cate that the changes in heme-protein interactions produced by the optical triggers are insufficient to do this. These optical triggers do, however, provide a unique opportunity to investigate the nanosecond-microsecond dynamics of the unfolded protein prior to surmounting the free energy barrier that separates the unfolded structures from the native state (9).

Chi-Kin V Chan James V Hofrichter William A. Eaton Laboratory of Chemical Physics, National Institute of Diabetes, Digestive, and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0520, USA

REFERENCES AND NOTES


5. As with many different types of single-laser-pulse, initiated processes, reversibility of the reaction puts limits on the longest times that can be studied. For cytochrome c, reversal of the photochemistry aborts folding at a few milliseconds. In the electron transfer experiment, reoxidation of the reduced cytochrome by the Ru^3+ complex occurs, while in the photodissociation experiment CO rebinds. Both of these problems could be overcome by using a continuous wave laser to maintain reduction or ligand dissociation after the initial excitation pulse.

6. The relaxation times corresponding to the rate constants obtained by Pascher et al. (2) for the kinetic scheme in Fig. 1 are -4 μs and -120 μs. After reduction in the electron transfer experiment, the heme is mainly six-coordinate, and the same two relaxation times should be observed as in the CO photodissociation experiment. At most wavelengths, however, the amplitude will be dominated by the slower relaxation time corresponding mainly to histidine dissociation and rebinding. Considering the small difference in solution conditions (for example, the slightly higher pH in the experiments of Pascher et al., which would result in less protonation of the histidines and faster binding rates) and the report of a single relaxation time in the electron-transfer experiment, we assume that the two techniques are giving consistent results, as also concluded by Pascher et al. (4). Thus, although the initial conditions are different in the two experiments, after -100 μs they should produce the same distribution of denatured structures.

7. The quantum yield of fluorescence relative to the quantum yield in the absence of heme quenching is given by Förster theory as [1 + (r_f/r)^2]^-1, where r is the heme-trypophan distance and r_f, which depends on the “overlap integral” of the normalized fluorescence spectrum of the tryptophan and the absorption spectrum of the heme, is ~3.5 nm. In a random coil distribution of heme-trypophan distances, where the mean distance is ~7 nm, the average relative quantum yield is 0.8, while at a distance of 2.0 nm the relative quantum yield is only 0.03. In the native conformation this distance is 1.0 nm.


11. We thank A. Szabo and E. Henry for helpful discussions.

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Response: In our report about the electron-transfer (ET)-initiated folding of ferrocyanochrome c (cyt c^I) (1), we noted rapid changes in the visible absorption spectrum corresponding to a process with a time constant of ~40 μs. We indicated that this observation was consistent with studies of cyt c^II folding initiated by CO dissociation where the fast dynamics were attributed to changes in heme ligation (2). We also suggested that these dynamics might correspond to the collapse of the protein into a compact denatured state, as has been proposed for apomyoglobin on the basis of laser-temperature double measurement experiments (3). Our transient absorption data could not distinguish between the two possibilities, and these fast folding dynamics were a relatively minor component of the study described in our report. Chan et al., with the use of tryptophan (Trp) fluorescence as a probe, found no significant collapse of the protein on the submillisecond time scale following dissociation of CO from unfolded cyt c^II. Clearly, multiple spectroscopic methods must be employed to study protein folding; accordingly, we are currently developing time-resolved Trp fluorescence as a probe for ET-initiated folding of cyt c^II.

A particularly significant difference between our work and that of Chan et al. is the nature of the unfolded form of the protein. The initial state in ET-triggered cyt c^II folding is guanidine hydrochloride (GuHCl) denatured ferricyanochrome c (cyt c^III) (1, 4); in contrast, Chan et al. start with CO-ligated cyt c^II in the presence of GuHCl (2). Higher GuHCl concentrations are required to unfold CO-bound cyt c^II than cyt c^III. Apparently, the positive charge on the heme in cyt c^II has a significantly greater destabilizing effect than does CO binding to the ferroheme. Given these differences, it is reasonable to question whether the two unfolded states are the same. Investigations of cyt c^III folding using stopped-flow kinetic spectroscopy have been interpreted in terms of a minor collapse during the mixing dead time (~2 ms) (5). Nevertheless, the reduction in Trp fluorescence observed in this burst phase is measurably greater than the changes found by Chan et al.; this reduction has been attributed to a decrease in the Trp to heme distance of ~5 Å. Given the possible differences between the unfolded forms of the proteins, we must use Trp fluorescence as an additional probe in our studies of ET-initiated cyt c^II folding.

The complex process of protein folding is believed to involve dynamics that span more than 12 orders of magnitude in time (picoseconds to minutes). The power of optical triggering methods (such as photo-induced CO dissociation and ET chemistry) is that they lay open a large part of this time regime for direct examination.

Jay R. Winkler
Harry B. Gray
Beckman Institute 139-74, California Institute of Technology, Pasadena, CA 91125, USA

REFERENCES


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Complementary DNA for 12-Kilodalton B Cell Growth Factor: Misassigned

The 12-kD B cell growth factor (BCGF), also known as low molecular weight-BCGF or BCGF, is a cytokine produced by activated T lymphocytes (1–4). Several functions have been implicated for this factor. Most importantly, 12-kD BCGF has been suggested to be a common progression factor for human B lymphocytes and to have an autocrine role in B cell neoplasms (5). Twelve-kilodalton BCGF was purified to homogeneity in 1985 (3), and cloning of the corresponding cDNA was reported 2 years later (4). However, these studies have not been confirmed, and the exact molecular connection between the widely used commercial BCGF (purified from the supernatants of mitogen-activated lymphocytes), natural 12-kD BCGF, and the reported cDNA has remained ambiguous. Recently, a genomic segment that clearly corresponds to the reported cDNA sequence was identified (6, 7). Sequencing of the genomic DNA of 12-kD BCGF by Żętkiewicz et al. (6)
Response: Optical Triggers of Protein Folding

Jay R. Winkler and Harry B. Gray

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