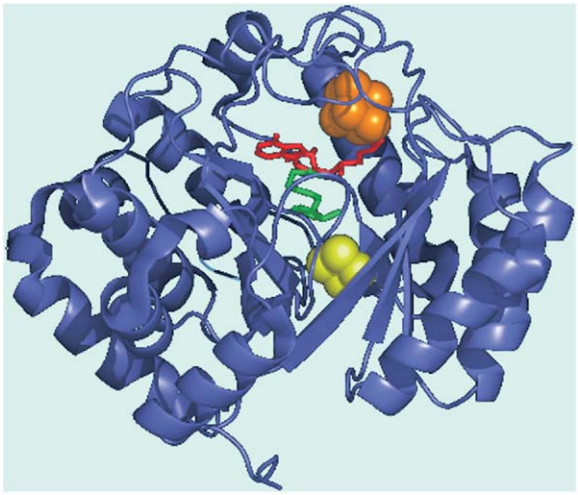

Figure 1. Ribbon Depiction of the Biotin Synthase Monomer

The protein backbone forms an $(\alpha\beta)_8$ barrel around the $[4Fe\text{-}4S]^{2+}$ cluster (orange), *S*-adenosylmethionine (red), DTB (green), and the $[2Fe\text{-}2S]^{2+}$ cluster (yellow) [8]. The C terminus is disordered and extends 31 residues from bottom center.

workers noted that the in vitro reconstituted $[2Fe\text{-}2S]^{2+}$ cluster is not vibrationally equivalent to the native cluster [12], suggesting minor differences in the surrounding protein fold or environment that could explain why in vitro cluster reconstitution does not correlate with activity. In vivo cluster assembly is likely carried out through the concerted action of several proteins from the iron-sulfur cluster (ISC) assembly system [13]. In the persulfide insertion mechanism, the inability to sustain multiple turnovers of biotin production is explained as due to severe product inhibition by biotin [10]. Under the conditions employed by Choi-Rhee and Cronan, this inhibition is presumably relieved by coupling of biotin to His_6-`AccB as catalyzed by BirA, and turnover would be limited only by the rate of biotin dissociation. It should be noted that biotin inhibition has not been observed by other groups working with biotin synthase.

Regardless of the mechanism, turnover of biotin synthase is clearly accompanied by increased degradation of the protein. This could be due to partial unfolding of the protein in the absence of the $[2Fe\text{-}2S]^{2+}$ cluster. A unique and unexpected feature of biotin synthase is the incorporation of an arginine guanidino group, as well as three cysteine thiolates, as ligands to this cluster [8]. In the absence of metal coordination, the arginine would likely become protonated and be repelled from the hydrophobic interior of the protein, providing a plausible role for this conserved residue in sensing the presence of the cluster. Unfolding of the protein may be an evolved feature that facilitates repair of the otherwise deeply buried $[2Fe\text{-}2S]^{2+}$ cluster. In the absence of efficient cluster repair, BioB degradation may facilitate more rapid downregulation of biotin production after only a few turnovers, a feature that could conserve metabolic energy and promote stationary-phase survival under nutrient-deprived conditions.

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Tissue Transglutaminase Inhibition

Khosla and coworkers report the synthesis of peptidic dihydroisoxazole derivatives, the in vitro evaluation of these novel compounds as inhibitors of recombinant human tissue transglutaminase (TG2), and their oral bioavailability and efficacy for the synergistic treatment of glioblastoma tumors [1].

In this issue, Chaitan Khosla and his coworkers report the synthesis of novel peptidic dihydroisoxazole derivatives, the results of their in vitro evaluation as inhibitors of human tissue transglutaminase (TG2), and the demonstration of their bioavailability and efficacy in an animal model [1]. Their inspiring results derive from an im-

pressively broad multidisciplinary research effort, as the title of the article suggests. To fully appreciate the extensive impact of this outstanding work, the context of three different aspects of the article merit particular attention: (1) the peptidic structure of their inhibitors, (2) the current lack of direct structural information that would facilitate the structure-based design of TG2 inhibitors, and (3) the clear implication of TG2 in diverse physiological disorders.

The novel inhibitors highlighted in Khosla's article feature a simple peptidic scaffold bearing a halo-dihydroisoxazole group. This particular pharmacophore has proven effective for the inhibition of a number of enzymes whose active sites resemble those of the cysteine proteases, including TG2 [2]. Likewise, the peptidic framework of Khosla's current series of dihydroisoxazole (DHI) inhibitors is also noteworthy. The importance of a Cbz protecting group for conferring affinity to TG2 substrates was demonstrated 40 years ago by Folk and Cole [3]. Around 20 years later, Krantz also published results suggesting that in a series of varied peptides bearing DHI groups, only Cbz-Phe- and Cbz-Tyr-based scaffolds were reactive as inhibitors [2]. Khosla's current work provides more detail, confirming that aromatic amino acids bearing certain aromatic protecting groups are simple, synthetically accessible scaffolds whose derivatives are among the few small molecules having submillimolar affinity constants for TG2. By way of comparison, previous work [4–6] featuring the use of Cbz-X scaffolds have shown similar affinity, where X is a glutamine analog bearing the inhibitory functional group. More recent work featuring nonpeptidic heterocyclic compounds further supports the hypothesis that an extended aromatic framework is important for TG2 affinity [7]. Moreover, certain peptides based on native protein sequences have been shown to have high affinity for TG2. These include PQPQLPY, identified by Khosla from the wheat protein gliadin [8], KVLDGQDP, deriving from the pro-elafin peptide [9], and PKPQQFM, from the Substance P protein [10], where the underlined glutamine residue indicates TG2 chemoselectivity.

Are there similarities between these molecules that can be accounted for by a common binding model with TG2? Certainly the hydrophobic nature of the residues flanking the active site-reactive functional group is immediately obvious in all of the most potent TG2 inhibitors reported to date. Cursory inspection of the proline-rich sequences of the peptide substrates further indicates that an extended hydrophobic rod-like conformation may be important for TG2 affinity. This speculative conformation and hydrophobic character would certainly be complementary to the shallow groove on the surface of TG2 that has been identified as the peptide-bound glutamine binding site [11, 12]. Unfortunately, a more comprehensive structural analysis allowing the rational design of high-affinity inhibitors is currently hindered by the lack of a single crystal structure of *active* TG2 from any source. For example, the structure of human TG2 has been published, but in its inactive form, with a bound GDP ligand [13]. The structure of the analogous red sea bream TG2 has also been solved, but in the absence of the Ca^{2+} ligand necessary for its activity [11]. Modeling studies based on the latter structure have shown that in its inactive form, the active site of TG2 is inaccessible to substrate and must be manipulated in silico to permit subsequent docking experiments [12]. Clearly, if we are to glean a more sophisticated level of understanding of the nature of the interactions between TG2 and effective inhibitors such as Khosla's, information deriving from crystallographic studies of human TG2 with bound active site inhibitors (or inactive TG2 mutants with bound substrate) is absolutely vital.

The urgency of this requirement is underlined by the critical role that TG2 plays in serious physiological disorders. Granted, the implication of TG2, a widespread enzyme, in a given physiological disorder based solely on its in vitro activity toward a particular native protein as a substrate is preliminary at best, given the enzyme's broad specificity with respect to the sequence of amino acids flanking the peptide bound glutamine and lysine residues that serve as acyl donor and acceptor substrates. However, many researchers, like Khosla, have recently undertaken the collaborative and multidisciplinary studies necessary to move beyond preliminary implication, to convincingly confirm the physiological benefit of TG2 inhibition in the context of specific disorders. Among others, one of these disorders is Huntington's disease, a neurodegenerative disease where TG2 appears to catalyze the transamidation of poly-Gln sequences of certain neuropeptides, leading to their aggregation [14, 15]. Another example is Celiac Sprue, an intestinal inflammatory disorder shown by Khosla and coworkers [8, 16] to involve the TG2-mediated deamidation of a glutamine residue to a glutamate residue, within a small peptide resulting from the partial proteolytic digestion of a gluten protein. The resulting peptide, now containing a glutamate residue, initiates in turn a T cell-mediated inflammatory response. Finally, previous work has implicated extracellular TG2 in mediating cellular adhesion and migratory processes, particularly relevant in the metastasis of tumor cells [17]. Herein, Khosla and his coworkers additionally demonstrate that glioblastoma cells can be rendered chemosensitive upon inhibition of TG2. Furthermore, the preparation of a fluorescent derivative of their irreversible inhibitor allowed them to localize this inhibitory activity on a cellular level.

In summary, Khosla and his coworkers have prepared a series of small-molecule, irreversible inhibitors of TG2, based on the structure of previous peptidic compounds having known in vitro inhibitory activity and without the benefit of an appropriate structural model of TG2. Evaluation of the in vitro activity of these novel inhibitors was followed-up by an evaluation of specific in vivo activity and the miscroscopic localization of a fluorescent derivative. In this way, the authors provide direct information regarding the physiological role of TG2 in Celiac Sprue, demonstrate the therapeutic validity of the use of TG2 inhibitors for the treatment of glioblastomas, and provide a diagnostic tool for evaluation of other TG2-dependent disease models. This thorough collaborative effort demonstrates visibly how multidisciplinary work at the interface of chemistry and biology can contribute so distinctively and significantly to both fields.

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