Topographical Mimicry of the Enzyme Binding Domain of Cytochrome c

Kimberly K. Clark-Ferris and Jed Fisher*

Department of Chemistry, University of Minnesota
Minneapolis, Minnesota 55455

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An extraordinary harmony must exist among all the components of an organism for life to persist. At the molecular level this is most directly seen in the existence of specific binary complexes between macromolecules, where the stability or nature of the complex is regulated by the momentary needs of the organism. Where a molecular description of such a complex has become available, its stability is seen to derive from the intimate contact of complementary structural features found on the molecular surfaces. Thus, should a molecule be found that duplicates these features, it too will complex to the macromolecule surface. Such a molecule may be described as a topographical mimic.

In the mitochondrial respiratory system, stable complex formation between protein pairs is necessary to the ordered transfer of electrons. Unquestionably the best studied of these proteins is cytochrome *c*; indeed the association of cytochrome *c* to its partners is arguably the best studied of all known macromolecule complexes.[1] From the exemplary crystallographic data[2] and exquisite protein modification studies,[3] the structural features used by cytochrome *c* in complex formation are identified as a cluster of lysine residues surrounding a hydrophobic surface domain, in the center of which is found an exposed edge of the heme. The lysines' orientation establishes a dipole, that complements an opposite dipole in the cytochrome *c* partner, derived from analogous carboxylate residues.[4] This dipole orientation not only provides specificity and stability in complex formation but also orientates the prosthetic groups for intracomplex electron transfer.[5]

It was our belief that this information was sufficient for the design of a topographical mimic of the cytochrome *c* enzyme binding domain. The four pertinent lysines (13,27,72,86) trace a squarelike pattern having approximately 1.5-nm edges.[6] Thus the intended mimic must complement, both in charge and dimension, this domain. With reference to this most important criterion and others (dimensional flexibility, spectroscopic character, synthetic ease) the molecule identified as the requisite mimic is *meso*-tetrakis(4-carboxyphenyl)porphyrin (**1**).[7]

The most straightforward, accurate, and reproducible method for the study of this porphyrin's association to cytochrome *c* is admixture within a split cell. By comparison of the spectrum before mixing (the linear sum of the two components) to that after (the spectrum of the complex) a characteristic difference spectrum is obtained. Examination of the porphyrin-cytochrome *c* pair at the Soret bands shows a substantial hypochromic effect on mixing ($\Delta\epsilon = -1.65 \times 10^5$ M^{-1} cm^{-1} at 414 nm; $\epsilon_{412\,nm}$ of the complex $= 2.98 \times 10^5$ M^{-1} cm^{-1}). Analogous changes persist into the visible region (Figure 1). Several control experiments indicate that these changes are indicative of complex formation and not of a trivial association of this porphyrin to the protein. Only exceedingly small alterations occur at the Soret band upon addition of *N*-α-*tert*-butoxycarbonyl-*L*-lysine (4 equiv) to the porphyrin, or benzoate (4 equiv) to cytochrome *c*. Likewise polyacetylated cytochrome *c*[8] provides no spectral evidence indicative of complex formation to the porphyrin nor does coproporphyrin I (also a tetraanionic porphyrin) when mixed with native cytochrome *c*.[9] On the other hand, analysis by continuous variance of the $\Delta\epsilon$ upon mixing is consistent with the formation of a one-to-one complex of *meso*-tetrakis(4-carboxyphenyl)porphyrin and cytochrome *c*, and the linearity of the observed $\Delta\epsilon$ with concentration (5-60 μM) indicates that the upper limit for K_d of the complex is 5 μM. Altogether these observations provide compelling circumstantial evidence in favor of specific complex formation between **1** and cytochrome *c*.

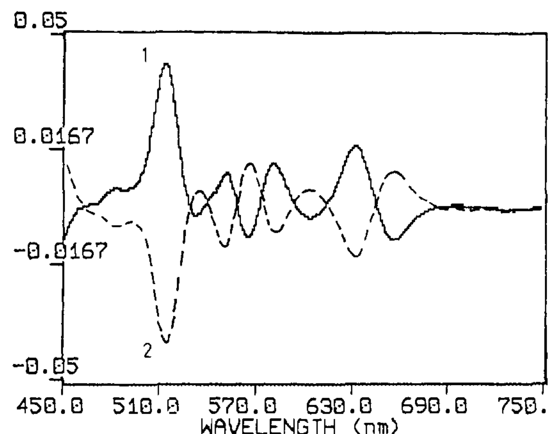

Figure 1. All experiments are done with horse heart ferricytochrome *c* in 5 mM Tris-HCl pH 7.1 buffer (30.0 °C) using a tandem mixing cell, having two 4.5-mm pathlength compartments. Spectrum 1 (—) is the difference spectrum obtained by substraction of the spectrum after mixing (the porphyrin-cytochrome *c* complex) from that before mixing (the sum of the porphyrin and cytochrome *c* spectra). The final concentrations of **1** and cytochrome *c* are equal, at 10.0 μM. Spectrum 2 (---) is the difference spectrum obtained by addition of 1 equiv of *Megasphaera elsdenii* flavodoxin to 1 equiv of the porphyrin-cytochrome *c* complex. The mirror-image relationship of the spectra indicates that association to the cytochrome *c* surface, by the porphyrin and flavodoxin, is mutually exclusive.

Proof that **1** associates to the lysine-defined enzyme binding domain of cytochrome *c* is obtained by the use of a protein partner for cytochrome *c*. The bacterial flavodoxins are known to form a particularly stable complex with cytochrome *c* ($K_d \sim 0.05$ μM at low ionic strength).[10,11] The details of this complex are known[11,12] and involve the complementary pairing of the cytochrome *c* lysines and flavodoxin carboxylates across hydrophobic domains. Should porphyrin **1** occupy the enzyme-binding domain

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(6) As measured between the α-carbons. This choice of reference points is arbitrary, and indeed if the ϵ-amino groups are chosen a considerably more irregular trapezium is obtained.

(7) 4,4′,4″,4‴-(21*H*,23*H*-Porphine-5,10,15,20-tetrayl)tetrakis(benzoic acid) tetrasodium salt. The distance between the carboxylates, estimated from Dreiding molecules, is 1.3 nm.

(8) Lambeth, J. D.; Lancaster, J. R., Jr.; Kamin, H. *J. Biol. Chem.* **1981**, *256*, 3674-3678.

(9) With the concentration of cytochrome *c* and coproporphyrin I after mixing equal to 5 μM. A possible explanation for the difference between this porphyrin and **1** is that the former's carboxylates reside on propionate side chains; hence complex formation may demand too great of a loss of their rotational degrees of freedom.

(10) Mayhew, S. G.; Massey, V. *J. Biol. Chem.* **1969**, *244*, 794-802. Mayhew, S. G.; Foust, G. P.; Massey, V. *Ibid.* **1969**, *244*, 1803-1810.

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of cytochrome *c*, and should the cytochrome *c*-flavodoxin complex be more stable than the porphyrin-cytochrome *c* complex, then addition of an equivalent of flavodoxin to the porphyrin-cytochrome *c* complex will displace ("unmix") the porphyrin and yield a mirror-image difference spectrum of that for complex formation. This proves to be so (Figure 1). The complete unmixing by a single flavodoxin equivalent places the boundaries on the porphyrin-cytochrome *c* stability to correspond to $0.05\ \mu M < K_d < 5\ \mu M$.[14]

It is anticipated that this topographical mimicry may be of value in examination of the surface dynamics of complex formation, the exploration of analogous domains on other proteins, and the kinetics of electron transfer among these proteins. In this respect **1** may complement the smaller redox-inert metal complexes.[13] Last, it provides a small yet satisfying vindication of the expectation that rational consideration of the structural elements of macromolecules may be used for the design of topographical mimics, as mechanistic principles now allow the design of mechanistic inhibitors.

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Supplementary Material Available: Experimental protocols and figures in support of the described experiments (4 pages). Ordering information is given on any current masthead page.

(13) Butler, J.; Chapman, S. K.; Davies, D. M.; Sykes, A. G.; Speck, S. H.; Osheroff, N.; Margoliash, E. *J. Biol. Chem.* **1983**, *258*, 6400-6404. Chapman, S. K.; Sinclair-Day, J. D.; Sykes, A. G.; Tam, S.-C.; Williams, R. J. P. *J. Chem. Soc., Chem. Commun.* **1983**, 1152-1154. Chapman, S. K.; Davies, D. M.; Vuik, C. P. J.; Sykes, A. G. *J. Am. Chem. Soc.* **1984**, *106*, 2692-2696.

(14) A more precise estimate of the K_d is precluded at present by additional absorption of **1** to cytochrome *c* when **1** is the reagent present in excess; this prohibits a quantitative interpretation of the titration experiments.

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Total Synthesis of (±)-4-Amino-4-deoxychorismic Acid: A Key Intermediate in the Biosynthesis of *p*-Aminobenzoic Acid and L-(*p*-Aminophenyl)alanine[1]

Chia-Yu P. Teng,[2a] Bruce Ganem,*[2a] Stella Z. Doktor,[2b] Brian P. Nichols,[2b] Raj K. Bhatnagar,[2c] and Leo C. Vining[2c]

Department of Chemistry, Baker Laboratory
Cornell University, Ithaca, New York 14853
Laboratory for Cell, Molecular and Developmental Biology, Department of Biological Sciences
University of Illinois at Chicago
Chicago, Illinois 60680
Department of Biology, Dalhousie University
Halifax, Nova Scotia, Canada B3H 4J1

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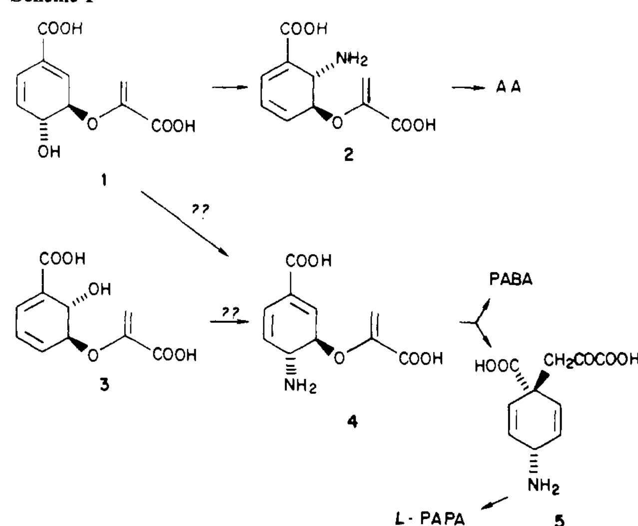
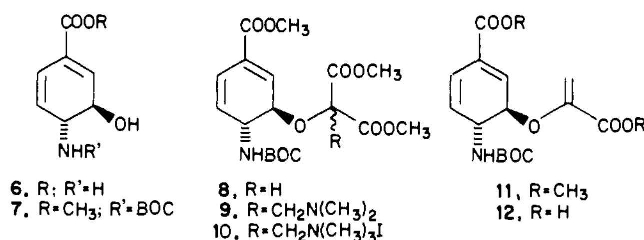
Chorismic acid (**1**), the branch-point metabolite of the shikimate pathway, is the biosynthetic precursor of both anthranilic acid (AA) and *p*-aminobenzoic acid (PABA).[3] In the mid-1970's, Dardenne[4] and Haslam[3c] independently suggested that (a) AA

Scheme I



and PABA both originated from chorismate or (b) AA arose from chorismate and PABA from isochorismate (**3**) by parallel 1,5-addition/elmination reactions of ammonia or glutamine (Scheme I). Amino enol pyruvates **2** and **4** were suggested as key "missing links". L-(*p*-Aminophenyl)alanine (L-PAPA), a microbial and plant precursor of chloramphenicol, could arise by the Claisen rearrangement of **4** to **5**, an amino analogue of prephenic acid.[4] These proposals gained support when we[5a] and others[5b] reported the conversion of **2** to AA by subunit I of anthranilate synthase (AS-I), purified from *Serratia marescens*. Here we describe the total synthesis of 4-amino-4-deoxychorismate (**±-4**) and 4-amino-4-deoxyprephenate (**5**). Enzymic studies indicate that **4** and **5** are bona fide intermediates between chorismate and PABA and L-PAPA and that isochorismic acid apparently plays no part in the biosynthetic scheme.

Racemic *trans*-4-amino-3-hydroxy-1,5-cyclohexadiene-1-carboxylic acid (**6**)[6] was smoothly N-protected with a BOC group



and then its sodium salt esterified ($NaHCO_3/CH_3I$/HMPA) to afford **7** in 46% overall yield. This substance was transformed to alkoxymalonate **8** [$N_2C(CO_2CH_3)_2$, $Rh_2(OAc)_4$, C_6H_6, 70 °C, 56%] and then alkylated with Eschenmoser's salt [$CH_2{=}N(CH_3)_2I$, CH_2Cl_2, Et_3N, 94%].[7] The resulting Mannich base **9** was quaternized (CH_3I/CH_2Cl_2, room temperature) to ammonium salt **10** which, upon treatment with base (1 equiv of NaOH, THF/H_2O), furnished dimethyl ester **11** in 47% yield. Further saponification of **11** with 2.5 equiv of NaOH gave **12** in >90% yield after acidification with Amberlite IR-120-H resin. Deprotection of **12** in neat CF_3CO_2H (0 °C, 15 min) furnished the desired **4** as its TFA salt. Less polar aromatic impurities were removed by silica gel chromatography, thus affording pure (±)-**4**-TFA in 68% yield (mp 110-115 °C).[8]

(1) Shikimate-Derived Metabolites. 15. For part 14, see: Teng, C.-Y. P.; Ganem, B. *Tetrahedron Lett.* **1985**, *26*, 21.

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