Spectroscopic Studies of a Transcriptional Activation Peptide

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The control of eukaryotic gene expression is crucial in a host of important processes including development and differentiation. In most cases, regulation is at the level of transcriptional initiation[1] and involves stimulation of RNA polymerase II activity by transcriptional activation proteins.[2] These species bind to specific DNA sequences, known as enhancers or upstream activation sequences, that can be located hundreds or thousands of base pairs from the promoter. The current model suggests that activation proteins contact the basal transcription complex through a discrete activation domain[3] and stimulate its assembly.[4] This interaction, which may be direct or facilitated by an adaptor protein,[3a,5] is thought to involve "looping out" of the DNA between the enhancer and the promoter.[6]

A major family of activation domains contains an abundance of acidic residues. On the basis of the lack of sequence homology between acidic activation domains (AADs) from various proteins (other than the acidic residues) and the observation that progressive deletions result in a stepwise loss of activity,[3b] it has been proposed that AADs have no well-defined structure and recognize some highly positively charged basal transcription factor via purely ionic interactions[7] (Figure 1). Alternatively, Ptashne and coworkers have suggested that AADs form amphipathic α-helices and that this structure is important in protein-protein interactions involving AADs.[8a] These hypotheses have been tested in vivo with mixed results.[8] However, the structures of AADs have never been investigated directly by spectroscopic methods.[8e] This report describes the first such study using a 19 amino acid peptide corresponding to residues 107-125 of the yeast GCN4 transcriptional activation protein.[3b] Webster et al. have shown that this peptide retains some transcriptional activation activity in vivo when fused to a heterologous, sequence-specific, DNA-binding domain,[9] demonstrating that this small fragment of the GCN4 protein can act *independently*. Therefore, in vitro studies of its solution structure are relevant to the mechanism of transcriptional activation in vivo and constitute a necessary first step toward understanding the specific protein-protein interactions that mediate gene expression.

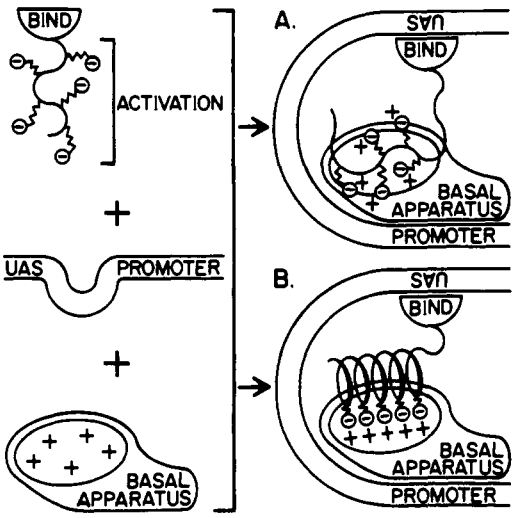

Figure 1. Two popular models for how eukaryotic transcriptional activators function in vivo. Model A suggests that the acidic activation domain makes ionic interactions with the basal transcription apparatus that do not require adoption of a specific secondary structure by the activation domain.[7] Model B suggests that the activation domain is an amphipathic α-helix.[8a] In both models, the interactions serve to increase the stability of the transcription complex. The experiments reported in this paper are relevant only to the structure of the activation domain in the free state.

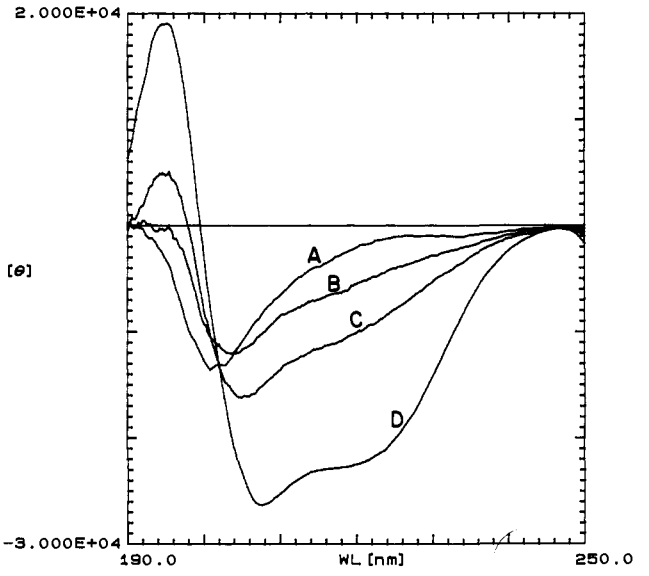

Figure 2. Circular dichroism spectra of GCN4(107-125) in various solvents. Spectrum A was recorded in a buffered aqueous solution.[11] Spectra B and C were recorded in the presence of 30% TFE and 75% TFE, respectively. Except for the alcohol, the three samples were identical. Also included for comparison is the spectrum of melittin in buffered aqueous solution (D). This peptide has been shown to contain some amphipathic α-helical secondary structure.[18] The fact that melittin is helical in our buffer demonstrates that the lack of structure in GCN4-(107-125) is not due to a poor choice of conditions.

Circular dichroism (CD) analysis of GCN4(107-125)[10] (NH_2-MFEYENLEDNSKEWTSLFD-$CONH_2$), under roughly physiological conditions,[11] failed to show a significant amount of

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(4) Ptashne, M. *Nature* **1986**, *322*, 697-701.

(5) (a) Flanagan, P. M.; Kelleher, R. J., III; Sayre, M. H.; Tschochner, H.; Kornberg, R. D. *Nature* **1991**, *350*, 436-438. (b) Kelleher, R. J., III; Flanagan, P. M.; Kornberg, R. D. *Cell* **1990**, *61*, 1209-1215.

(6) Ptashne, M. *Nature* **1988**, *335*, 683-689.

(7) Sigler, P. B. *Nature* **1988**, *333*, 210-212.

(8) (a) Giniger, E.; Ptashne, M. *Nature* **1987**, *330*, 670-672. (b) Ma, J.; Ptashne, M. *Cell* **1987**, *51*, 113-119. (c) Ruden, D. M.; Ma, J.; Li, Y.; Wood, K.; Ptashne, M. *Nature* **1991**, *350*, 250-252. (d) Cress, W. D.; Triezenberg, S. J. *Science* **1991**, *251*, 87-90. (e) Loret, E. P.; Vives, E.; Ho, P. S.; Rochat, H.; Van Rietschoten, J.; Johnson, W. C. *Biochemistry* **1991**, *30*, 6013-6023. Loret et al. do report a CD study of peptides corresponding to regions of the HIV Tat protein, a transactivator. However, these workers were attempting to localize the activation domain by determining the degree of α-helicity exhibited by a particular peptide. Given the results reported here, this may not be a reasonable criterion. The domain responsible for activation in this protein has not been mapped precisely by biochemical methods.

(9) Webster, N.; Jin, J. R.; Green, S.; Hollis, M.; Chambon, P. *Cell* **1988**, *52*, 169-178.

(10) GCN4(107-125) corresponds to amino acids 107-125 of the yeast GCN4 protein. It was synthesized at Multiple Peptide Systems (La Jolla, CA) using Fmoc chemistry and deprotected with trifluoracetic acid. The C-terminus of the peptide is a primary amide. The peptide was purified by reverse-phase HPLC on a Vydac C_{18} column. Amino acid sequencing confirmed the identity of GCN4(107-125). Peptide concentrations were measured by UV spectroscopy using an extinction coefficient of 6970 $(M\ cm)^{-1}$ (see: Edelhoch, H. *Biochemistry* **1967**, *6*, 1948-1954).

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secondary structure, as evidenced by the single minimum at approximately 200 nm, indicative of a random coil (Figure 2, spectrum A). Reduction of the sample temperature to 4 °C did not change the spectrum.

We also employed a two-dimensional (2D) ROESY experiment[12a] to address the question of structure in GCN4(107-125). Under conditions similar to those employed for the CD studies, interresidue ROEs were not observed. If a significant fraction of the population was α-helical, strong ROEs should have been observed between the α and β protons of the i and $i + 3$ residues.[12b] In addition, the optical spectrum of GCN4(107-125)[13] was identical under native and denaturing conditions (data not shown), indicating that the native conformation does not place the aromatic residues in a solvent-restricted environment. Thus, CD, UV, and NMR experiments all demonstrate that GCN4(107-125) does not adopt a stable secondary structure in aqueous solution at neutral pH.

Can secondary structure be induced in GCN4(107-125)? Figure 2 shows the results of adding increasing amounts of trifluoroethanol (TFE).[14] Although changes in the CD spectrum are apparent, they are not consistent with a large increase in α-helicity. The spectrum recorded in 75% TFE (C in Figure 2) suggests at most 20% α-helix content.[15] Other additives were also tested: monovalent salts (100 mM KCl), divalent salts (100 mM $MgCl_2$), and poly-L-lysine (a crude model for the putative positively charged protein GCN4 contacts in vivo). All failed to induce significant secondary structure as judged by CD (data not shown).

In summary, GCN4(107-125) does not adopt a stable secondary structure in solution nor does it have much helix-forming potential. However, our findings do not preclude the possibility that this peptide adopts a specific conformation in the presence of its in vivo target protein. Many peptides of this size or smaller exhibit significant secondary structure,[16] so the lack of structure in GCN4(107-125) is not simply due to its modest size. We believe these results are biologically relevant since a fusion protein containing GCN4(107-125) and no other GCN4 residues does activate transcription.[9] Nonetheless, it is important to note that GCN4(107-125) is a weak activator compared to the intact GCN4 protein and represents only a fragment of the true activation domain.[3b] This modest activation potential is typical of a variety of short acidic peptides that have been examined in vivo.[8a,b,17] It may be that they are weak activators precisely because they lack structural elements found in potent AADs. This view is supported by our preliminary studies with GCN4(107-144), a peptide that corresponds more closely to the full activation domain and is a much more potent activator in vivo. The CD spectrum of GCN4(107-144) suggests a significant amount of structure, but mostly β-sheet rather than α-helix (M.V.H. and T.K., unpublished results). Detailed structural studies of GCN4(107-144) are underway.

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(11) Each sample contained 0.8 mM GCN4(107-125) and 20 mM $NaPO_4$ (pH 7.6). Samples were scanned 10 times in a J600 spectropolarimeter using a 0.01 cm path length cell at 23 °C. Each spectrum was corrected for background by subtraction of an identical sample lacking peptide.

(12) (a) GCN4(107-125) (2 mM), 5 mM $NaPO_4$ (pD 7.6). All of the protons were assigned by a standard strategy[12b] involving a double-quantum phase-sensitive COSY experiment in D_2O in conjunction with ROESY and double-quantum phase-sensitive COSY experiments in DMSO-d_6. A ROESY experiment was employed because the 2D NOESY spectrum did not reveal any cross peaks. (b) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986.

(13) The sample contained 0.042 mM GCN4(107-125) and 24 mM $NaPO_4$ (pH 7.6) for the native study and was scanned from 400 nm to 240 nm in a 1.0 cm path length cell at 23 °C. Samples for denaturing studies were identical to that for the native study except that they contained up to 8.0 M urea, 5.7 M guanidinium hydrochloride, or 0.75 M sodium dodecyl sulfate.

(14) Lehrman, S. R.; Tuls, J. L.; Lund, M. *Biochemistry* **1990**, *29*, 5590-5596.

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(16) (a) O'Neil, K. T.; DeGrado, W. F. *Science* **1990**, *250*, 646-651. (b) Bradley, E. K.; Thomason, J. F.; Cohen, F. E.; Kosen, P. A.; Kuntz, I. D. *J. Mol. Biol.* **1990**, *215*, 607-622. (c) Lyu, P. C.; Liff, M. I.; Marky, L. A.; Kallenbach, N. R. *Science* **1990**, *250*, 669-673.

(17) Ma, J.; Ptashne, M. *Cell* **1988**, *55*, 443-446.

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A New Photochemical Reaction and Its Mechanism: Rearrangement of Acyl and Imino Cyclopropenes[1]

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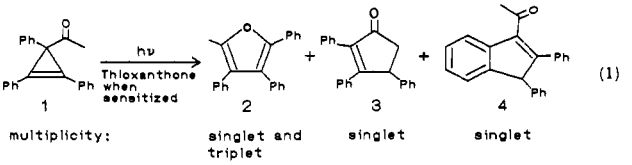
The search for fundamentally new photochemical reactions is arduous and only occasionally successful. However, this has remained one of our main goals. Our previous studies of the photochemistry of vinylcyclopropenes[2] led us to explore the chemistry of acyl- and imino-substituted cyclopropenes, both of which have been postulated[4] to be intermediates in the photochemical isomerizations of furans and pyrroles. We now report (1) a remarkable dependence of the photochemistry of acylcyclopropenes on multiplicity wherein the triplets rearrange smoothly to furans, (2) evidence for a reaction mechanism of the triplet rearrangement of the acylcyclopropenes different from that of the corresponding vinylcyclopropenes, (3) a striking dependence of the photochemistry on the substituent at C-3, (4) the first synthesis of an iminocyclopropene and the general stability of the acyl compounds, and (5) the triplet rearrangement of the iminocyclopropenes to pyrroles.

In our studies[2,3] of the photochemical rearrangements of vinylcyclopropenes to cyclopentadienes, we determined that four mechanisms were possible for the rearrangement and that the triplet excited state utilized an unusual mechanism quite different from the singlet. Hence we were interested in the behavior of the isoconjugate acyl- and imino-substituted cyclopropenes. One example is illustrated in eq 1 for acylcyclopropene **1**.



(1)

In contrast to the direct irradiation which affords a multiplicity of products,[4] thioxanthone (or (*N*,*N*-dimethylamino)benzophenone) sensitization led exclusively to 2,3,4-triphenyl-5-methylfuran (**2**); cf. eq 1. This initial success with the triplet photochemistry of an acylcyclopropene prompted us to attempt a synthesis of the hitherto unknown iminocyclopropene counterparts. The (benzoylimino)cyclopropene **5** was obtained by phenyllithium addition to 3-cyano-1,2,3-triphenylcyclopropene

(1) (a) This is publication No. 162 of our photochemical series. (b) For paper 161 see: Zimmerman, H. E.; Heydinger, J. A. *J. Org. Chem.* **1991**, *56*, 1747-1758.

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(4) (a) Padwa, A.; Akiba, M.; Chou, C. S.; Cohen, L. *J. Org. Chem.* **1982**, *47*, 183-191. (b) We have confirmed this singlet behavior.

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