Hydrogen Sulfide Concentration in the Milieu of the Hydrated Alanine Dipeptide Determines its Polyproline II-Beta Propensity: Main Chain Contribution to the Energetic Origin of the Formation of Amyloid

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ABSTRACT

In view of the observation that the concentration of hydrogen sulfide in brains with Alzheimer's disease (AD) is lower than that in normal brains and in line with our previous studies indicating that additional content in the aqueous environment (milieu) of a peptide can change its local energetic preference from a polyproline II (P) to a β conformation (and therefore its tendency to form the β-chain structures that lead to the amyloid plaques associated with the disease), we have studied the effect of H_2S concentration on such propensity in a simple model peptide, the alanine dipeptide (ADP). The two concentration states are represented by $ADP(H_2O)_{18}(H_2S)$ and $ADP(H_2O)_{18}(H_2S)_2$. Ab initio calculations of these structures show that the lowest energy of the former is a β conformation while that of the latter is a P, mirroring the observed AD results and strengthening our proposal that amyloid diseases are better viewed in the context of a protein milieu-folding paradigm.

INTRODUCTION

Although inhalant hydrogen sulfide (H_2S) has long been known for its high toxicity, it has recently become evident that the H_2S produced in the brain plays a significant functional role as a neuromodulator.[1] While the mechanisms in this role are still being investigated,[1] it is currently known that the concentration of H_2S in the human brain has an important effect: low levels are associated with a high incidence of Alzheimer's disease (AD).[2] This correlation is strengthened by the determination that increasing the concentration of H_2S in the brains of transgenic AD mice is associated with a reduction in the size of the amyloid plaques

associated with their symptoms of AD.[3] These plaques are formed from *ca*40-residue hydrogen-bonded β AD-peptide (Aβ) chains, produced by enzymatic cleavage of a large precursor protein, that would normally be in an unordered (and thus soluble) functional state.[4] It is natural to expect that the molecular origin of this structural change in the peptide would be sought, and from a Raman spectroscopic study of amyloids of hen egg white lysozyme it was concluded that the formation of trisulfide bridges by H_2S is the origin of the effect.[5]

While not questioning this correlation, we start from a different perspective in establishing the source of the underlying H_2S connection. This derives from our recent studies,[6,7] in which we showed that the addition of other species (in these cases an acid molecule, HCl) to the environment (milieu) of a hydrated peptide can invert the relative energies (the propensity) of the two local peptide conformations that are relevant to the formation of plaques: the (3-fold left-handed helical) polyproline II (P), which we demonstrated[8,9] and has been substantiated,[10] is the basic local conformation in the aqueous unordered state and the (extended) β. Hydrogen bonding of the latter to other such Aβ peptides can account for the plaque formation in AD. It is therefore appropriate to determine if this propensity

in peptides is sensitive to the concentration of H_2S added to the aqueous milieu. In this study we do so through ab initio calculations of relevant hydrated alanine dipeptides (ADP).

CALCULATIONS

The possible sensitivity of the P-β propensity of the hydrated ADP molecule, $CH_3-CONH-CH(CH_3)-CONH-CH_3$, to the concentration of environmental H_2S was determined by the following protocol: 1) An ADP molecule was surrounded by 18 water molecules, 6 placed in hydrogen-bonded positions at the CO and NH groups and the other 12 distributed in various likely bonding positions to these waters. 2) Starting with canonical values of φ,ψ (P, $-75°,145°$ and β, $-134°$, $145°$) these preliminary structures were ab initio minimized, resulting in φ,ψ of P, $-84°,147°$ and β, $-154°,146°$ and of course the finding that the energy of P is lower than that of β (by 2.44 kcal/mol). 3) The low-concentration case was modelled by the interaction of a single H_2S molecule with this system, and the sampling of the P and β energy regions was done by placing this molecule in a variety of initial positions (a total of some 20 on the surfaces of, within the water

interior of the ADP$(H_2O)_{18}$, and hydrogen bonding to the peptide) and, starting with the above φ,ψ, minimizing each structure. 4) The high-concentration case was assumed to be represented by the interaction of two H_2S molecules with the hydrated ADP, and with a similar set of starting positions the new energy minimizations were obtained. These sets of results provided the lowest and associated higher-energy minima in each of the P and β energy basins (which, obviously, are not smooth). For descriptive purposes we designate the global energy minimum for each H_2S concentration as zero and present those of the higher energies that were less than about 7 kcal/mol.

Calculations were done with Gaussian 09,[11] with the dispersion-corrected wB97X-D functional[12] and the 6-31++G** basis set. A polarized continuum model (PCM) reaction field was included at the start to ensure that polarization effects from a surrounding medium, particularly relevant for the H_2S molecules, were incorporated during the energy minimization. Atomic charges were determined as natural population analysis (NPA) charges. The P-β propensity is determined by the relative energies of the lowest minima in the P and β regions of the energy landscape.

RESULTS AND DISCUSSION

In Table 1 we present calculated energies in the two conformational basins and the charges on the peptides in the respective lowest minimum in each. It is clear that the β conformation prevails in the single H_2S form whereas the P conformation prevails in the double H_2S state. In the light of our previous studies[6,7] it is not surprising that in the first case this milieu (as well as the complex environment in the brain) would enhance the prevalence of the local β conformation, thus providing our previously demonstrated energetic basis for forming the hydrogen-bonded structures that result in amyloid plaques.[7] What is perhaps unexpected is that the P propensity in the second case (and in the comparably higher H_2S concentration in the normal brain environment) can result just from the addition of another H_2S molecule interacting with this ADP system, returning it to the individual unordered, and therefore soluble, chain structures that prevail for these peptides in the normal brain. It is also of interest that the change in peptide charge in the H_2S case from a P minimum (–27 me) to a β minimum (12 me) is in the same positive direction but is very much larger than the similar positive change, from 28 me to 36 me, in the case of a hydrated alanine tripeptide with an added

HCl in the milieu.[7] This reflects the close relationship between charge transfer and the hydrogen-bond energetics of the water structures interacting with the peptide.[13] Detailed examination of the molecular structures of the two conformations in each of the H_2S states indicates that the propensity difference resides in the different peptide-water organizations that result from the nearby H_2S-water interactions (including those originating from initial H_2S-peptide hydrogen-bonded placements) that are analogous to those associated with the solubilization of H_2S in water.[14]

While our present analysis encompasses the basic molecular energetic interactions characteristic of the conformations of hydrated peptide chains, its application to the study of specific properties of real peptide systems must depend on the ability to understand the behavior of longer chains and of other side chains. Such information cannot come from the current computationally limited ab initio approach used in this study and will have to involve extensions to free energy results and to the use of molecular dynamics simulations. However, the latter still cannot be depended on to include in their energy functions all of the physics that is inherent in the ab initio approach. As we pointed out,[7] even using simplified approximations, such as the rigid waters that are often assumed, can lead to gross errors. Repeating our present computations with rigid water constraints, while they

maintain the structure and P propensity for the $2H_2S$ case they invert the propensity

in the single H_2S case, favoring P over β by 4.64 kcal/mol and with a peptide

charge of -17me (compared to 12me for the flexible water). Clearly, all of the

relevant properties of hydrogen-bond interactions that are necessary for

determining energies and charge transfers must be incorporated in such simulations

in order to accurately predict the subtle structural preferences in such molecular

systems[15] (i.e., "to get the physics right"[16]). Despite this absence it should be

expected that the basic physical properties revealed here for a simple peptide

system are also operational in more general peptide structures.

CONCLUSIONS

Our demonstration that a low H_2S concentration in the hydrated environment of a

peptide can favor a local β over a P chain conformation whereas a high

concentration favors P over β has more general implications. The number of

amyloid diseases, neurodegenerative and not, is very large (a recent review

describes about 70[17]) and although the initial bonding leads to insoluble fibrils[17]

that can then aggregate in many polymorphic forms[18] (such as the amyloid plaques

of AD) it is now evident that the basic source of this outcome is the physical drive

from the environment for the peptide to adopt a specific (i.e. β) local conformation. Since the peptide is thus undergoing a natural response to its environment, we prefer to characterize these ailments as "protein milieu-folding diseases."[7] (Such results can also occur in nominally non-disease situations, as evidenced in the finding of amyloid in human cataracts.[19])

Adopting the milieu-folding paradigm also points to potentially useful research directions in preventing amyloid illnesses. This is in the spirit that rather than efforts to destroy the plaques after they have been formed it makes more sense to prevent them from forming in the first place. In the present case an obvious path would be to investigate the causes that determine an onset of the H_2S depletion in the normal brain that we now know is associated with the development of AD (genetic? lifestyle? environmental? etc.).

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CONFLICTS OF INTEREST

The authors have no conflicts of interests.

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Table 1. Relative Energies of Minimized P and β Conformations of $ADP(H_2O)_{18}(H_2S)$ and $ADP(H_2O)_{18}(H_2S)_2$

P			β		
φ,ψ	ΔE[a]	q(pep)[b]	φ,ψ	ΔE[a]	q(pep)[b]
$ADP(H_2O)_{18}(H_2S)$					
-103°, 143°	2.51	0.1	-162°, 144°	0	12
-77°, 156°	3.13		-164°, 133°	1.07	
-100°, 110°	6.05		-151°, 145°	5.75	
$ADP(H_2O)_{18}(H_2S)_2$					
-86°, 128°	0	-27	-143°, 140°	4.22	11
-89°, 138°	6.92		-128°, 141°	7.06	

[a] Energy in kcal/mol above minimum. [b]Charge in millielectrons (me).
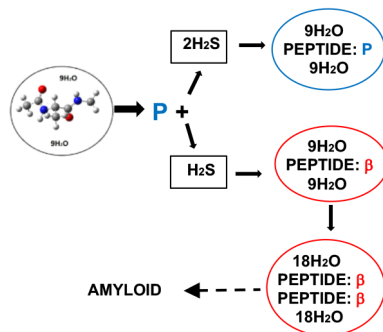
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