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Enhancement of intrinsic fluorescence of human carbonic anhydrase II upon topiramate binding: Some evidence for drug-induced molecular contraction of the protein

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ABSTRACT

In this report, the effect of topiramate (TPM), an anticonvulsant sulfamate drug, on the structure of human carbonic anhydrase II (hCA II) was investigated by spectroscopic techniques. The intrinsic fluorescence experiments indicated that TPM binding causes enhancement of enzyme fluorescence via decreasing the internal quenching and energy transfer efficiency, the result supported by molecular dynamics simulation. Thermodynamic analysis of the binding process suggested that hydrogen bonding and van der Waals interactions are the major forces in the interaction of TPM with hCA II. The far-UV circular dichroism (CD) results showed that TPM caused increment in α-helical and β-sheet content of hCA II whereas, near-UV CD experiments in the presence of the drug showed induction of some compactness in the enzyme tertiary structure. The number of accessible tryptophans and protein surface hydrophobicity index of the enzyme were reduced in the presence of TPM which confirms the enzyme structural compactness upon drug binding. In addition, the enzyme thermal stability was increased in the presence of the drug. It seems that the induction of compactness in the enzyme structure upon drug binding may be responsible for increment of its conformational stability.

1. Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) are ubiquitous zinc containing metalloenzymes which catalyze the interconversion of CO_2 and bicarbonate [1,2]. These enzymes are involved in vital physiological processes associated with respiration and transport of CO_2/bicarbonate, pH homeostasis, electrolyte secretion, some biosynthetic reactions, bone resorption, renal acidification, tumorigenicity, production of cerebrospinal fluid and gastric acid, and possibly cell growth and proliferation [2,3]. Carbonic anhydrases are encoded by six distinct and evolutionarily unrelated gene families including α-, β-, γ-, δ-, ζ- and η-CAs [4]. The α-class possesses at least 16 known isoforms or CA-related proteins in higher vertebrates such as humans. Each isoform differs in relative catalytic activity, tissue distribution and subcellular localization, and susceptibility to inhibition [2,3]. The most intensive studied of the α-CA isozymes, human CA II (hCA II), is a single polypeptide chain containing 259 amino acid residues with a molecular weight of ~29 kDa [5]. This enzyme is known as a cytoplasmic isozyme and has a high catalytic activity (k_{cat} greater than 10^6 s^{-1}) under physiological conditions and very high affinity for sulfonamides [3,6]. It contains one zinc ion in the active site coordinated with three histidine residues (His-94, His-96 and His-119) and a water molecule or OH^- in tetrahedral geometry [7]. CA II appears to be almost universally expressed in some cell types of all major mammalian tissues, such as erythrocytes, kidney tubules, the digestive system, eye and central nervous system [6]. Most of the CAs can be inhibited by aromatic and heterocyclic sulfonamides (R-C-SO_2NH_2), specific and strong inhibitors of CAs, which form the basis for many therapeutic drugs [3]. Variations on the sulfonamide structure may also yield carbonic anhydrase inhibitors (CAIs), such as sulfamates (R-OSO_2-NH_2), hydroxysulfonamides (R-SO_2NH(OH)) and hydroxamates (R-CO-NH-OH) [6]. CAIs are clinically used agents

Abbreviations: ANS, 1-anilinonaphthalene-8-sulfonate; CA, carbonic anhydrase; CD, circular dichroism; hCA II, human carbonic anhydrase II; NBS, N-bromosuccinimde; PSH, protein surface hydrophobicity; TPM, topiramate.

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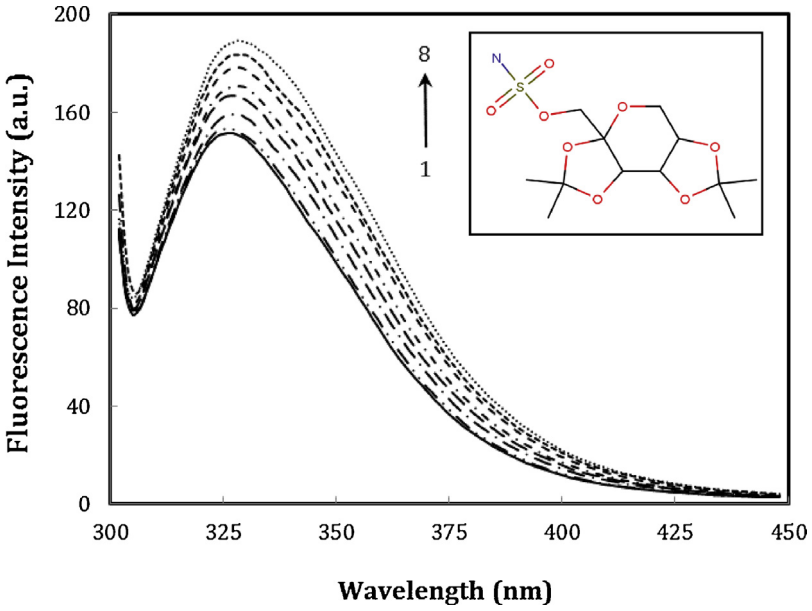

Fig. 1. The intrinsic fluorescence spectra of hCA II (0.68 μM) in the absence (1) and presence of 5 (2), 50 (3), 100 (4), 150 (5), 300 (6), 450 (7) and 600 (8) μM TPM (1 → 8) in 50 mM Tris–sulfate, pH 7.75. The enzyme was excited at 295 nm. For more details please see Section 2.3.1. Inset: the chemical structure of TPM.

for treatment of a variety of disorders associated with CAs such as glaucoma, increased intracranial pressure, epilepsy, genetic hemiplegic migraine, essential tremor, Parkinson's disease, edema due to congestive heart failure or drug-induced edema, in addition to their applications as antiobesity, diuretics, anti-infective and antitumor agents [3,8,9]. Topiramate (TPM), designated chemically as 2,3:4,5-di-*O*-isopropylidene-β-*D*-fructopyranose sulfamate (inset of Fig. 1), is particularly interesting from a structural point of view because it is derived from a monosaccharide and bears a sulfamate functional group which is considered to be responsible for its medicinal properties [2]. TPM is an antiepileptic drug with a well-established efficacy as monotherapy or adjunctive therapy agent in the treatment of epilepsy in adults and children [10]. Its potent anticonvulsant effects is due to multiple and complex mechanisms of action. Pharmacological actions of TPM are diverse and include: (i) reduction of epileptiform discharges through a voltage-dependent block of Na^+ channels, (ii) enhancement of the activity of γ-aminobutyric acid (GABA)-ergic transmission at some subtypes of GABA receptors, (iii) antagonism of non-*N*-methyl-*D*-aspartate (NMDA) glutamate receptors and (iv) inhibition of CAs [2,6]. The crystal structure of hCA II–TPM adduct has been reported in details [11], but since the crystal structure of proteins may have subtle differences with their dynamic structure in the solution (as their native environment) [12,13], we attempted to further characterize the TPM binding to hCA II using various biophysical methods, in combination with a molecular dynamics (MD) simulation technique. The UV–vis spectroscopy, intrinsic and extrinsic fluorimetry and circular dichroism (CD) techniques were used for explaining the mode of binding, determination of binding constant and probable structural alterations of hCA II upon the drug binding. Furthermore, MD simulation study was used for justification and rationalization of some of the spectroscopic data.

2. Materials and methods

2.1. Chemicals

Pure TPM was obtained as a generous gift from Tehran Darou Co. (Tehran, Iran). 1-Anilino-naphthalene-8-sulfonate (ANS) and *N*-bromosuccinimide (NBS) were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). All other reagents (analytical grade) were obtained from Merck (Darmstadt, Germany). Double distilled water was used for preparation of solutions. All of the experiments were performed in 50 mM Tris–sulfate, pH 7.75 as the buffer, except CD experiments which were carried out in 50 mM Tris–HCl, pH 7.75 in order to decrease the amount of noise on CD spectrum. Reproducibility of the data was confirmed by performing the experiments in triplicate.

2.2. Carbonic anhydrase purification

Human CA II was purified from human erythrocytes according to the method described by Lindskog [14] except the final zone electrophoresis step which was replaced by chromatography on DEAE-Sepharose. The enzyme purity was assessed by SDS–PAGE and estimated by densitometry using PROCESS-24 (Helena, France). The protein concentration was determined by the method of Lowry et al. [15].

2.3. Fluorescence measurements

2.3.1. Intrinsic fluorescence measurements

Fluorescence emission spectra of the enzyme (0.68 μM) in the absence and the presence of different concentrations of TPM (5, 50, 100, 150, 300, 450 and 600 μM) were recorded on a Cary Eclipse (Varian, Australia) spectrofluorimeter equipped with a jacketed cell holder in which temperature was controlled by an external thermostated water circulation. The fluorimetry experiments were carried out in a 1.0 cm quartz cell. The excitation wavelength was set at 295 nm, to avoid the contribution from tyrosine residues [16], and the emission spectra were recorded at 300–450 nm. The excitation and emission slit widths were 5 and 10 nm, respectively.

2.3.2. Mechanism of fluorescence enhancement

To elucidate the mechanism of fluorescence enhancement of hCA II by TPM, the fluorescence experiments were carried out at 293, 298, 303 and 308 K where hCA II does not undergo any considerable thermal denaturation. Analogous to the Stern-Volmer