- ' - weak decrease,**
'+' - weak increase, **'+++' - very strong increase,** **'- - ' - strong decrease**

We have shown that the origin of diffuse ring (4,5 nm) is not "lipid" but most likely the glycoprotein one.

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2. A.A. Vazina et al. *International Conference " Current Status of Synchrotron Radiation in the World"* (March 9-10, 2000) pp.32, 114, Moscow, Russia

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Aslan M. Aksirov *aslan_a@mail.ru*

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ЭЛЕМЕНТНЫЙ РФА-СИ АНАЛИЗ ВОЛОС И ЕГО КОРРЕЛЯЦИЯ С РЕНТГЕНОДИФРАКЦИОННЫМИ ИССЛЕДОВАНИЯМИ

Трунова В.А.[1], Вазина А.А.[2], Сергиенко П.М.[2], Ланина Н.Ф.[2] Кондратьев В.И.[3]

[1]*Институт неорганической химии СО РАН, Новосибирск*

[2]*Институт теоретической и экспериментальной боифизики РАН, Пущино*

[3]*Институт ядерной физики им. Г.И.Будкера СО РАН, Новосибирск*

Обнаружено, что для каждого элемента наблюдается бимодальное распределение концентраций по образцам волос. Природа бимодальности основывается на следующих пунктах: в 1-й пул входят образцы с минимальным кол-во кальция, стронция и т.п., что соответствует их внутриклеточному держанию. Эти элементы определяются гомеостазом клетки, эта величина меняется мало от образца к образцу, т.е. она инвариантна. Здесь наблюдается узкая избирательность клетки по элементам, которые необходимы только для самой клетки. Во 2-й пул входят образцы, имеющие гораздо более высокие концентрации и большее разнообразие элементов, т.к. они сорбируются во внеклеточный матрикс, который не под контролем клеточной регуляции, но отражает наличие элементов в поте, крови, в коже, также металлы внешней среды. В этом матриксе взаимодействие с элементами неспецифично и зависит только от количества матрикса на клетку, его афинных свойств и структурного состояния. На него могут также влиять элементы окружающей среды, если защитная мантия волоса (волос покрыт разными жирными кислотами - продуктами сальной железы) нарушена действием детергентов - аэрозолей, пыли и т.д.

Выполненный элементный РФА-СИ анализ волос коррелирует с рентгенодифракционными картинами тех же образцов. Корреляция между элементами Ca, Sr и Br показывает:

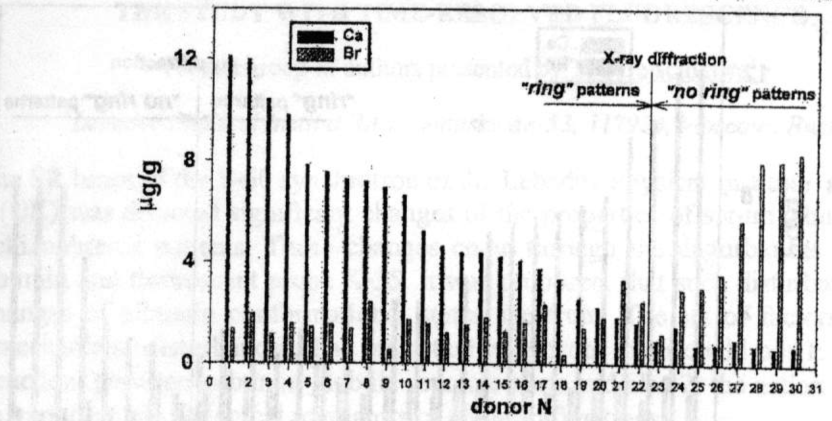
присутствие дифракционного "кольца"- низкие концентрации Ca и Sr и высокие концентрации Br;

отсутствие дифракционного "кольца" – высокие содержания Ca и Sr и низкие Br (рис. ниже).

Найдено, что функция интеграла интенсивности концентраций хим. элементов монотонно возрастает по длине волоса от корней к концам. Её вид индивидуален для каждого человека.

Работа в данном направлении продолжается и носит исследовательский медико-биологический характер.

Content of Ca(×450) & Br in hairs of 30 donors from Novosibirsk&Pushchino



Валентина Александровна Трунова *valna@mail.nsk.ru*

SRXRF ELEMENTAL ANALYSIS OF HAIR AND ITS CORRELATION WITH X-RAY DIFFRACTION THE INVESTIGATION

[1]V.A.Trounova, [2]A.A.Vazina, [2]P.M.Sergienko, [2]N.F.Lanina, [3]V.I.Kondratyev

[1]*Institute of Inorganic Chemistry, SD RAS, Novosibirsk, Russia*
[2]*Institute of Theoretical and Experimental Biophysics RAS, Pushchino, Moscow Region, Russia*
[3]*Budker Institute of Nuclear Physics SD RAS, Novosibirsk, Russia*

Institute of biophysics (Puschino) participated in the works on stuty of hair for solution biological tasks. Over 100 hair samples were measured at the elemental analysis station in oder to determine the micro-content of 14 elements.

The distribution of the element content along the hair and/or among a great number of samples has a bimodal character: there are two pools in the distribution of element content.

The first pool is due to minor amount of intrinsic elements of in the cell that has endogenous origin. The second pool with the maximal level of element contents is due to the specific nature of the extracellular matrix (ECM). The element contents in the ECM are variable and have the exogenous origin. Two levels of protection conserve the ordered structure of hair tissue. On the first level, cellular membranes protect the structure of cell as such. On the second one, the extracellular matrix structure is isolated from the environment by acidic sebum protective mantle that is the product of the sebaceous gland. There are many different detergents, for example, soap, shampoo and other means for hair care which may strip the protective mantle from skin and hairs and let the aggressive environment to attack the exposed tissue.

Thus, the protective mantle of skin and hair plays an essential role in metal absorption by the ECM assembly.

An X-ray fluorescent element analysis of hair samples giving various types of diffraction patterns is carried out. A correlation between the character of X-ray patterns and the element contents for Ca, Sr and Br is shown:

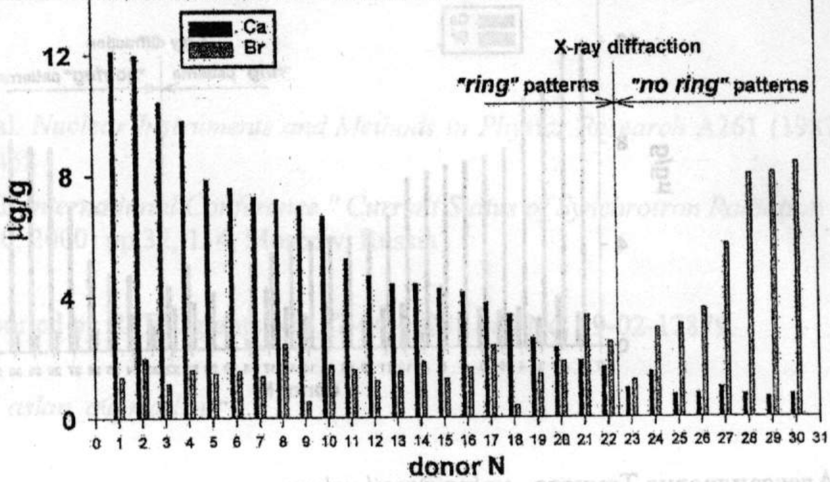
"*no ring*" type - low Ca and Sr concentration and high Br concentration;

"*ring*" type – high Ca and Sr concentration and low Br concentration (picture down).

The function of intensive integral of chemical elemental concentration was found monotone increasing on length of hair from beginning to end. It is the sort the individual for every human..

Activity on this subject continued and is of both applied and investigation medicine-biological character.

Content of Ca(×450) & Br in hairs of 30 donors from Novosibirsk&Pushchino



Valentina Trounova *valna@mail.nsk.ru*

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КОНФОРМАЦИОННЫЕ ИЗМЕНЕНИЯ СЫВОРОТОЧНОГО АЛЬБУМИНА ЧЕЛОВЕКА ПРИ ПСИХИЧЕСКИХ ЗАБОЛЕВАНИЯХ. ИЗУЧЕНИЕ С ПОМОЩЬЮ РАЗРЕШЕННОЙ ВО ВРЕМЕНИ ФЛУОРЕСЦЕНЦИИ.

Т.И.Сырейщикова[1], М.Н.Якименко[1], Ю.А.Грызунов[2], М.Н.Комарова[2], Г.Е. Добрецов[2] Д.Кларк[3], Г.Джонес[3], Я.Монро[3],

[1]) Физический институт им.П.Н.Лебедева РАН, Москва

[2]) НИИ физико-химической медицины минздрава РФ, Москва

[3]) Лаборатория Дарсбери,Варрингтон, Соединенное Королевство

Разрешенная во времени спектроскопия в пучке синхротронного излучения (СИ) и метод флуоресцентных зондов используются для исследования динамических свойств сывороточного альбумина человека. В пучке СИ синхротрона ФИАН С-60 и далее на SRS Daresbury было обнаружено, что некоторые заболевания человека, в частности психические, сопровождаются резким изменением свойств альбумина. Эти изменения проявляются как нарушение взаимодействия альбумина с флуоресцентным зондом К-35, специфичным к альбумину. Было сделано предположение, что нарушения связаны с изменениями спектра конформационных состояний альбумина. Молекулярный механизм таких нарушений может определяться рядом факторов. Данная работа посвящена изучению важнейших из них: взаимодействия между участками альбуминовой глобулы и действия насыщенных жирных кислот- важнейших физиологических регуляторов функции альбумина.

Проведено измерение законов затухания интенсивности и анизотропии флуоресценции при различных длинах волн испускания. Анализ экспериментальных данных с помощью развитого нами метода амплитудного стандарта, позволил выделить четыре конформационных состояния альбуминовых связывающих центров и рассчитать конформационный спектр- заселенность каждого из конформационных состояний. Показано, что как при изменении pH, так и при связывании с альбумином насыщенных жирных кислот времена в функции затухания флуоресценции зонда не изменялись. Следовательно, конформационные состояния центров альбумина не изменялись.Однако, заселенность этих состояний менялась существенно.

Приводятся различные варианты объяснения результатов измерения.

Татьяна Ивановна Сырейщикова *syrej@sci.lebedev.ru*