(11) J. Shapira, *J. Org. Chem.*, **27**, 1918 (1962).
(12) M. Świerkowski and D. Shugar, *J. Med. Chem.*, **12**, 533 (1969).
(13) J. R. Geigy, British Patent, 833 573, Apr 27, 1960, *Chem. Abstr.*, **55**, 1652*h* (1961).
(14) R. U. Lemieux, *Can. J. Chem.*, **39**, 116 (1961).
(15) H. J. Minnemeyer, H. Tieckelmann, and J. F. Holland, *J. Med. Chem.*, **6**, 602 (1963).

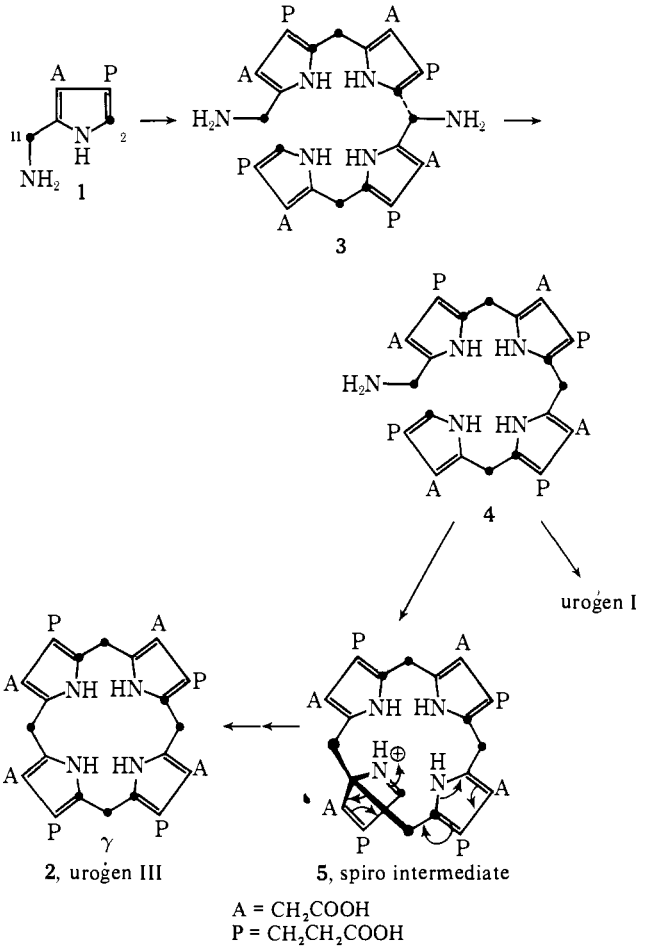
Donald E. Bergstrom,* Jerry L. Ruth
Department of Chemistry, University of California
Davis, California 95616
Received October 6, 1975

Biosynthesis of Uroporphyrinogen III from Porphobilinogen. Resolution of the Enigmatic "Switch" Mechanism

Sir:

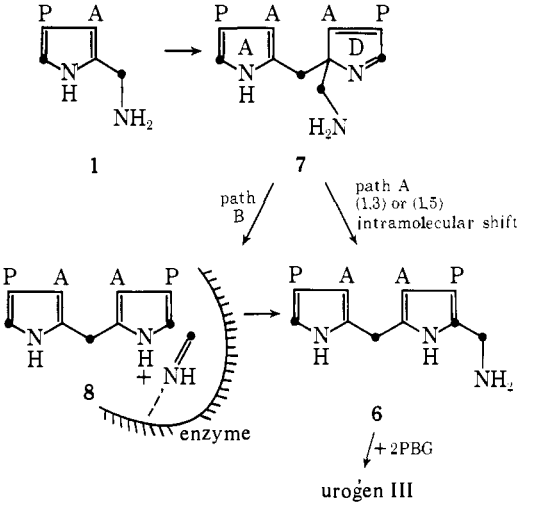
A long-standing problem in the biosynthesis of the ubiquitous porphyrins, which play a vital role in the architecture of heme, chlorophyll, cytochrome *c*,[1] and vitamin B_{12},[2] concerns the fascinating mechanism[1d] whereby the combined action of two enzymes, porphobilinogen deaminase and modifying cosynthetase, transforms the monomeric pyrrole (PBG, **1**) to the unsymmetrical uroporphyrinogen (uro'gen) III (**2**). In the type III structure one of the PBG units (ring D) appears to have been "switched" with respect to a head-to-tail polymerization sequence, an event which has given rise to more than 20 published speculations[3] and some 200 experimental papers[1] designed to solve this mystery. The stage has now been reached where the evidence for what happens is secure; i.e., an *intra*molecular process (clearly defined by the elegant kinetic work of Bogorad)[4] is responsible for transforming four molecules of PBG to a mixture of uro'gens I and III and the liberation of 4 mol of ammonia. Within the experimental error (ca. 10%) of a ^{13}C NMR experiment, the work of Battersby[5] has confirmed not only the intramolecular nature of the switch mechanism, but has rigorously defined the new location of the mobile amino methyl carbon from C-11 of one PBG unit as it finds its way into the γ position of protoporphyrin IX, according to Scheme I. In order to recognize the timing and nature of the mechanism, the use of specifically labeled dipyrrylmethanes has been undertaken recently in two laboratories. According to the Cambridge group,[6] the dipyrrylmethane (DPM) **3** serves as a good precursor for the type III porphyrins via the linear bilane **4**, and the spiro intermediate **5**; i.e., the switch takes place after the head-to-tail condensation of 4 PBG units. On the other hand, the Frydmans' work supports a mechanism in which "switching" occurs at the first head-on encounter of two PBG molecules under the influence of the deaminase-cosynthetase system to afford the DPM **6** (Scheme II) followed by addition of two more PBG units to complete the type III macrocycle.[1d] Thus although both groups have secured evidence that aminomethyldipyrrylmethanes are involved, different conclusions were reached about the timing of the rearrangement process, and it was clearly recognized that verification of either the Corwin (Scheme I) or Rimington-Johnson (Scheme II) hypothesis[7] was fraught with an overwhelming experimental difficulty associated with the in vitro chemistry of the DPM systems **3** and **6** which necessitates not only subtraction of a chemical blank (up to 80% in some cases) from the enzymatic yield, but also the development of analytical systems for the biologically irrelevant or "nonsense" type IV porphyrins generated from **3**.[11] We have devised a new approach toward the solution of this problem which, by removing the in vitro chemistry of the aminomethyl side chain, provides for the first time a clear mechanistic distinction between Schemes I and II and sets the stage for more advanced enzymological studies.

Scheme I. The Corwin Hypothesis[a]



[a] Heavy circles in Schemes I and II denote the sites of ^{13}C enrichment from a sample of 2,11-[$^{13}C_2$]PBG.[5]

Scheme II. Rimington–Johnson Hypothesis



Consideration of a mechanism such as that depicted in Scheme II reveals that, although the reaction may be deemed *intra*molecular in the chemical sense, i.e., via concerted rearrangement **7** → **6** (path A), the operation of enzymatic intramolecular transfer **7** → **8** → **6** (path B) cannot be excluded. We were, therefore, led to a simple experiment in which a *labeled* sample of DPM **8**[12,16] which has

Table I

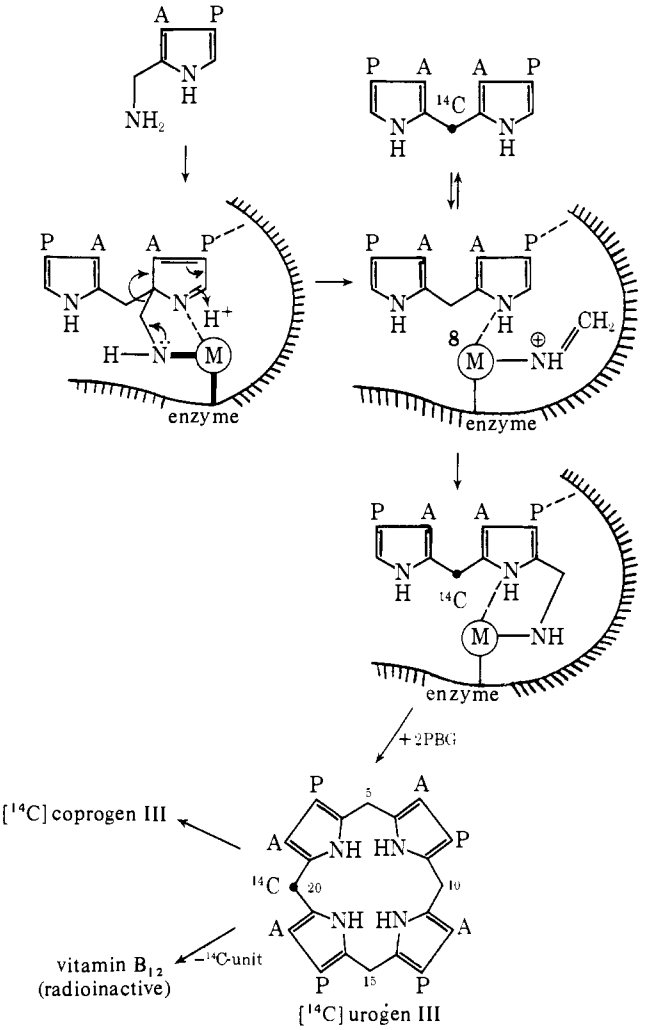
Experiments	Radioactivity in copro III methyl ester, dpm	Radioactivity in [^{14}C]DPM **8**, dpm	Percentage of total radioactivity[d] incorporated into type III isomer	Ratio of porphyrin amounts formed copro III:copro I	Ratio of radioactivity found in copro III:copro I
(I) PBG deaminase[a] + uro'gen III cosynthetase	11 153	6.4×10^6	0.15	42:58	9:1
(II) *Clostridium*[b] *tetanomorphum* Cell free	77 250	12.8×10^6	0.55	22:78	9:1
(III) *P. shermanii*[c] Whole cell	150 680	17.7×10^6	0.89	~100% type III	

[a] PBG deaminase and uro'gen III cosynthetase isolation, purification and incubation were carried out according to Bogorad's procedures.[21a,22] Incubation mixture of final volume of 7 ml contains PBG, 1.2 mg in 1.2 ml of Tris·HCl buffer, pH 8.2, 0.1 M; [^{14}C]DPM **8**, 1.2 mg in 1.2 ml of Tris buffer; EDTA, 0.2 ml, 0.1 N; glutathione, 2 mg in 1.8 ml of Tris buffer; 2 ml of wheat germ enzyme fraction 40-50% (80 mg of protein/ml); 0.6 ml of PBG deaminase (8 mg/ml); incubated anaerobically at 30 °C for 3 h in the dark. [b] Incubation with crude enzymes of *Clostridium tetanomorphum* in a final volume of 7 ml of Tris·HCl buffer, pH 8.2, 0.1 M, containing the same amounts of additives and substrates as in footnote *a* but with 2 ml of *clostridium* enzymes (45 mg of protein/ml). [c] 5 mg of [^{14}C]DPM (**8**) was used for feeding to *P. shermanii* bacteria (28 g, wet cells) in 100 ml of phosphate buffer, pH 7.6, 1/15 M, together with the following additives: $CoCl_2 \cdot 6H_2O$, 1 mg; DMBI, 25 mg; 50% glucose, 3.2 ml. The mixture was incubated anaerobically for 52 h, with seven adjustments of pH and addition of glucose, affording radioactive copro III and radioinactive vitamin B_{12}.[23] [d] In experiments a and b boiled enzyme controls using identical conditions and same amounts of additives and substrates were run. Copro III isomer from the control experiments contains some radioactivity (ca. 10% that of copro III from the enzymic incorporation). This blank was incorporated into the final calculation of total radioactive incorporation, and corresponds to *post*-incubation reactions of PBG and DPM **8** at pH 3.5, 16 h, during the standard procedure of porphyrin extraction.

temporarily lost its aminomethyl "head" could be exchanged with an identical molecule during operation of the formal 1,3 (or 1,5) shift shown in Scheme II, path B. In other words, the enzyme cleft may be "leaky" with respect to exchange of the headless DPM **8** during the switching mechanism[19] (Scheme III). Accordingly, a synthetic sample[16] of ^{14}C-meso labeled DPM **8** was incubated with both enzyme and whole cell preparations listed in Table I, *in the presence of PBG*. After 1-3 h, the resultant porphyrin mixture was analyzed by standard procedures[21-23] (conversion to copro I and III methyl esters, crystallization to constant radioactivity). The results (Table I) reveal that within the accepted[24] (10%) experimental error for separation of the types III and I isomers, DPM **8** is indeed exchanged and is specifically incorporated into uro'gen III in a selection of organisms (*P. shermanii, C. tetanomorphum,* wheat germ), thereby providing the first unambiguous proof that, as adumbrated by the work of Frydman, type III synthesis is controlled by the formation of DPM **6**. In contradistinction to previous methodology, *there is virtually no chemical blank* in these experiments; i.e., in presence of boiled enzyme, no porphyrin formation is observed spectroscopically because only under the influence of uro'gen III cosynthetase can the aminomethyl group (or its equivalent) from PBG become available at the active site for condensation with the free α position of DPM **8**. Moreover, under these conditions PBG does not polymerize chemically. Most importantly, these experiments exclude a concerted mechanism for the 1,3(or 1,5) shift and provide clear evidence for the discrete intermediate **8** in the enzyme mechanism. Thus we have an unusual mechanistic probe in that the synthetic substrate is "incomplete" and the reaction only proceeds when the active site is loaded with the switching C_1 unit from C-11 of PBG (**1**). The virtual absence of radioactivity in the type I porphyrin produced precludes trivial substrate degradation and provides an excellent built-in control, while the absence of radioactivity in vitamin B_{12} isolated in the *P. shermanii* whole cell experiment indirectly locates the ^{14}C label in the uro'gen III formed, since C-20 of uro'gen III has been shown to be extruded in the biosynthesis of corrin nucleus.[26]

A general mechanism in accord with these observations is depicted in Scheme III which takes into account the effect of Mg^{2+} ion on the soybean enzyme system[3b] and implicates the possibility of a divalent cation at the catalytic site

Scheme III. The "Headless" DPM Postulate



of the enzyme. It is also reasonable to postulate that the —Ⓜ—NH_2 in Scheme III is an amine or imidazole group on the enzyme.[27] Further obvious extensions of this work, e.g., trapping of the "C_1 unit" in the form of imine or Schiff base, trapping of the enzymatically formed DPM (**8**),[25] and investigation of "headless" tripyrrylmethane and tetrapyrrylmethane as possible "intermediates", are in progress.

Acknowledgments. This work was generously supported by grants from NSF-MPS72-04601 and NIH (AM 17014). We thank Mr. A. Brown for cultures of *C. tetanomorphum* and *P. shermanii*, Dr. W. K. Chan for the preparation of DPM **8**, and Professor L. Bogorad for a generous gift of uro'gen III cosynthetase.

References and Notes

(1) (a) J. Lascelles, "Tetrapyrrole Biosynthesis and Its Regulation", W. A. Benjamin, New York, N.Y., 1964, p 38; (b) B. F. Burnham, in "Metabolic Pathways", Vol. III, 3rd ed, D. M. Greenberg, Ed., Academic Press, New York, N.Y., 1969, p 403; (c) L. Bogorad in "The Chlorophylls", L. P. Vernon and G. R. Seely, Ed., Academic Press, New York, N.Y., 1966, p 481 (d) B. Frydman and R. B. Frydman, *Acc. Chem. Res.*, **8**, 201 (1975).

(2) A. I. Scott, B. Yagen, and E. Lee, *J. Am. Chem. Soc.*, **95**, 5761 (1973); A. I. Scott, *Ann. N. Y. Acad. Sci.*, **244**, 356 (1975); A. I. Scott, N. Georgopapadakou, K. S. Ho, S. Klioze, E. Lee, S. L. Lee, G. H. Temme, III, C. A. Townsend, and I. M. Armitage, *J. Am. Chem. Soc.*, **97**, 2548 (1975); A. R. Battersby, M. Ihara, E. McDonald, F. Satoh, and D. C. Williams, *J. Chem. Soc., Chem. Commun.*, 436 (1975); H. O. Dauner and G. Müller, *Hoppe-Seylers Z. Physiol. Chem.*, 1353 (1975).

(3) (a) J. H. Mathewson and A. H. Corwin, *J. Am. Chem. Soc.*, **83**, 135 (1961); (b) E. B. C. Llambias and A. M. C. Batlle, *Biochem. J.*, **121**, 327 (1971).

(4) L. Bogorad and G. S. Marks, *J. Biol. Chem.*, **235**, 2127 (1960).

(5) A. R. Battersby, E. Hunt, and E. McDonald, *J. Chem. Soc., Chem. Commun.*, 442 (1973).

(6) A. R. Battersby, K. H. Gibson, E. McDonald, L. N. Mander, J. Moron, and L. N. Nixon, *J. Chem. Soc., Chem. Commun.*, 768 (1973).

(7) In the Weizmann lectures[8] Robinson offered the mechanism shown in Scheme II, but considered three such "switches" to account for the observed type III pattern. Recent ^{13}C experiments[5] using double labeling apparently exclude more than one such switch during the process. Johnson et al.[9] favored an intramolecular rearrangement again involving a single switch, based on the earlier ideas of Cookson and Rimington.[10]

(8) R. Robinson, "Structural Relations of Natural Products", Oxford University Press, Oxford, 1955, p 25.

(9) E. Bullock, A. W. Johnson, E. Markham, and K. B. Shaw, *J. Chem. Soc.*, 1430 (1958).

(10) G. H. Cookson and C. Rimington, *Biochem. J.*, **57**, 476 (1954).

(11) R. B. Frydman, A. Valasinas, H. Rapoport, and B. Frydman, *FEBS Lett.*, **25**, 309 (1972); B. Frydman, R. B. Frydman, A. Valasinas, S. Levy, and G. Feinstein, *Ann. N. Y. Acad. Sci.*, **244**, 371 (1975); R. B. Frydman and B. Frydman, *FEBS Lett.*, **52**, 317 (1975).

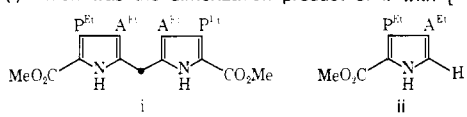
(12) The suggestion that DPM **7** is a kinetically favored product during in vitro condensation of PBG was made in the germinal study of uro'gen chemistry by Mauzerall.[13] It is interesting to note that two groups[14,15] have reported on the lack of porphyrin formation by the action of PBG deaminase on DPM **8**. In neither case, however, was PBG added to the incubation.

(13) D. Mauzerall, *J. Am. Chem. Soc.*, **82**, 2605 (1960).

(14) D. S. Hoare and H. Heath, *Biochim. Biophys. Acta*, **39**, 167 (1960).

(15) A. T. Carpenter and J. J. Scott, *Biochim. Biophys. Acta*, **52**, 195 (1961).

(16) Methylene ^{14}C-labeled DPM **8** tetraacid was obtained as the decarboxylation product[17] of the corresponding ^{14}C-labeled "PAAP" hexa ester DPM (i) which was the dimerization product of ii with [^{14}C]formaldehyde in presence of HI, acetic anhydride, and hypophosphorus acid.[18] The hexa ester i was characterized spectroscopically and had mp 134–135 °C.



i

ii

(17) G. P. Arsenault, E. Bullock, and S. F. MacDonald, *J. Am. Chem. Soc.*, 4384 (1960).

(18) M. W. Roomi and S. F. MacDonald, *Can. J. Chem.*, **48**, 139 (1970).

(19) Further evidence for the discrete or "leaky" nature of the switch comes from Shemin's early work[20] concerning the isolation of radioactive formaldehyde (as the dimedon derivative) in cell-free extracts of enzymatic conversion of PBG to porphyrins.

(20) D. Shemin, C. S. Russell, and T. Abramsky, *J. Biol. Chem.*, **215**, 613 (1955).

(21) (a) L. Bogorad in "Methods in Enzymology", S. Colowick and N. O. Kaplan, Ed., Academic Press, New York, N.Y., 1953, p 885; (b) J. Falk, "Porphyrins and Metalloporphyrins", Elsevier, Amsterdam, 1964, pp 147, 193.

(22) L. Bogorad, *J. Biol. Chem.*, **233**, 510 (1958).

(23) A. I. Scott, C. A. Townsend, K. Okada, and M. Kajiwara, *J. Am. Chem. Soc.*, **96**, 8054 (1974).

(24) E. Stevens and B. Frydman, *Biochim. Biophys. Acta*, **151**, 429 (1968).

(25) M. Higuchi and L. Bogorad, *Ann. N. Y. Acad. Sci.*, **244**, 401 (1975), have obtained evidence that in a purified preparation of PBG deaminase and uro'gen III cosynthetase enzyme–enzyme interaction takes place during the synthesis of uro'gen III from PBG. The effect of DPM **8** on the modifying action of the cosynthetase on the deaminase is also under investigation.

(26) A. I. Scott, C. A. Townsend, K. Okada, M. Kajiwara, R. J. Cushley, and P. J. Whitman, *J. Am. Chem. Soc.*, **96**, 8069 (1974).

(27) C. S. Russell, *J. Theor. Biol.*, **47**, 145 (1974). Three "switches" are involved[8] in this theory which can still be accommodated by the results of ref. 5.

A. Ian Scott,* K. S. Ho, M. Kajiwara, T. Takahashi
Sterling Chemistry Laboratory, Yale University
New Haven, Connecticut 06520
Received October 29, 1975

Matrix Reactions of Alkali Metal Fluoride Molecules with Fluorine. Infrared and Raman Spectra of the Trifluoride Ion in the $M^+F_3^-$ Species

Sir:

The trihalide anions, X_3^-, have generated considerable synthetic and theoretical interest; spectroscopic studies of these anions have been carried out in solids and solutions[1,2] and in inert matrices.[3,4] While Cl_3^-, Br_3^-, and I_3^- have been investigated thoroughly, the most reactive member of this group, F_3^-, has never been observed. It is known that CsF catalyzes oxidative fluorination reactions,[5,6] and it is possible that this reaction proceeds through an F_3^- intermediate species. Several recent studies have shown that matrix reactions of alkali halide salt molecules with halogen molecules in argon matrices form the $M^+X_3^-$ ion pair, for X = Cl, Br, I.[3,4,7] Analogous studies of the reaction of MF salt molecules with F_2 should provide a method of stabilizing the elusive F_3^- species and obtaining spectroscopic information and an indication of the nature of the bonding in the F_3^- anion.

Salt-molecule reactions were carried out in a manner similar to those reported in earlier papers.[3,8] The argon and fluorine mixture was deposited on a CsI window held at 15 K, while the alkali fluoride salt vapor was evaporated from a Knudsen cell and deposited simultaneously with the gas sample. Approximately 585 °C was required to produce the necessary vapor pressure of KF, while 640 and 495 °C were required for RbF and CsF, respectively. Infrared spectra were recorded after 20–30 h of deposition on a Beckman IR-12 infrared spectrophotometer. In Raman experiments, the salt and gas mixture was deposited on a polished copper block for 3–5 h. Raman spectra were obtained using argon ion laser excitation and a Spex Ramalog spectrometer.

KF, RbF, and CsF were each deposited in argon matrices without added reagent, and three infrared bands were observed in each experiment, at 396, 306, and 275 cm^{-1} with KF, at 345, 266, and 230 cm^{-1} with RbF, and at 313, 248, and 207 cm^{-1} with CsF. In each case, the highest energy band was most intense, and it appeared slightly below the gas phase wavenumber value for the MF monomer;[9] this band can be assigned confidently to the MF monomer vibration in an argon matrix. KF has been observed at 395 cm^{-1} in an argon matrix,[10] in agreement with the value obtained here. The two lower energy bands were of comparable intensity, and they can be assigned to two modes of the cyclic $(MF)_2$ dimer species. The $(KF)_2$ values of 306 and 275 cm^{-1} are in agreement with reported values,[10] and the band positions for $(RbF)_2$ are reasonable for the heavier alkali metal species. Also, in these experiments a weak band was observed near 350 cm^{-1}, which is labeled A in Figure 1 and is likely due to a complex between the MF salt and im-