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Original Article

CHIRAL SWITCHING CONTROL OF PHARMACEUTICAL SUBSTANCES

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ABSTRACT

Objective: The aim of this study was to demonstrate that chiral switching should be recognized as a widespread phenomenon that extends beyond the production of pure enantiomeric drugs.

Methods: To investigate the optical activity of substances from various chemical classes, enantiomers of chiral compounds (Sigma-Aldrich, USA) were chosen: valine and its racemic form (D-valine, L-valine, and racemic valine with optical purity ≥ 99%), L-ascorbic acid (content ≥ 99%), carbohydrates (D-glucose, D-galactose, L-galactose, contents ≥ 99.5%). Solutions were prepared using deuterium-depleted water (DDW–"light" water, D/H=4 ppm), natural deionized high-ohmic water (BD, D/H=140 ppm), and heavy water (99.9% D_2O; Sigma-Aldrich). Optical activity was measured using the Atago POL-1/2 polarimeter.

Results: One of the components in the racemic medication mixture can act as an inert agent, exhibit toxicity, or undergo undesirable biotransformation mechanisms, resulting in the formation of products with unknown properties. It has been established that a change in the deuterium/protium (D/H) ratio in water leads to a change in the equilibrium and kinetic characteristics of optically active compounds across various chemical classes, such as amino acids, carboxylic acids, and carbohydrates. An inequality was observed in the absolute values of the optical rotation of the L-and D-isomers of valine and galactose, depending on the D/H isotope ratio. The impact of chiral water clusters on optical rotation accounts for the sudden shift in the specific rotation of dilute solutions (less than 0.5%) of L-ascorbic acid in water, based on the D/H ratio. The influence of the isotopic composition of water was confirmed by studying the temperature-dependent mutarotation kinetics of D-glucose and L-and D-galactose in Arrhenius coordinates.

The mutarotation process in natural high-resistivity water is characterized by an activation energy (E_a) of 40.8±1.4 kJ mol^{-1}, while in deuterium-depleted water, E_a = 63.6±3.5 kJ mol^{-1}. This results in a kinetic isotope effect for deuterium (KIED) of 1.6.

Conclusion: Methodological approaches have been developed to control chiral switching based on the isotopic composition of water *in vivo* and *in vitro*. The study of changes in the optical activity of hierarchical structures in the human body, the influence of solvent properties on the mechanisms of optical rotation, as well as the use of KIED values, can be utilized to monitor various chiral transitions *in vitro* and living organisms.

Keywords: Kinetic isotope effect, Deuterium/protium ratio, Chiral switching

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INTRODUCTION

The renowned J.-B. Biot established the principles of dynamic stereochemistry [1], which explain various processes involving optically active compounds. The term "chiral switch" was first used to refer to the extraction of the active enantiomer from a racemate or mixture of diastereoisomers (epimers) patented as drugs [2]. Later, this term began to be used for the synthesis of new enantiomers of pharmaceutical substances that had not previously been used as drugs [3]. The stereochemical properties of drug enantiomers contribute to differences in pharmacokinetic and pharmacological profiles, as well as the ways they interact with chiral biological targets [4]. The use of a single pure enantiomer instead of a racemate improves the effectiveness and/or safety of treatment [5] and promotes the development of new drugs across various pharmacological classes [6].

The significance of chiral switching extends beyond the development and production of enantiopure drugs. This concept has significantly expanded in scope. Over the past decades, scientific knowledge has accumulated regarding the change in the chirality of drugs during biotransformation (stereoselective metabolism), leading to the formation of numerous optically active products with unknown properties [7, 8]. These degradation products can interact with the naturally occurring alternating hierarchical structures in the body [9].

Changes in the pharmacokinetic properties of chiral compounds are observed at molecular, cellular, and organismal levels (fig. 1). For example, deuterated drugs are new compounds in which protium (H) atoms are replaced by deuterium (D) atoms [10]. The kinetic isotope effect of deuterium (KIED) results in a reduction in the drug's dosage and toxicity and also prevents the epimerization of optically active substances. Dose-response diagrams for a unicellular organism demonstrate the importance of deuterium as an essential trace element. Deficiency or excess of this element can reduce the organism's vitality. The homeostasis area is tightly regulated by the ratio of deuterium to hydrogen at various temperatures [11]. Another example that highlights the significance of broadening the concept of "chiral switching" is the shift in the direction of optical rotation that occurs as hierarchical structures develop in living systems. This makes it possible to use chirality as a tool for the stratification of hierarchical systems in living organisms and non-living objects [12, 13].

Chiral switching occurs during the process of inducing chirality in achiral molecules and giant heterogeneous water clusters [14], facilitated by small optically active structures known as "sergeant-soldier" [15]. The accumulated experimental data necessitate the generalization and development of new approaches to control chiral switching based on the kinetic isotope effect of deuterium [16, 17].

Deuterium is one of the essential elements [18]. Its main role in living organisms and laboratory settings is to participate in chiral switching at different hierarchical levels within the body. The presence of deuterium (D) in a drug molecule or solvent, in various ratios with protium (H), enables the control of various types of chiral switching. The behavior of optically active compounds from various chemical classes (such as the amino acid valine, ascorbic acid, and carbohydrates like glucose and galactose) in waters with different deuterium/protium ratios is discussed.

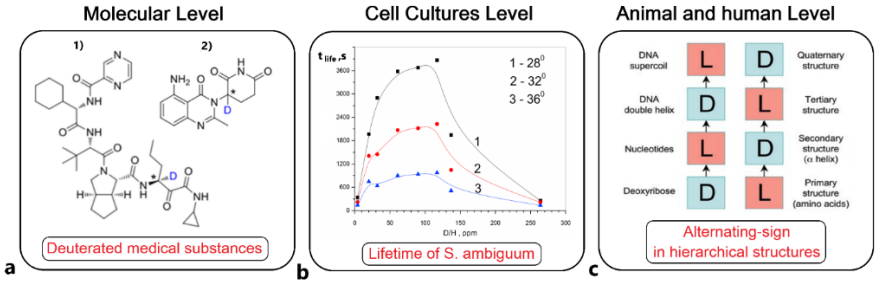

Fig. 1: Types of chiral switches on different hierarchical structures of the human organism. a–molecular level exchange protium by deuteriumind_1-telaprevir (1) and d_1-CC-122 (analog of Thalidomid (2); b–area of homeostasis of a biological object depending on the deuterium/protium) ratio; c–dynamic helical chirality (Tverdislov's law)

MATERIALS AND METHODS

To investigate the optical activity of substances from various chemical classes, enantiomers of chiral compounds (Sigma-Aldrich, USA) were chosen: valine and its racemic form (D-valine, L-valine, and racemic valine, with optical purity of ≥ 99%), L-ascorbic acid (content ≥ 99%), and carbohydrates (D-glucose, D-galactose, L-galactose, with content of ≥ 99.5%). Solutions were prepared using deuterium-depleted water (DDW–"light" water, D/H=4 ppm), natural deionized high-ohmic water (BD, D/H=140 ppm), and heavy water (99.9% D_2O; Sigma-Aldrich). Deionized high-ohmic water with a specific electrical resistivity of 18.2 MΩcm at 25 °C was prepared using the Milli-Q system (Millipore, Great Britain). Deuterium-depleted water was produced at the Research and Production Association "Almaz" using the vacuum rectification technique. Optical activity was determined using the Atago POL-1/2 polarimeter (Japan) with a 100 mm cell, providing a measurement accuracy of±0.002 ° and a resolution of 0.0001°. The electronic Peltier module was used to set the required temperatures.

The mutarotation rate of carbohydrates at various temperatures was determined, enabling the calculation of activation energies using Arrhenius kinetics. The kinetic effects of deuterium in different types of water were also evaluated.

To regulate the impact of water clusters on optical activity, their size distribution was determined using laser light diffraction spectroscopy (LALLS) (MALVERN Instruments, UK) [19]. Hexane was used as a solvent. Before the experiment, the hexane solutions were filtered through 0.22 μm filters (Millipore, Great Britain).

Statistics

The findings were analyzed using statistical methods and software packages from OriginPro 2021 (USA). Each value on the fig. represents the mean±standard deviation (SD).

RESULTS AND DISCUSSION

Deuterium is one of the essential elements. Its primary role *in vivo* and *in vitro* is to participate in chiral switching at different hierarchical levels of the body. The presence of deuterium (D) in a drug molecule or solvent, in various ratios with protium (H), enables the control of different types of chiral switching. The behavior of optically active compounds from various chemical classes (such as the amino acid valine, ascorbic acid, and carbohydrates like glucose and galactose) in waters with different deuterium/protium ratios is discussed below.

Valine in water with varying deuterium/protium ratios

The size and structure of giant heterogeneous clusters (GHCs) of water depend on the pH and isotopic composition, especially the D/H ratio [14]. For instance, the volumetric distribution of water clusters in deuterium-depleted water (DDW) is illustrated (refer to fig. 2a). A hypothetical model for the formation of a chiral center in a water cluster (see fig. 2c) demonstrates that this phenomenon only occurs in water containing both protium and deuterium isotopes (HDO). In water that does not contain the heavy hydrogen isotope H_2O, such as in heavy water D_2O, a chiral center is not formed. The GHCs of water have their own chirality, which is formed in the presence of optically active compounds following the "sergeant-soldier" model [20]. This is why GHCs of water can make a significant contribution to the measured optical activity, the magnitude of which depends on the nature of the optically active compound acting as "sergeants" (chiral inducers). For example, the study found a difference in the absolute values of optical rotation for L-and D-isomers of valine, depending on the deuterium/hydrogen (D/H) isotope ratio (refer to fig. 2b and table 1).

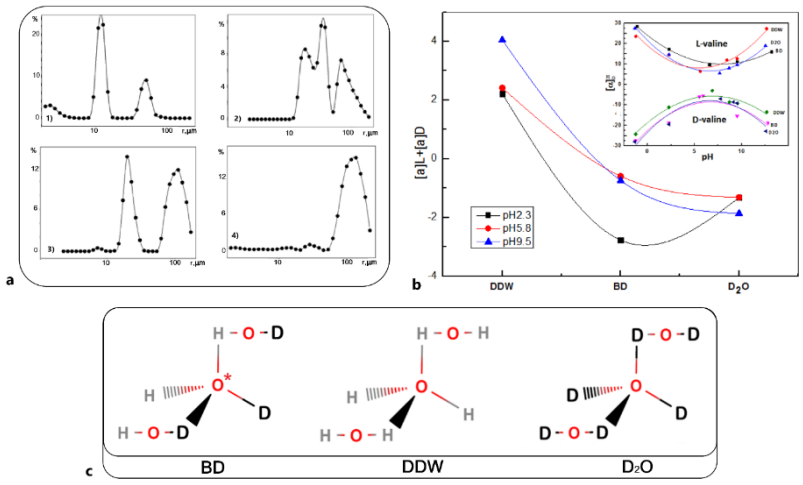

Fig. 2: Valine optical isomers in aqueous solutions with varying isotopic compositions, a-dimensional spectra of giant heterogeneous clusters (GHCs) of water in deuterium-depleted water (DDW) solution of D-valine at pH 2.3–1), pH 6.3–2), L-valine at pH 2.3–3), andpH 6.6–4). b–the total values of the specific rotation of 4% valine stereoisomer solutions at pH 2.3, pH 5.8, and pH 9.5 (BD: D/H=140 ppm; DDW: D/H=4 ppm; D_2O: 99.9% D_2O). Insert-the initial results for F(pH); n=5, mean±SD. c-represents the theoretical prediction of chiral center formation in waters with different D/H ratios (hypothetical model)

Table 1: Optical characteristics of water with different deuterium/hydrogen (D/H) isotope ratio and their effect on the valine optical activity

D/H, ppm	(1-T) LALLS, 633 nm	Frequency of occurrence of intrinsic optical activity maxima, n=880 (10 cm³ layer)	$[a]_D^{20}$(pH=6)	
			L-valine	D-valine
4	0.003	-0.020	9.5	-10
140	0.020	-0.015 and 0.015	7.0	-7.8
D_2O	0.005	+0.015	5.8	-7.5

As shown in fig. 2 and table 1, the absolute values of the specific optical rotation for L-and D-valine do not match.

L-Ascorbic acid in polar solvents

According to Biot's law, the specific rotation of solutions of optically active substances at a selected temperature (T) and wavelength (λ) does not depend on the concentration (C). However, in quantum-mechanical calculations, it has been shown that in some cases, this relationship may be violated [21]. In the non-linear function describing the specific optical rotation for 0.03-4 mol/l solutions of D-levoglucosan, the authors explain the presence of heterogeneous water structures, which increase in size upon dilution. The combined impact of optically active substances and solvent associates has been termed "solvent imprinting" (or sergeants-and-soldiers-type), referring to the influence of supramolecular structures on the specific optical rotation value. There is also evidence that the water surrounding biomolecules also exhibits chirality [22].

The presence of "solvent imprinting" is confirmed by an increase in the specific optical rotation in low-concentration solutions of L-ascorbic acid (fig. 3). This is evident in a significant shift in the specific optical rotation in waters with varying isotopic compositions and in other polar solvents. This deviation cannot be observed when measuring the optical rotation dependence within the concentration range recommended by pharmacopoeias for identifying pharmaceutical substances.

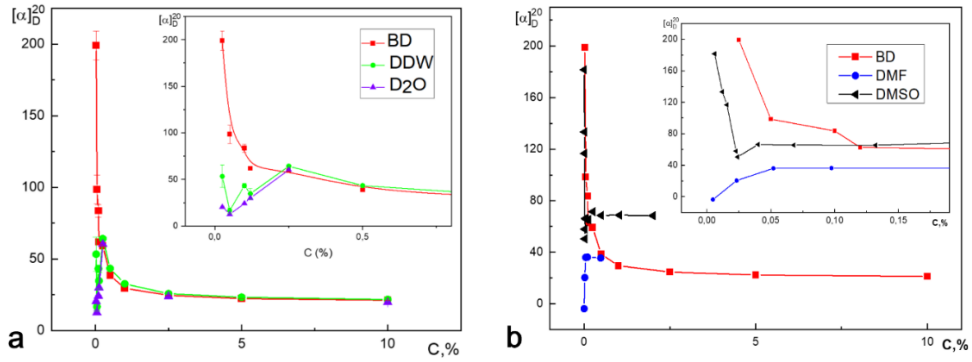

Fig. 3: The relationship between the specific optical rotation and the concentrations of L-ascorbic acid solutions in various solvents: a–BD (D/H=140 ppm); DDW (D/H=4 ppm) D_2O (99.9%); b–BD, DMF, DMSO; insets–concentration range below 0.5%; n=5, mean±SD

The specific optical rotation of low concentrations of L-ascorbic acid solutions (0.025% to 0.12%) in water with varying D/H ratios, as well as in dimethylformamide (DMF) and dimethyl sulfoxide (DMSO), exhibits significant deviations from the expected lines in the coordinates $[a]_{\square}^{\square\square}$ = F(C). Simultaneously, the LALLS method was used to control the presence of giant hydration clusters (GHCs) in water. The size spectrum changed depending on the D/H ratio. Considering the abundant information on the impact of achiral solvents on the optical rotation of chiral compounds and the induction of chirality in supramolecular structures in water, we can infer that the deviation from Biot's law is evident due to the presence of optically active water GHCs. The heterogeneity of water is arranged in a sequence where natural water (140 ppm)>deuterium-depleted water (4 ppm)>D_2O. Therefore, it was expected that the L-ascorbic acid solutions with natural isotopic composition would make the strongest contribution to the specific optical rotation value. Indeed, the specific optical rotation was highest for high-resistivity water with natural isotopic composition.

Carbohydrates in water with varying deuterium/protium ratios

Carbohydrate mutarotation is characterized by statistically different rate constants for natural (D/H = 140 ppm), deuterium-depleted (D/H = 4 ppm) waters, and D_2O. The kinetic isotope effect (KIE) calculated for these types of water is particularly significant when comparing the rate of L-galactose mutarotation in deuterium-depleted water or in water of natural isotope composition with heavy water, where GHCs are not formed (table 2).

The Arrhenius kinetics demonstrates the variation in activation energies for the mutarotation processes of monosaccharide stereoisomers in waters with different isotopic compositions (fig. 4). For example, in the case of D-glucose, the kinetic isotope effect (KIE) in relation to the activation energies of the mutarotation process varies by more than 1.5 times between natural high-ohmicwater and deuterium-depleted water (fig. 4a). Undoubtedly, this reflects the influence of optically active GHCs. Optical antipodes of galactose, acting as "sergeants," induce various types of GHCs ("soldiers"). This explains the differences in the activation energy for mutarotation of D-galactose and L-galactose isomers in water with varying D/H ratios (fig. 4b). Therefore, the observed experimental variations in chiral compounds from different chemical classes in various solvents confirm the emergence of chirality in water clusters. The size and quantity of the particles depend on the ratio of deuterium to hydrogen in water and the properties of polar organic solvents.

Table 2: Kinetic isotope effect of deuterium for galactose enantiomers mutarotation

Carbohydrate	Kinetic isotope effect, T=22 °C		
	k(DDW)/k(D_2O)	k(BD)/k(D_2O)	k(DDW)/k(BD)
D-galactose	2.42	2.32	1.04
L-galactose	5.01	6.42	0.78

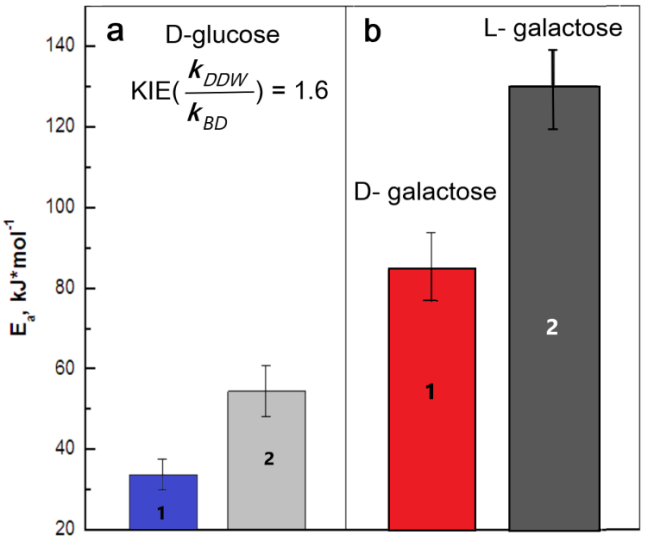

Fig. 4: Activation energy (Ea) of monosaccharide mutarotation (n=3, mean±SD): a–D-glucose in BD (1) and DDW (2) solutions. D/H: BD–140 ppm, DDW–4 ppm; b–D-galactose (1) and L-galactose (2) in DDW

The results obtained can be interpreted within the framework of the theory of induction of additional chiral structures by optically active substances, as described by the sergeants-and-soldiers-type, where chiral molecules act as the "sergeants" and GHCs act as the “soldiers”.

The key role in chiral switching *in vivo* and *in vitro* is played by deuterium, which should be attributed to the group of essential elements. Various ratios of deuterium to protium (D/H) in water can lead to changes in the equilibrium and kinetic characteristics of optically active compounds in different chemical classes, such as amino acids, carboxylic acids, and carbohydrates. An inequality was observed in the absolute values of the optical rotation for the L-and D-isomers of valine and galactose, which was dependent on the D/H isotope ratio. It is important to consider the contribution of chiral water clusters on optical rotation. These clusters differ in number and type for deuterium-depleted water (DDW) with a D/H ratio of 4 ppm, and high-resistivity water of natural isotopic composition (BD) with a D/H ratio of 140 ppm. This also explains the sudden change in the specific rotation of dilute solutions of L-ascorbic acid (less than 0.5%) in water, based on the D/H ratio, as well as in polar organic solvents. The kinetic isotope effect of deuterium (KIED) is particularly notable for L-galactose, reaching 5.0-6.4 compared to heavy water at 22 °C. The influence of the isotopic composition of water was confirmed by studying the temperature-dependent mutarotation kinetics of D-glucose and L-and D-galactose in Arrhenius coordinates. The mutarotation process in natural high-resistivity water is characterized by an activation energy (Ea) of 40.8±1.4 kJ mol^{-1}, and in deuterium-depleted water, Ea = 63.6±3.5 kJ mol^{-1}, resulting in a KIED value of 1.6.

CONCLUSION

The significance of chiral switching extends beyond the development and production of enantiopure drugs. This concept has significantly expanded in scope. Methodical approaches have been developed to control chiral switching based on the isotopic composition of water *in vivo* and *in vitro*. The study of changes in the optical activity of hierarchical structures in the human body, the influence of solvent properties on the mechanisms of optical rotation, as well as the use of KIED values can be utilized to monitor various chiral transitions *in vitro* and living organisms.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

Olga V. Levitskaya–The development of research design, obtaining experimental data, and writing the article text.

Tatiana V. Pleteneva–Writing the article and designing the article.

Daria A. Galkina–Obtaining experimental data.

Nadezda A. Khodorovich–Review of current publications on the topic.

Elena V. Uspenskaya–Review of current publications on the topic and statistical data processing.

Anton V. Syroeshkin–Significant revision incorporating valuable intellectual content.

CONFLICTS OF INTERESTS

The authors have declared no conflict of interest.

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