(18) (a) "Spontaneous" addition of triplet oxygen are known. For a review see D. H. R. Barton, R. K. Haynes, G. Leclerc, P. D. Magnus, and I. D. Menzies, *J. Chem. Soc., Perkin Trans. 1*, 2055 (1975). These authors provide a discussion of mechanisms for oxidation with triplet oxygen which "avoid the spin barrier". (b) Some diradical character may contribute to the actual hybrid structure of **7**.
(19) The same product of oxidation of TME is found when singlet is employed (NMR spectroscopy) and has been identified as the alcohol derived from the expected allylic hydroperoxide.
(20) The ring opening of **9** to **2** has been classified as thermally "forbidden" if a plane of symmetry is preserved during the reaction: J. R. Swenson and R. Hoffmann, *Helv. Chim. Acta*, **53**, 2331 (1970).

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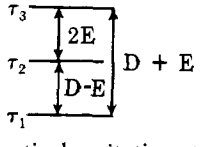
Optical Detection of Magnetic Resonance of Glucagon and Glucagon Peptides in Solution. A Conformational Change Related to Critical Length of the Peptide Chain

Sir:

The x-ray crystal structure of glucagon, a 29 residue[1] adenylate-cyclase-stimulating hormone,[2-4] recently reported by Blundell and co-workers,[5] shows that the polypeptide chain is largely helical. By contrast, circular dichroism studies[6,7] on dilute solutions of glucagon indicate that, near neutral pH, there is 15–20% α-helical structure in the polypeptide. This is consistent with the theoretical predictions (31% α-helix) of Chou and Fasman,[8] made on the basis of a set of empirical rules deduced from examination of a number of protein crystal structure models. We wish to report the first evidence that there is specific nonrandom structure in glucagon, in dilute solid solution at neutral pH, in the region of the single tryptophan residue (Trp-25). Further, our investigation of glucagon peptide *fragments* suggests that a distinct conformational transition occurs in peptide fragments containing Trp-25, which are longer than some minimum critical length.

Glucagon was obtained from Elanco Products and was purified by ion-exchange chromatography.[9] Glucagon peptides were generously donated by Dr. W. W. Bromer of Eli Lilly and Dr. A. Fontana of the Institute for Organic Chemistry, Padova. The polypeptides were dissolved in 1:1 ethylene glycol/water (EGW) buffered with 0.1 M K_xPO_4 at pH 7.4, at final polypeptide concentrations of less than 10^{-5} M. The samples were investigated using optical detection of magnetic resonance (ODMR)[10-12] which provides a sensitive means for determining the zero-field splittings (zfs)[13] in the lowest excited triplet state of phosphorescent molecules. The zero-field splitting parameters which characterize the disposition of the triplet sublevels are defined as shown below.[14] The triplet states



were populated by optical excitation at 297 nm at temperatures around 1.3 K. The zfs parameters and the sublevel decay and spin-lattice relaxation rate constants in tryptophan have been reported by Zuclich et al.[15] and by Rousslang and Kwiram.[16,17] All three of the zero-field transitions of tryptophan were observed in the *N*-acetylamide derivative and the peptides. The D − E and the 2E transitions were readily detected, but the D + E transition could only be detected by a double resonance experiment. Since both the 2E and D − E transitions have the principal radiative level, τ_2, in common, the D + E transition can be observed by saturating either the 2E or D − E transitions, and simultaneously sweeping through the frequency range corresponding to D + E. The same values for D + E were obtained in both cases, providing a check on the accuracy of the 2E and D − E transitions. To ensure the greatest accuracy for extracting the ODMR transition frequencies, microwaves were swept slowly through resonance such that the line shape of the response was unaltered by the lifetimes of the tryptophan triplet sublevels.

Table I. Zero-Field Transition Frequencies, ν (GHz)[a]

Sample	D − E	2E	D + E
Tryptophan	1.82	2.61	4.43
N-Acetyltryptophanamide	1.79	2.62	4.41
Glucagon (22–26)	1.79	2.62	4.41
Glucagon (22–29)	1.79	2.62	4.41
Glucagon (18–29)	1.72	2.68	4.40
Glucagon	1.72	2.68	4.40

[a] Zero-field splittings of samples at 1.3 K, dissolved in pH 7.4 EGW, and excited at 297 nm. The frequency values are usually averages of more than three observations (±0.01 GHz).

The zfs of the hormone, peptides, tryptophan, and *N*-acteyltryptophanamide as measured by ODMR are shown in Table I. There is a small shift in the zfs in going from the zwitterion form of tryptophan to that of *N*-acetyltryptophanamide. However, the latter, because of the presence of amino- and carboxy-terminal peptide groups, is a more suitable model for tryptophan incorporated in a polypeptide chain. The ODMR results demonstrate that the zfs of tryptophan in glucagon are *significantly different* than those of *N*-acetyltryptophanamide alone.

Moreover, this change in the tryptophan zero-field transition frequencies takes place as one goes from the eight residue fragment (22–29) to the 12 residue fragment (18–29). Whereas the zfs of fragments 22–26 and 22–29 are identical with those of *N*-acetyltryptophanamide, fragment 18–29 apparently furnishes an environment for tryptophan equivalent to that found in glucagon.

Two possibilities were considered responsible for the large change in the zfs. A careful examination of residues adjacent to tryptophan in the hormone and peptide fragments suggested that perhaps the sulfur-containing methionine residue (Met-27) could be perturbing Trp-25 through increased spin-orbit interaction with the heavy sulfur atom. However, no shift is observed for fragment 22–29 which contains the methionine residue. Moreover, preliminary calculations suggest that this effect is too small to account for the observed 70-MHz shift in D − E.

An alternate explanation may be found by assuming an altered solvent accessibility of Trp-25 or, in more general terms, a change in the configuration of localized charges. If the 18–29 peptide forms an α-helical structure, clustering of hydrophobic residues and/or binding of the side chain to the helix backbone could alter the environment of the tryptophan. Chou and Fasman predict that residues 19–27 fulfill the necessary requirements for helical folding in glucagon, while residues 28 and 29 are either indifferent or inhibit α-helix formation. Consistent with this prediction and with the general theory[18,19] for random coil-helix transitions in polypeptides, our data suggest that whereas fragments 22–26 and 22–29 are probably not structured, it is possible that fragment 18–29 is folded into an α-helix. Although we have no classical proof that helices per se are involved we believe that it is the formation of secondary structure in the 18–29 peptide and in glucagon that is responsible for the dramatic shift in the zfs of Trp-25. Our

results are also consistent with the observed critical length for acquisition of secondary structure in angiotensin-II,[20] an octapeptide hormone, and agree with critical length estimates for helix formation in γ-ethyl L-glutamate oligopeptides.[21] Further studies are underway to elucidate the origins of the observed shifts, and to obtain quantitative estimates of the magnitudes of the shifts due to local electric fields.

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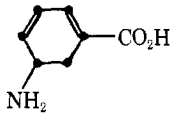
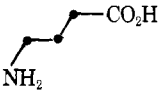
The Irreversible Inhibition of Mouse Brain γ-Aminobutyric Acid (GABA)-α-Ketoglutaric Acid Transaminase by Gabaculine

Sir:

There are a large number of natural products that are specific, irreversible enzyme inhibitors. These molecules are of diverse structural types but are linked by a common mechanism of action.[1] This general mechanism of action requires these molecules, which are chemically unreactive, to be substrates for the target enzyme. In the process of catalytic turnover, the enzyme becomes inactive.[2] That is, the target enzyme catalyzes its own destruction. The fact that these molecules are chemically unreactive before turnover is the key to the specificity of these inhibitors. We would like to report here a further example of a naturally occurring irreversible inhibitor of this type. Specifically, we show that gabaculine (5-amino-1,3-cyclohexadienylcarboxylic acid), a natural product isolated from *Streptomyces toyocaensis*,[3] is a potent irreversible inhibitor of mouse brain, pyridoxal phosphate linked γ-aminobutyric acid (GABA)-α-ketoglutarate transaminase and that the mechanism of action of this inhibitor requires its catalytic turnover.



gabaculine



GABA

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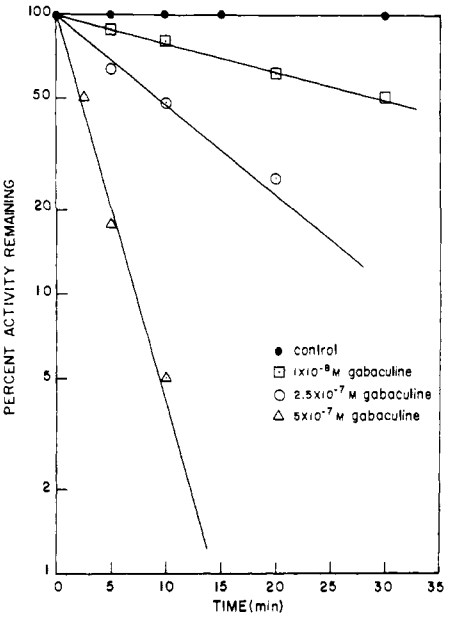

Figure 1. Irreversible inhibition of GABA-transaminase by gabaculine; 0.05 units of mouse brain enzyme (specific activity = 0.03 units/mg) was incubated with gabaculine at the indicated concentrations (based on *l*-gabaculine) in 0.1 ml of 0.1 M potassium phosphate buffer, pH 8.3 at 37 °C. A unit of enzyme is defined as the amount of enzyme required to catalyze the formation of 1 μmol of product per min under standard conditions.[5] At the indicated times aliquots of the enzyme were removed and the remaining activity determined by the method of Wu which uses a coupled assay with glutamate dehydrogenase and acetyl-NAD.[5] The activity of the inactive enzyme would not be increased in the slightest by extended dialysis against the phosphate buffer.

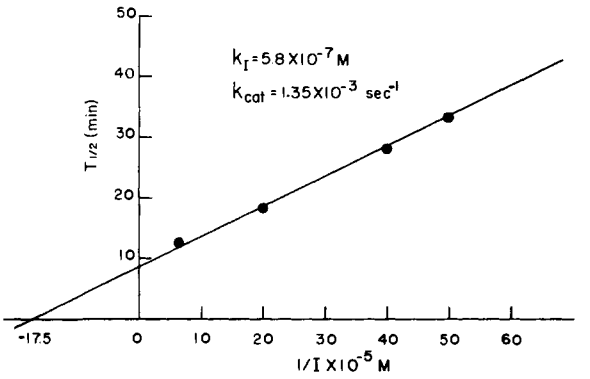

Figure 2. Determination of K_I and k_{cat} for gabaculine. The rates of transaminase inhibition were determined for the indicated concentrations of gabaculine at 15 °C. Assuming the scheme

$$E + I \overset{K_I}{\rightleftarrows} EI \xrightarrow{k_{cat}} EI' \xrightarrow{k_{inh}} E - I'$$

with $d(EI)/dt = 0$, $I \gg E$ and $k_{inh} \gg k_{cat}$ the following equation is derived $t_{1/2} = (0.69/k_{cat} + 0.69/k_{cat})K_I/I$[4]. A plot of $t_{1/2}$ vs. $1/I$ yields a $K_I = 5.8 \times 10^{-7}$ M and a $k_{cat} = 1.35 \times 10^{-3}$ s^{-1} at 15 °C.

In the presence of gabaculine the mouse brain enzyme is progressively and irreversibly inactivated (Figure 1). The inhibited enzyme cannot be reactivated by gel filtration or by extended dialysis against buffer. Mercaptoethanol (10 mM) has no effect on the rate of inactivation suggesting that an affinity labeling mode of inactivation is not occurring. Furthermore, derivatives of gabaculine incapable of being substrates for the enzyme are not inhibitors of it. For example, the *tert*-butylcarbamate derivative of gabaculine in the micromolar range is neither an irreversible nor a competitive inhibitor of the enzyme. In addition, synthetic *d,l*-gabaculine has one-half the activity of the naturally occurring *l*-isomer.[3] These results, taken together, argue against a direct chemical