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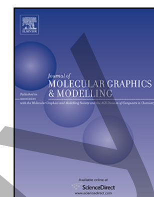

Topical Perspectives

Ethanol binding sites on proteins

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ABSTRACT

This study is on the analysis of ethanol binding sites on 3D structures of nonredundant proteins from the Protein Data Bank. The only one amino acid residue that is significantly overrepresented around ethanol molecules is Tyr. There are usually two or more Tyr residues in the same ethanol binding site, while residues of Thr, Asp and Gln are underrepresented around them. Residues of Ala and Pro are significantly underrepresented in ethanol binding surfaces. Several residues (Phe, Val, Pro, Ala, Arg, His, Ser, Asp) bind ethanol significantly more frequent if they are not included in beta strands. Residues of Ala, Ile and Arg preferably bind ethanol when they are included in an alpha helix. Ethanol molecules often make hydrogen bonds with oxygen and nitrogen atoms from the main chain of a protein. Because of this reason, the binding of ethanol may be associated with the decrease of the length of alpha helices and the disappearance of 3/10 helices. Obtained data should be useful for studies on new targets of the direct action of ethanol on enzymes, receptors, and transcription factors.

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1. Introduction

Ethanol is a small molecule that has a short hydrophobic part (CH_3—CH_2—) and a hydroxyl group (—OH) that is hydrophilic. Because of its amphiphilic nature, ethanol can participate in different types of contacts with proteins. The motif for ethanol binding has been suggested by D.S. Dwyer and R.J. Bradley [1] based on the analysis of several X-ray structures of proteins with bound ethanol molecules and theoretical considerations. According to their opinion, ethanol prefers to bind N-terminal parts of alpha helices, making both hydrogen bonds and hydrophobic contacts with amino acid residues. The number of available 3D structures of proteins with bound ethanol molecules has grown, and there is a need to perform statistics-based search for structural motifs. Previously it was suggested that ethanol should be prone to make cation-pi contacts with aromatic residues [2]. These data are confirmed by mutagenesis studies of *N*-methyl-D-aspartate (NMDA) receptors [3,4] in which phenylalanine residues from the transmembrane alpha helix are playing the most important role in ethanol binding. In glycine receptors the most important amino acid residues for ethanol action are situated both in the transmembrane helix (serine) and in the loop from extracellular domain (alanine) [5]. The attention of researchers has also been concentrated on the odorant receptor LUSH of *Drosophila melanogaster* that is highly sensitive to ethanol [6]. The importance of multiple hydrogen bonds for the binding of ethanol has been highlighted [6]. The aim of this study was to analyze the set of 3D structures of nonredundant proteins able to bind ethanol and find characteristic structural motifs for that molecule. The importance of this work is in the fact that the obtained data should help to identify previously unknown targets for the ethanol action.

Alcoholism is an actual health care problem. Chronic alcoholic intoxication leads to the drastic changes in the metabolism of human body. Many effects of ethanol are known as “indirect”. Indeed, ethanol is metabolized by alcohol dehydrogenases into acetaldehyde [7]. This process leads to the overproduction of reduced nicotinamide adenine dinucleotide (NADH) that dysregulates the metabolism [8]. Acetaldehyde itself binds proteins, makes conjugates with neurotransmitters [9], and disturbs the main biochemical pathways. Ethanol metabolism catalyzed by cytochrome-P450 2E1 produces reactive oxygen species and causes oxidative stress [8]. Specific influence of ethanol on the central nervous system causes the changes in metabolism and hormonal regulation [8]. Namely, the intake of an average or a high dose of

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ethanol leads to the decrease of dopamine in the brain due to its massive release, as it was approved in rats [10]. Ethanol withdrawal also leads to some characteristic changes in concentrations of neurotransmitters in the brain: the level of glutamate is growing on the first day after the stop of ethanol intake [11]. Activity of phosphofructokinase in liver is inhibited by average and high doses of ethanol [12], while the activity of GABA catabolizing enzymes is activated by low doses of ethanol and inhibited by high doses [13]. Moreover, ethanol can change the fluidity of the lipid bilayer, while the effects of this action of ethanol become prominent at a dose of ethanol that is much higher than pharmacologically relevant ones [5].

There are several targets for the direct action of ethanol identified to date responsible of its effects on the central nervous system. Ethanol binds nAChRs (nicotinic acetylcholine receptors) [14] and exhibits different effects that depend on the concentration of ethanol and the nature of nAChR subunits. For example, α7nAChRs showed a potentiation effect after the application of 100 mM ethanol that decreased after the application of 300 mM ethanol [15]. In contrast, α3β2nAChRs are inhibited by 100 mM concentration of ethanol [16].

Pharmacologically relevant concentrations of ethanol potentiate the action of GABA (gamma-aminobutiric acid) and glycine on GABA and glycine receptors, respectively [17,18]. Moreover, ethanol activates G protein-gated inwardly rectifying K^+ channels (GIRK) [19]. GIRK2 channels are activated by ethanol in the dose-dependent manner at concentrations from 10 to 200 mM, and then they become saturated [20].

Ethanol inhibits NMDA receptors (*N*-methyl-D-aspartate receptor) in a subunit-dependent way [21]. At least partially this effect has been explained by the decrease of phosphorylation of the receptor by brain-specific tyrosine phosphatase in response to the increase of ethanol concentration [22]. However, later studies confirmed the existence of ethanol action site directly on NMDA receptors [4].

Other proteins (that are not involved in neurotransmission) can also bind ethanol. Among these proteins we can mention enzymes and transcription factors. Ethanol consumption leads to the characteristic changes in gene expression patterns [8]. Probably, some of them may be explained by the direct binding of ethanol by some proteins regulating gene expression.

Even though ethanol can bind a protein in many different ways, some of the characteristic properties of ethanol binders have been described in this work: the avoidance of beta strands by Phe, Val, Pro, Ala, Arg, His, Ser and Asp residues binding ethanol; the preference to be situated in alpha helices for Ala, Ile and Arg residues binding ethanol; and the lack of 3/10 helices around 14 out of 20 types of proteinogenic amino acid residues binding ethanol.

2. Materials and methods

As the material for this study we used the set of 63 3D structures of proteins with bound molecules of ethanol from the Protein Data Bank (www.pdb.org). The set (see Supplementary material PDB IDs) was built from proteins with the level of similarity lower than 33% according to the Decrease Redundancy algorithm (http://web.expasy.org/decrease_redundancy/) and it includes proteins from all the domains of life. In other words, we used all the available 3D structures of proteins with bound ethanol molecules, and then excluded all the similar structures. The number of ethanol molecules included in the study was equal to 110, since several ethanol molecules can be bound by the same protein.

Each PDB file has been analyzed with the help of the Protein-Ligand Interaction Profiler (PLIP) server (https://projects.biotec.tu-dresden.de/plip-web/plip/) [23]. That server described the types of contacts between all the atoms of ethanol molecules (including hydrogen atoms) and amino acid residues from proteins (once again, including hydrogen atoms). From the obtained data we calculated the usage of each amino acid in ethanol binding sites, as well as the numbers of all the types of contacts for each residue.

Additionally, with the help of MS Excel algorithm, we calculated the distances between each atom of every ethanol molecule (excluding hydrogen atoms) and the atoms of proteins (excluding hydrogen atoms). In case if the distance between an atom of ethanol and any atom of an amino acid residue was less than 5 Å, we included that amino acid in the set of residues situated near the corresponding atom of ethanol. Using this technique, we significantly increased the number of possible ethanol binders. For each amino acid residue found in this way we collected an information on the distribution of other amino acids and the elements of secondary structure (See Supplementary Material Secondary Structure file) around it (from the position "-5" to the position "+5" in the primary sequence of a protein).

The usages of amino acid residues situated near ethanol molecules have been compared with their usages in all other regions of analyzed proteins with the help of two-tailed *t*-test. In the same manner, we compared the distribution of other amino acids and the elements of secondary structure around residues interacting with ethanol molecules and those that are not interacting with them. We considered the preferences in structural motifs strong enough in case if the same element is significantly overrepresented (or underrepresented) at least in three from eleven positions (in more than one quarter of the sequence) around the binder. This additional cutoff criterion has been used throughout the text because it is hard to suggest that alpha helix or beta strand may be overrepresented or underrepresented in just one or two positions around the binder in the specific structural motif. However, we provide the information on all the significant differences in secondary structure distribution around residues binding ethanol in the Supplementary Material Secondary Structure file.

To characterize the combinations of amino acids binding the same ethanol atom, we compared amino acid usages in binding sites containing each amino acid with amino acid usages in binding sites that do not contain that amino acid.

For residues of tyrosine we conducted additional in silico experiment with the aim to find out what amino acid residues are forming ethanol binding sites together with them. Using the MS Excel algorithm we found all the amino acid residues situated at a distance less than 5 Å from each atom of each Tyr residue in our set of proteins. We separately studied interactions between close residues (5 positions around Tyr in a primary sequence) and distant residues. Then we checked what amino acid residues are over- and underrepresented around Tyr residues binding ethanol, relatively to those that are not involved in ethanol binding, using two-tailed *t*-test.

With the help of "sequence similarity" resource from the PDB web page we found 100% identical structures to those from our data set but without ethanol molecules. Then we compared the secondary structure of regions surrounding ethanol binding residues (from −5 to +5 position) in two 3D-structures (with and without ethanol). Comparisons have been made according to the DSSP [24] description of secondary structure.

3. Results

3.1. The nature of contacts between ethanol and proteins

With the help of the PLIP server we determined the nature of contacts between ethanol and amino acid residues from studied 3D structures of proteins. From 181 contacts found by the PLIP server, 52 are hydrogen bonds of ethanol with main chain (MC)

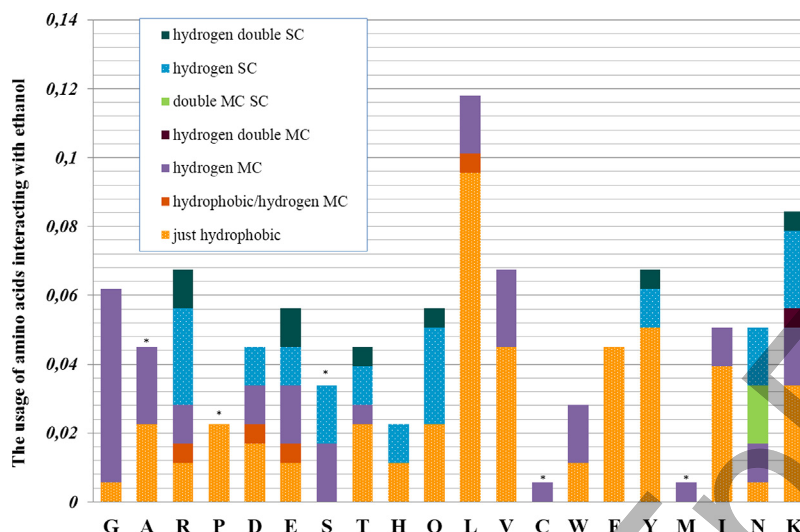

Fig. 1. The usage of ethanol binding amino acid residues according to the PLIP results. Concrete types of interactions are shown for each amino acid residue. "MC" means main chain hydrogen bond, "SC" means side chain hydrogen bond. Significantly underrepresented amino acid residues are shown by asterisks.

atoms: oxygen and nitrogen from the backbone of a protein (those from peptide bonds). The number of the hydrogen bonds of ethanol with atoms from side chains (SC) of amino acid residues is lower (41 contacts) than that with MC atoms. The number of hydrophobic contacts between atoms of ethanol and hydrophobic parts of amino acid residues is equal to 88. From this data one can conclude that ethanol molecules are usually making both hydrophobic contacts and hydrogen bonds with proteins (especially those with MC atoms). As it is shown in Fig. 1, such amino acid residues that can make both MC and SC hydrogen bonds, as Arg, Asp, Glu, Ser, Thr, Asn, and Lys, were found to form both types of hydrogen bonds in different cases. Other residues able to make both MC and SC bonds, made just SC hydrogen bonds (His, Gln, and Tyr), or just MC hydrogen bonds (Cys, Trp, and Met). Hydrophobic amino acid residues can make just MC hydrogen bonds with ethanol, sometimes along with hydrophobic contacts. However, hydrophobic contacts prevail for such hydrophobic residues as Leu, Val, and Ile. Pro and Phe were found in hydrophobic contacts only. Ala has made the same number of hydrogen bonds and hydrophobic contacts with ethanol, while Gly has made mostly MC hydrogen bonds.

The most frequent binder of ethanol is leucine (12.36%). However, we cannot say that it is overrepresented in ethanol binding sites. The frequency of its usage among residues that do not make contacts with ethanol (8.59%) is not significantly lower than among those that make such contacts ($P > 0.05$). There are no overrepresented residues among ethanol binders, but there are five significantly underrepresented ones: Ala, Pro, Ser, Cys, and Met. Based on the data just from the results of PLIP calculations it is hard to make any conclusion about structural motifs in ethanol binding sites.

Some of the binding sites can be described by a scenario in which hydrocarbon chain of ethanol makes hydrophobic contacts with hydrophobic side chains of amino acid residues, while —OH group dives inside the protein and makes a hydrogen bond with one of the main chain oxygen or nitrogen atoms (See Graphical Abstract for samples of ethanol binding sites).

In other cases, hydrophobic ethyl group of ethanol dives inside the hydrophobic regions of a protein, while its —OH group makes hydrogen bonds with atoms from side chains of amino acid residues situated on a surface.

Such hydrophilic (by the definition) amino acid residues as Glu and Arg possess relatively long hydrophobic fragments in their side chains. Sometimes ethanol makes hydrophobic contacts with hydrophobic parts of hydrophilic amino acids.

Even though ethanol can bind many different types of sites, there should be specificity at least for some of them. To search for specific patterns, we decided to collect the data about all amino acid residues situated at the distance lower than 5 Å from each of the three ethanol atoms visualized with the help of X-ray analysis (two carbon atoms and one oxygen atom). This strategy allowed us to increase the number of interacting amino acid residues.

3.2. Amino acid residues situated around ethanol molecules

In Fig. 2 one can see the distribution of amino acid residues around (at the distance shorter than 5 Å) two carbon atoms of ethanol molecules (C1 is the carbon from CH_3— group, C2 is the carbon from —CH_2— group), as well as around oxygen atoms (O3) from —OH groups of ethanol molecules. These distributions are compared with the one for all the amino acid residues situated at the long distance (≥5 Å) from the mentioned atoms of ethanol molecules.

As well as in Fig. 1, the usages of Ala and Pro are significantly lower around all the atoms from ethanol molecules. Residues of Ser are underrepresented around carbon atoms of ethanol, but not around oxygen, since sometimes ethanol makes hydrogen bond with —OH group of Ser. The usages of Cys and Met are not significantly different around ethanol molecules relative to other parts of proteins. Residues of Thr are underrepresented around C2 atoms of ethanol molecules, isoleucine residues are underrepresented around oxygen atoms of ethanol molecules.

The most intriguing result of the search of amino acid residues situated around ethanol molecules is as follows: residues of Tyr are more than two times overrepresented around both carbon and oxygen atoms of ethanol. It means that some of the ethanol binding sites are enriched by Tyr residues. However, PLIP algorithm did not find all of those Tyr residues.

3.3. Residues of Tyr binding ethanol molecules

To find out why Tyr residues are overrepresented around ethanol molecules we performed additional in silico experiment with our set of proteins. We found out which amino acid residues are situated around Tyr residues that are involved, and those that are not involved in ethanol binding. We separated the contacts

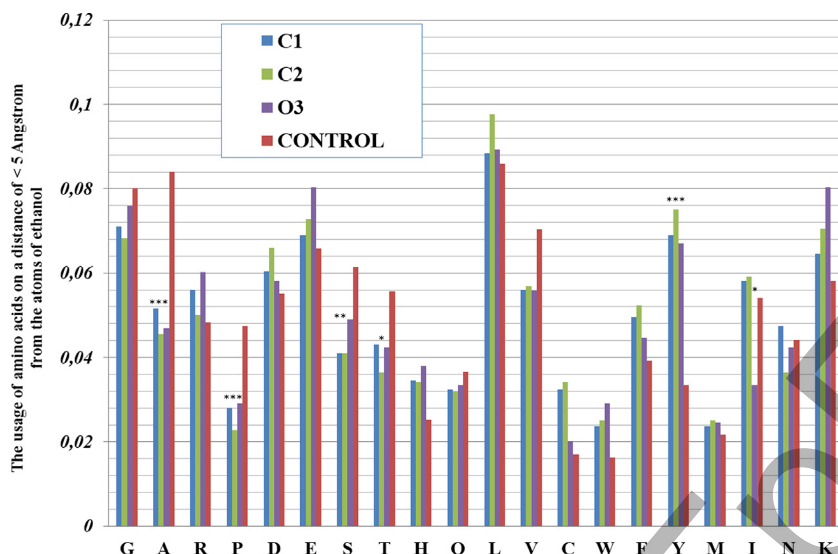

Fig. 2. The usage of amino acid residues situated at the short distance (less than 5 Å) from ethanol molecules. Distributions of amino acids situated near C1, C2 and O3 atoms of ethanol (near CH_3—, —CH_2— and —OH groups, respectively) are given separately. Amino acid usage in other parts of proteins is given as well. Significant differences with the amino acid usage in those parts of proteins that are not interacting with ethanol molecules are shown by asterisks.

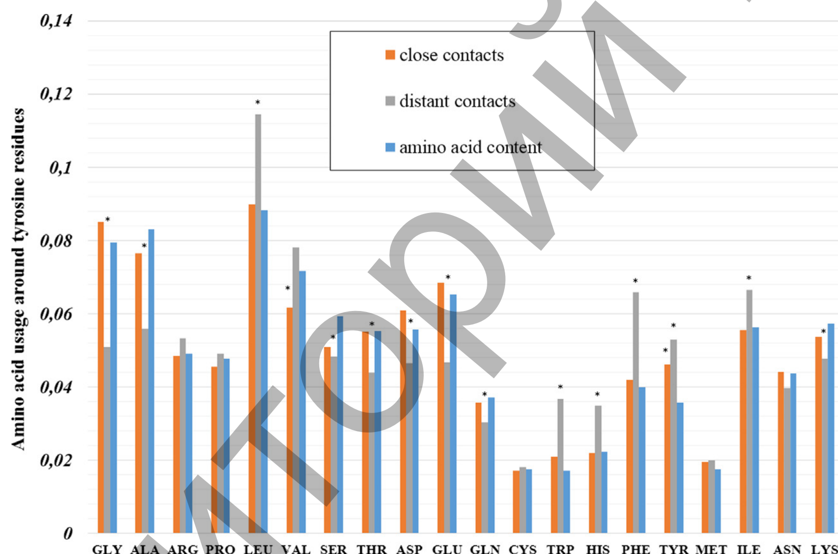

Fig. 3. The usage of amino acids situated at a short distance (less than 5 Å) from Tyr residues that do not bind ethanol. Close and distant contacts are shown separately. Significant differences with the overall amino acid content are shown by asterisks.

of Tyr residues into two groups: contacts with amino acids situated close to Tyr in the primary sequence (from −5 to +5 position) and contacts with residues situated in distant parts of the primary sequence. In Fig. 3 the distribution is shown for Tyr residues that are not involved in ethanol binding. At the close distance we can find that residues of Val are underrepresented around Tyr, but other Tyr residues are overrepresented. Distant contacts showed some stronger trends. Aromatic residues (Trp, His, Phe, and Tyr) prefer to be situated near Tyr residues in the 3D structure of a protein [25]. Two hydrophobic amino acid residues (Leu and Ile) are also more frequently found near Tyr residues. Such hydrophilic amino acids, as Ser, Thr, Asp, Glu, Gln, and Lys are significantly underrepresented around Tyr residues, as well as Gly and Ala. These trends may be explained by the aromaticity and hydrophobicity of Tyr residues: they prefer to form clusters of hydrophobic and aromatic residues with other Tyr residues and with side chains of amino acids with similar physical properties. Hydrophobic regions frequently make beta structure [26]. That is why Ala as the strong alpha-helical residue [27] is underrepresented around Tyr residues.

The next step was to compare the "control group" of Tyr residues with ethanol binding ones. Results showed that Tyr residues binding ethanol avoid contacts with Asp and Gln situated nearby, as well as with Thr residues situated at a long distance. Ethanol molecule is known to be able to make cation-pi interactions with aromatic rings [2]. The same type of interaction with Tyr can be made by side chains of Thr, Asp and Gln (in their protonated forms) [2,28]. Probably, there is a concurrence between ethanol molecules and Thr, Asp and Gln side chains for Tyr aromatic rings. Moreover, —OH group of Tyr residue can make hydrogen bonds with either ethanol, or side chains of residues like Thr, Asp and Gln [29].

Ethanol binding residues of Tyr are making contacts with other Tyr residues from distant regions of a protein significantly more frequently than Tyr residues that are not involved in ethanol binding. Representation of ethanol binding site containing multiple Tyr residues (4EJO: a bacterial transcriptional regulator) is given in

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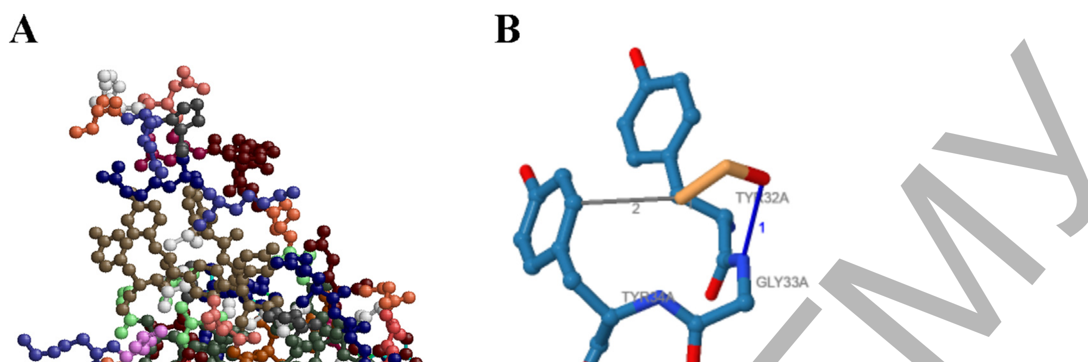

Fig. 4. A sample of the structure of ethanol binding site with multiple Tyr residues: a) RasMol view (Tyr residues are brown), b) PLIP view. Hydrogen atoms are not shown.

Fig. 4. In this 3D structure ethanol molecule is situated between four Tyr residues (Fig. 4A). Interestingly, PLIP software found contacts with just two Tyr residues and a hydrogen bond with Gly main chain atom (Fig. 4B). Probably, ethanol molecules can fluctuate between Tyr residues in different moments in time getting closer to one of them and moving to a little longer distance from another one.

Comparison of secondary structure elements distribution around Tyr residues binding ethanol revealed just one significant difference: random coil is strongly underrepresented around Tyr residues contacting with carbon atoms of ethanol. In many cases instead of random coil there is an alpha helix, while it is not significantly overrepresented. In the +1 position after Tyr residue interacting with C1 and C2 atoms of ethanol the usage of Gly is significantly higher than for Tyr residues that are not binding ethanol.

3.4. Residues of Leu binding ethanol

There are no specific features of secondary structure for Leu residues involved in interactions with ethanol molecules, if we compare them with other Leu residues. Interestingly, the usage of Tyr is significantly higher around (in positions from −5 to +5) Leu residues binding C2 atom of ethanol. Especially high abundance of Tyr residues is in the +5 position after Leu. Residues of Lys are, in general, overrepresented around Leu situated near oxygen atoms of ethanol molecules. If we compare amino acid content of the binding sites containing Leu residues and those without Leu, then we will find out that Asp residues are overrepresented in the areas with Leu situated near oxygen atoms of ethanol molecules. These data are obviously not enough to say that Leu residues binding ethanol have some specificity. As the most "popular' amino acid residue in proteins (especially in those that are encoded by genes with average GC-content) [26], Leu is usually somewhere around ethanol no matter where exactly this molecule was bound.

3.5. Residues of Arg, Lys and His binding ethanol

Arginine residues binding ethanol in the group of proteins we studied are usually situated in the beginning of alpha helices. That element of secondary structure is overrepresented in positions +1–+5 after Arg binding oxygen atom of ethanol (See Supplementary Material Secondary Structure file). Beta strand is underrepresented in positions −2 to 0 around such Arg residues. One can compare the distribution of secondary structure elements around arginine residues from the 'control' group and around Arg that bind oxygen atom of ethanol (Fig. 5). Arginine residues in beta strand should usually be unavailable for the binding of ethanol. Side chains of Arg should be more suitable for interactions with ethanol when the residue is included in alpha helix (in its hydrophilic surface). Other Arg residues are overrepresented around the one that is binding ethanol, as well as Gln. Val is overrepresented in +1 position around Arg interacting with C1 of ethanol. Arginine residues preferably bind C2 atom of ethanol together with Ser.

In contrast to Arg residues, residues of Lys binding ethanol avoid alpha helices. Alpha helix is underrepresented in +3–+5 positions after Lys binding oxygen atoms of ethanol. 3/10 helices are also underrepresented in +1–+5 positions after Lys residues binding ethanol. Residues of Gly are overrepresented in the position +2 (for Lys binding C2 and O atoms). Residues of Arg are overrepresented in the position +3 (for Lys binding C1 and O atoms).

Another amino acid classified as the one with a positively charged side chain is His. Just like for Lys residues, helices 3/10 are underrepresented in −2 to +1 positions around His residues binding oxygen atoms of ethanol molecules. Beta strand is significantly underrepresented before His residues (in positions from −5 to −3) binding C2 atoms of ethanol. Pro and Met residues are, in general, overrepresented around ethanol binding His residues. So, we cannot say that there is a common structural preference for all positive charged residues binding ethanol: each amino acid has its own characteristic motifs that can help it to bind C_2H_5OH.

3.6. Residues of Glu, Asp, Ser, Thr, Gln, Asn, Cys, Gly, and Met binding ethanol

Residues of Glu binding ethanol do not demonstrate any specific features of secondary structure around them, just like Leu. Interestingly, residues of Leu are overrepresented near Glu binding ethanol. Glu can provide both hydrophobic body of its side chain and hydrophilic carboxylic group, or main chain oxygen and nitrogen atoms for ethanol binding. However, it is involved in ethanol binding just because of its relatively high usage in proteins [26]. Asp residues binding ethanol show some structural preferences only if we consider just those surrounding C1 atoms of ethanol molecules: beta strand is underrepresented in positions +3, +4 and +5 after them. Residues of Asp preferably bind ethanol together with Ala residues, Arg residues are overrepresented in +4 position after Asp binding C2 atoms of ethanol molecules. Ethanol binding residues of Ser show some structural preferences only if we consider those around C2 atoms of ethanol molecules. Beta strand is underrepresented in the positions +1, +2 and +3 around them. Helices 3/10 are underrepresented around residues of Ser binding ethanol if we consider each of the ethanol atoms, while for Thr residues the same tendency works only if we consider C2 atoms of ethanol (See Supplementary Material Secondary Structure file).

Residues of Gln and Asn binding ethanol demonstrate the same tendency. The usage of 3/10 helices is significantly lower around them, than around the same residues that are not involved in ethanol binding. We can also admit that residues of Ile are overrepresented around Gln residues binding ethanol. Lysine is overrepresented in +3 position after Asn residues binding oxygen

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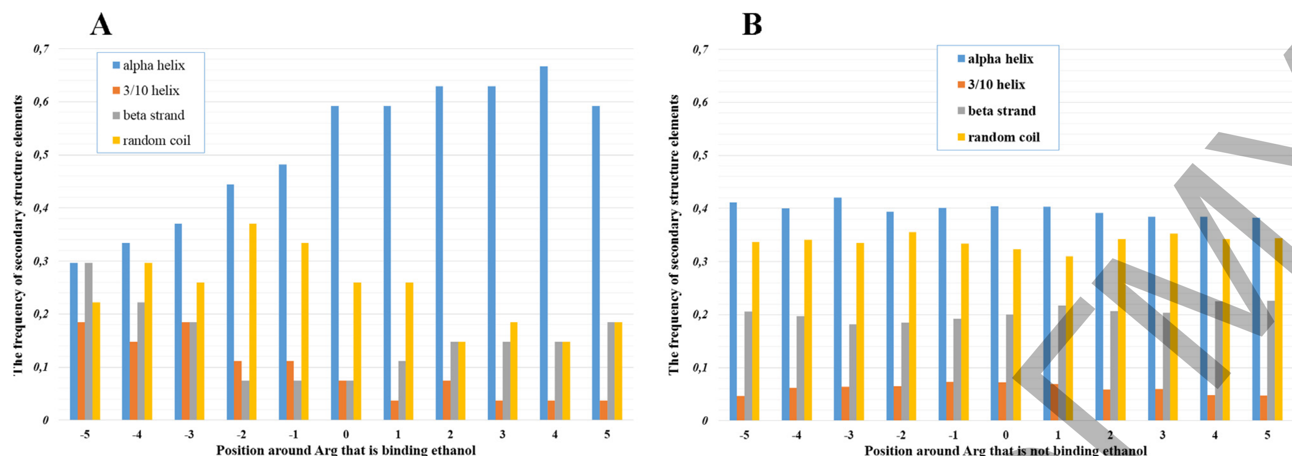

Fig. 5. Distribution of secondary structure elements around Arg residues a) that bind ethanol; and 2) those that do not bind ethanol.

atoms of ethanol molecules. Helices 3/10 are also underrepresented around Cys, Gly and Met residues binding ethanol.

3.7. Residues of Ala and Ile binding ethanol

Residues of Ala involved in ethanol binding are significantly more frequently situated in alpha helices than those that are not involved in interactions with ethanol molecules. Moreover, beta strand is underrepresented in positions from 0 to +3 around Ala residues binding ethanol. Helices 3/10 are underrepresented around Ala making bonds to ethanol. It is known that Ala is the strongest former of alpha helices [26,27]. However, it still can be found in random coil and beta strands, but in those structural elements it seems to be not available for ethanol binding. It is not surprising that residues of Pro are underrepresented around residues of Ala binding ethanol.

Residues of Ile demonstrate a strong structural motif if we consider those which are binding carbon atoms of ethanol, and not its oxygen atom. Alpha helix is overrepresented around those Ile residues, while random coil is underrepresented. If residues of Ile from the "control group" are found more frequently in beta strands (like they should) [27], residues making contacts with ethanol are more frequently included in alpha helices. Helices 3/10 are significantly underrepresented around residues of Ile that are situated around ethanol molecules.

3.8. Residues of Phe, Val, Pro, and Trp binding ethanol

Beta strand is significantly underrepresented around residues of Phe binding ethanol that results in the growth of the frequency of alpha helix (not significantly). Residues of Val and Pro binding ethanol are also showing decreased frequencies of beta strands around them. The last two amino acid residues prefer to bind ethanol if they are included in random coil. Ethanol binding residues of Trp also prefer to be included in the random coil, while the frequency of alpha helix, and not beta strand, is decreased around them.

3.9. The influence of the ethanol binding on the secondary structure of proteins

As one can see in Table 1, several amino acid residues (Ala, Ile, and Arg) prefer to bind ethanol when they are included in alpha helices. Several other amino acids prefer to bind ethanol when they are included in random coil and not in beta strands (Pro, Val), or not in alpha helices (Trp). The strongest trend among ethanol binding

Table 1
Significant trends in the distribution of secondary structure elements around amino acid residues binding the molecules of ethanol.

Amino acid residue	Major elements of secondary structure around ethanol binding residues	Minor elements of secondary structure around ethanol binding residues
Ala	Alpha helix body (not beta strand)	Not 3/10 helix
Ile	Alpha helix body (not random coil)	Not 3/10 helix
Arg	Alpha helix N-terminus (not beta strand, not random coil)	
Phe	Not beta strand	
Tyr	Not random coil	
Pro	Random coil (not beta strand)	Not 3/10 helix
Val	Random coil (not beta strand)	Not 3/10 helix
His	Not beta strand	Not 3/10 helix
Ser	Not beta strand	Not 3/10 helix
Asp	Not beta strand	
Trp	Random coil (not alpha helix)	Not 3/10 helix
Lys	Not alpha helix	Not 3/10 helix
Asn		Not 3/10 helix
Cys		Not 3/10 helix
Gln		Not 3/10 helix
Gly		Not 3/10 helix
Thr		Not 3/10 helix
Met		Not 3/10 helix
Glu		
Leu		

amino acid residues is the avoidance of beta strands (Ala, Arg, Phe, Pro, Val, His, Ser, Asp). Even more impressing fact is that 3/10 helices are underrepresented around 14 out of 20 amino acid residues. So, it is possible that the binding of ethanol may destroy 3/10 helices because of the formation of hydrogen bonds with the main chain of a protein. The typical lengths of 3/10 helices are 3 and 5 residues [30]. So, a small change in the position of a main chain atom may result in their destruction. To test this hypothesis, we analyzed 3D structures of proteins without co-crystallized ethanol molecules that are 100% identical to those with ethanol molecules.

In 19 out of 31 cases (61.29%) the structure of a protein without ethanol was different from that with ethanol. We considered changes in the rage from −5 to +5 position around each binding residue. It is important to mention that we registered 13 cases when the length of alpha helix has been decreased because of the ethanol binding and just 4 cases when it has been increased. For 3/10 helices

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the tendency is the same, but the number of observations is lower: 4 cases of the decrease of a length and 1 case of the increase. It means that the decrease of 3/10 helices frequency around amino acids binding ethanol may be explained by the influence of ethanol on the secondary structure of proteins, while the number of such cases is low in the PDB. However, the prevalence of alpha helices around some ethanol binding amino acid residues is an evidence that alpha helices are making main chain and side chain atoms of those residues available for ethanol, but ethanol itself is not promoting alpha helix formation at concentrations used in crystallographic experiments. Interestingly, ethanol at high concentration (higher than 0.5 M) has been shown to stimulate formation of alpha helices [1]. At lower concentrations ethanol has been shown to decrease stability of alpha helical peptides in molecular modelling experiments [31].

As to beta strands, their lengths increased in 7 cases, and they decreased in just 2 cases. It means that the avoidance of beta strands that we found for many residues (see Table 1) is not associated with their destruction by the ethanol binding. Certain amino acid residues should be unavailable for ethanol binding if they are included in relatively long beta strands.

4. Discussion and conclusions

Interestingly, there is no general overrepresentation for all ethanol binding residues in alpha helices. The preference for being included in alpha helices among amino acids binding ethanol suggested previously [1] is residue-specific. In contrast, the trend of the avoidance of beta strands for residues binding oxygen atom of ethanol is significant even if we take into considerations all amino acids. The data described in this study may be useful for finding more or less suitable sites for ethanol binding on 3D structures of proteins. As we found out, such amino acids as Ala, Arg, Phe, Pro, Val, His, Ser, and Asp are usually not included in beta strands if they bind ethanol molecules. This trend is especially useful for those residues that normally prefer to be included in beta strands – for Phe and Val [27]. So, if one predicted Phe and/or Val residues that are not included in beta strands to bind ethanol using molecular docking or other methods, then that prediction will be strengthened by the knowledge on structural motifs for ethanol binding amino acid residues.

In the same manner, residues of Ile in alpha helices (usually they form beta strands) and residues of Lys not included in alpha helices (usually they are included in them) should be strong binders of ethanol, as well as Trp residues situated in random coil (usually they form beta strands).

The data from Table 1 cannot be explained by hydrophobicity of amino acid residues. For example, among residues that bind ethanol being not included in beta strands there are four hydrophobic residues (Ala, Phe, Pro, and Val) and four hydrophilic residues (Arg, His, Ser, and Asp). These residues also show different preferences for secondary structure formation: Ala and Arg are alpha helix formers, Phe and Val are beta sheet formers, while Pro, His, Ser, and Asp are usually found in random coil. So, we can suggest that discovered preferences for secondary structure motifs include some useful information on "markers" of ethanol binding sites.

Other "marker" of ethanol binding sites should be multiple residues of Tyr in the same area on the surface of a protein. Moreover, the presence of such amino acids as Ala and Pro among predicted residues interacting with ethanol should be an evidence of a weak ethanol binding. This information can be checked on several proteins that are known to possess specific sites for ethanol binding.

If we consider the structure of the LUSH protein from *Drosophila melanogaster* that is a specific receptor for ethanol [32], then some characteristic properties of ethanol binding sites described above will be observed. There are two Phe residues around the ethanol molecule. Both of them are not included in beta strands. Phe64 residue is situated in random coil exactly before an alpha helix, while Phe113 is situated in the body of an alpha helix. Trp123 residue is situated in random coil. Ser52 is situated in alpha helix. Val58 is situated in random coil. Ala55 is also included into the binding site (alanine residues are, in general, avoided by ethanol molecules), while it is situated in alpha helix (in the favorable structural motif for ethanol binding). Other residues show neither prohibited, nor favorable structural motifs: Thr57 – is in random coil, Thr109 and Val106 – are in alpha helices.

In the structure of alcohol dehydrogenase [7] we can find three amino acids that "escaped" beta strands in consistence with the data from Table 1. Phe93 and Val294 are situated in random coil that starts right after beta strand, His67 is in the random coil between two beta strands, Ser48 is in alpha helix. Other amino acids are showing neutral structural motifs: Leu57, Leu116 and Leu141 are in random coil regions.

Previously we described possible binding sites for ethanol on the models of rat and human liver and muscular phosphofructokinases [33]. In the recently published [34] 3D structure of human muscle phosphofructokinase (4OMT) residues predicted by us to bind ethanol molecule follow the structural pattern described in this study. Phe308 and Phe671 are situated in alpha helices, as well as Asp543, while Phe538 is situated in the random coil region.

The position of ethanol molecule in models of liver phosphofructokinase from human and rat is similar, but different from that in muscle enzymes [33]. In addition to Phe538 and Asp543, Val546 participates in ethanol binding (coordinates are given according to the muscle enzyme 3D structure). Val546 residue is situated in the alpha helix.

Ethanol inhibited activity of rat muscle phosphofructokinase isoform (it has become 25% lower) at the concentration of 500 mM, but the activity of rat liver phosphofructokinase isoform has been inhibited (it has become 42% lower) at the concentration of 100 mM [33]. Both concentrations are high. However, the concentration of 100 mM can be established after the fast intake of a high dose of alcohol. Probably, the binding with Ala542 (in muscle isoform) and Ser542 (in liver isoform) is not favorable (see Figs. 1 and 3). According to the results from Fig. 3, alanine is much more underrepresented residue in ethanol binding sites than serine. Interestingly, the decomposed free energy of binding for Ala542 and ethanol was positive according to the docking results [33]. That is how one can use the data from the current study to enhance the performance of molecular docking.

Another important issue from the current study is in the fact that ethanol molecules can cause local changes of secondary structure mostly because of the formation of hydrogen bonds with oxygen and nitrogen atoms from the main chain of a polypeptide. If the binding occurs in regions of a protein that are not involved in catalytic activity or the binding of other substrates, such small structural changes as the disappearance of 3/10 helix or the elongation of a beta hairpin will not cause any significant changes in the function of a protein. However, if a length of an alpha helix or 3/10 helix decreases in an active site of an enzyme, or if the length of a beta hairpin becomes longer in such functionally important region, the consequences may be unpredictable Structural changes can be explained by local dehydration, delocalization of electron density, altered flexibility, resulting in the modification of hydrogen bonds pattern [1,31]. In this way ethanol may influence the structure (and the function) of certain transcription factors, receptors and enzymes providing alternative explanations for many ethanol-caused phenomena like the modulation of K^+ channels activity [35] or sedation after the ethanol intake [36].

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jmgm.2017.10.017.

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