dropwise with stirring at room temperature. To complete the reaction, the mixture was allowed to stand overnight. Then it was diluted with 300 ml. of water; the precipitated heavy oil was washed with 100 ml. of a 4% aqueous sodium hydroxide solution and dissolved in a minimum amount of hot benzene–heptane mixture. From the solution 6.5 g. (53%) of phenylsulfinylacetophenone, m.p. 79–80°, crystallized on standing at room temperature.

Acknowledgment.—The authors wish to acknowledge helpful discussions with Professor G. A. Russell and Dr. P. V. Smith, Jr. They also wish to thank A. M. Palmer and T. J. Jermansen for valuable technical help, and D. E. Bachert for recording the infrared spectra.

[CONTRIBUTION FROM THE RESEARCH DIVISION, POLAROID CORPORATION, CAMBRIDGE 39, MASS.]

Spectral Shifts in Anthraquinone Dyes Caused by Non-conjugated Substituents

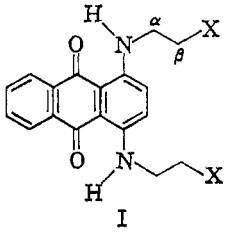
BY MYRON S. SIMON

RECEIVED JANUARY 30, 1963

Anomalous absorption spectra in the 400–700 mμ region caused by β-amino groups on the alkyl chains of 1,4-bis(N-alkylamino)-anthraquinone dyes are reported and discussed.

In a study of N-alkylated 1,4-diaminoanthraquinones an interesting anomaly was observed in the visible spectra of β-amino ethyl substituted derivatives. The normal absorption spectra of 1,4-bis(N-alkylamino)-anthraquinones show peaks close to 642 and 595 mμ and an inflection around 555 mμ in 2-methoxyethanol, benzene or hexane. The data for 1,4-bis-(isopropylamino)-anthraquinone (m.p. 179–180°) or 1,4-bis-(*sec*-butylamino)-anthraquinone (m.p. 155–158°) are typical, and correspond to type I of Fig. 1. The position of these peaks and over-all shape of the absorption envelope is unchanged by substituting hydroxyl groups for a β-hydrogen on the N-alkyl substituents. 1,4-Bis-(β-hydroxyethylamino)-anthraquinone (I, X = OH; m.p. 242.5–244°) has a spectrum identical with those cited above.

Replacement of a β-hydrogen by an amino group leads to a marked change in the visible absorption spectrum.



I

The peaks are shifted to shorter wave length, but peak height is not altered, and a new, broad absorption band of relatively low intensity appears in the 400–500 mμ region. The curve of 1,4-bis-(β-aminoethylamino)-anthraquinone (I, X = NH_2; m.p. 207–208°) dissolved in 2-methoxyethanol is shown in Fig. 1 and is labeled a type II spectrum for easy reference. The new peak (435 mμ, ε 4500, $MeOCH_2CH_2OH$) is shifted in less polar solvents to lower wave length (419 mμ, 4400, benzene; 405 mμ, 4800, hexane), while the high intensity bands are shifted in the opposite direction (605 mμ, ε 21400; 563 mμ, ε 16800, $CH_3OCH_2CH_2OH$; 616 mμ, ε 21400; 571 mμ, ε 17000, benzene; 613 mμ, ε 21400; 568 mμ, ε 17800, hexane) (I, X = NHC_2H_5). Spectral data are summarized in Table I.

In an effort to explain this phenomenon a number of related compounds were synthesized: (1) It was found that the bridge of two carbon atoms between the nitrogens of the side chain is essential for the spectral shift: 1,4-bis(γ-aminopropylamino)-anthraquinone (m.p. 135.5–137°) and similar dyes with a longer bridge between nitrogens of the side chain have the type I curve of the model compounds.

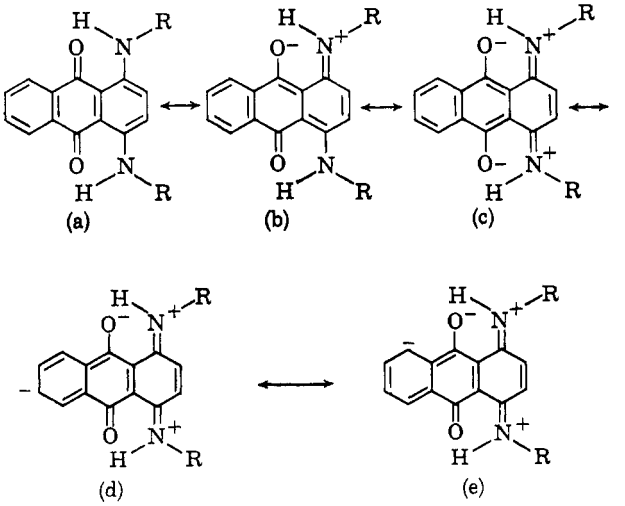
(2) Substitution of one hydrogen atom on the β-amino group by an alkyl group does not cause this phenomenon to disappear: 1,4-bis-(β-N-ethylamino ethylamino)-anthraquinone (I, X = NHC_2H_5; m.p 120.5–122°) has the type II spectrum.

(3) At least one hydrogen on the β-amino group is necessary: 1,4-bis-(β-N-diethylaminoethylamino)-anthraquinone (m.p. 112–112.5°) has a spectrum identical with that shown by compounds of type I.

(4) Basicity of the β-nitrogen is a requirement for appearance of the type II spectrum: 1,4-bis-(β-acetamidoethylamino)anthraquinone (m.p. 218–219°) has a normal, type I, spectrum.

(5) This phenomenon appears to be limited to the 1,4-diamino series: 1,5-bis-(β-aminoethylamino)-anthraquinone (m.p. 195–199° dec.) has the same spectrum as simpler 1,5-bis-(alkylamino)-anthraquinones while 5,8-dihydroxy-1,4-bis-(β-aminoethylamino)-anthraquinones are shifted in the same manner as the simpler 1,4-diamino series described above, and have a type II spectrum with maxima at 642, 591, ~549 and 443 mμ (2-methoxyethanol).

The 1,4-diaminoanthraquinone absorption system appears to have unique properties which are imperfectly understood. A detailed discussion of the problem may be found in the papers of Egerton and Roach[1] and Peters and Sumner,[2] which refer to earlier work. Thus, while the 1,5-diaminoanthraquinone chromophore seems to be the arithmetical sum of two 1-aminoanthraquinone chromophores,[3] the 1,4-system exhibits a more complicated spectrum which has not been related in a



(a) (b) (c) (d) (e)

(1) G. S. Egerton and A. G. Roach, *J. Soc. Dyers and Colourists*, **74**, 405 (1958).
(2) R. H. Peters and H. H. Sumner, *J. Chem. Soc.*, 2101 (1953).
(3) C. J. P. Spruitt, *Rec. trav. chim.*, **68**, 329 (1949).

TABLE I

Anthraquinone, m.p., °C.	2-Methoxyethanol λ, mμ	2-Methoxyethanol ε	Benzene λ, mμ	Benzene ε	Hexane λ, mμ	Hexane ε
1,4-DIAMINOANTHRAQUINONES						
1,4-Bis-(isopropylamino)-, 179–180°	642	19,400	646	16,800	642	16,400
	594	16,000	598	14,400	594	14,400
	~556	7,300	~561	7,000	~557	7,200
1,4-Bis-(*sec*-butylamino)-, 155–158°	643	21,200	647	18,800	644	17,600
	595	17,200	600	15,800	595	15,200
	~560	8,000	~560	7,400	~560	7,600
1,4-Bis-(β-hydroxyethylamino)-, 242.5–244°	640	19,000	638	....[a]	...[b]	....
	594	16,200	593	....	...	....
	~556	8,100	~553	....	...	....
1,4-Bis-(β-aminoethylamino)-, 207–208°	608	20,600	616	....[a]	...[b]	....
	566	15,600	573	....	...	....
	~531	7,000	~540	....	...	....
	435	4,560	415	....	...	....
1,4-Bis-(γ-aminopropylamino)-, 131–132°	642	18,000	646	16,400	643	[a]
	594	15,200	599	14,400	595	....
	~554	7,200	~558	7,000	~558	....
1,4-Bis-(β-N-ethylaminoethylamino)-, 120.5–122°	605	21,400	616	21,400	613	21,400
	563	16,800	571	17,000	568	17,800
	~529	7,900	~537	7,800	532	8,600
	437	4,500	~499	2,700	~495	3,000
			419	4,400	~427	3,800
					405	4,800
1,4-Bis-(β-N-diethylaminoethylamino)-, 112–112.5°	642	17,600	646	16,400	644	15,400
	595	15,200	600	14,400	596	14,000
	~560	7,200	~562	7,200	~558	7,000
1,4-Bis-(β-acetamidoethylamine-, 218–219°	636	18,600	638	16,200	...[b]	....
	590	16,200	591	14,700	...	....
	~553	8,400	~553	7,500	...	....
			~512	2,600	...	....
1,5-DIAMINOANTHRAQUINONES						
1,5-Bis-(β-aminoethylamino)-, 195–199° dec.	519	12,600				
	541	11,800				
1,5-Bis-(γ-aminopropylamino)-, 165–168°	522	12,600				
	544	11,800				
1,5-Bis-(β-N-diethylaminoethylamino)-, 147–150°	518	13,000				
	544	12,000				
1,5,Bis-(β-hydroxyethylamino)-, 256–261°	518	13,200				
	545	12,000				
1,5,Bis-(α-methyl-β-aminopropylamino)- (isomer mixture), 90–92° with prior softening	524	13,800				
	550	13,000				

[a] Sample poorly soluble. [b] Sample insoluble.

simple manner to less highly substituted anthraquinone derivatives. This may be rationalized by observing that the 1,5-system can be written as a resonance hybrid of various forms, none of which shows interaction between the aromatic rings. On the other hand, more extensive charge separation can be postulated for the 1,4-series, as shown in the resonance forms drawn.

In both series hydrogen bonding between nitrogen and oxygen probably plays a major part in the chromophore. Attention has been directed to the phenomenon of two-headed peaks in this series.[4] The regularity of the separation of the peaks and inflections (1150–1300 cm.$^{-1}$ in the cases presented) suggests that the lower wave length peaks are vibrational overtones of the main peak[5] (Table III).

Several hypotheses can be suggested to account for the effect on the spectrum caused by the insulated amino group of the side chain. One possibility is that the aliphatic amine group reacts with the carbonyl group, forming a carbinolamine.

That the molecule is not completely in the form of the carbinolamine is indicated by the fact that the type II spectrum is still very similar to the type I spectrum.

(4) Ref. 2, p. 2106; C. F. H. Allen, C. V. Wilson and G. F. Frame, *J. Org. Chem.*, **7**, 179 (1942).

(5) Personal communication from Dr. George Bird.

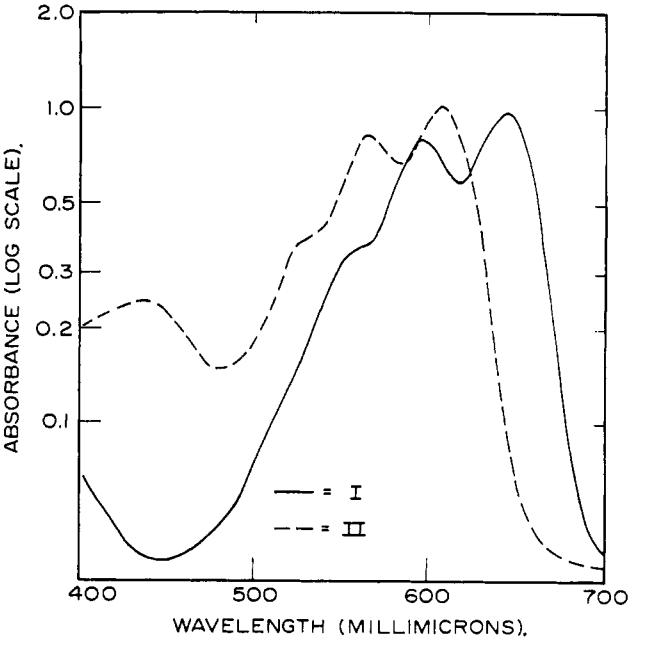
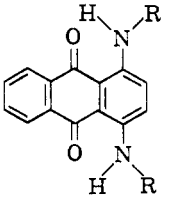


Figure 1.

TABLE II

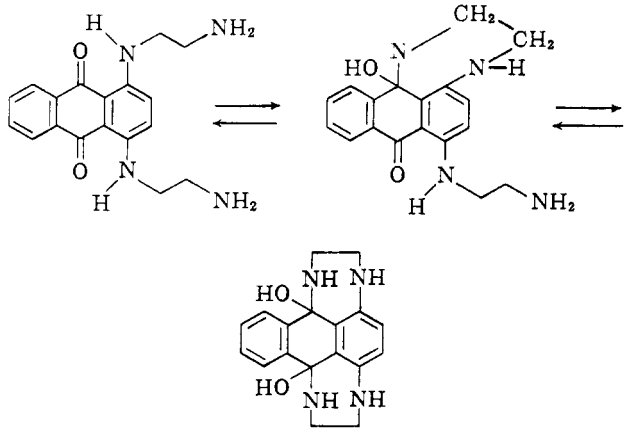


Anthraquinone	R =	M.p., °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
1,4-Bis-(isopropylamino)-	$-CH(CH_3)_2$	179–180	74.51	74.23	6.88	6.78	8.69	8.65
1,4-Bis-(*sec*-butylamino)-	$-CH(CH_3)CH_2CH_3$	155–158	75.40	75.53	7.48	7.40	7.99	7.91
1,4-Bis-(β-hydroxyethylamino)-	$-CH_2CH_2OH$	242.5–244.0	66.24	66.68	5.56	5.54	8.58	8.54
1,4-Bis-(β-aminoethylamino)-	$-CH_2CH_2NH_2$	207–208	66.6	66.7	6.2	6.1	17.3	17.1
1,4-Bis-(γ-aminopropylamino)- dihydrochloride hemihydrate	$-CH_2CH_2CH_2NH_2$	251–253	55.30	55.32	6.27	6.53	12.90	12.70
1,4-Bis-(β-N-ethylaminoethylamino)-	$-CH_2CH_2NHC_2H_5$	120.5–122	69.5	69.4	7.4	6.8	14.8	15.1
1,4-Bis-(β-N-diethylaminoethylamino)-	$-CH_2CH_2N(C_2H_5)_2$	112–112.5	71.6	71.7	8.3	8.3	12.9	12.9
1,4-Bis-(β-acetamidoethylamino)-	$-CH_2CH_2NHCOCH_3$	218–219	64.0	64.4	5.9	5.8	13.7	13.2
				64.4		5.9		

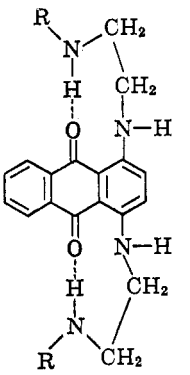
TABLE III

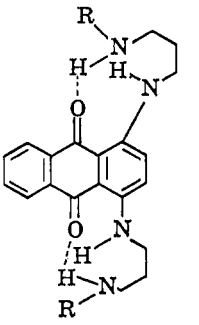
λ, mμ	ν, cm.⁻¹	Δν, cm.⁻¹	λ, mμ	ν, cm.⁻¹	Δν, cm.⁻¹
642	15,600	1200	613	16,300	1300
595	16,800		568	17,600	
555	18,000	1200	532	18,800	1200
608	16,450		495	20,200	1400
566	17,700	1250	427	23,400	
531	18,850	1150	405	24,700	
435	23,000				

The identity of spectra from I, X = NH_2, and I, X = NHEt, argues against an equilibrium situation where the carbinolamine is responsible for the new, broad band in the blue transmission region, since the substitution of an ethyl group for hydrogen should alter the position of this equilibrium. Furthermore, the γ-aminopropyl side chain might be expected to show carbinolamine formation to some extent also.



The explanation we favor is that the aliphatic amino group is hydrogen bonding with the anthraquinone carbonyl group, in competition with the 1-amino group on the anthraquinone ring. This would reduce the strength of the hydrogen bond to the 1-amine. Furthermore, for the aliphatic amine to approach within hydrogen bonding distance of the carbonyl group requires rotation around the bond between the anthraquinone carbon atoms (1 and 4) and the 1-nitrogen, leading to less effective overlap of the free electron pair of the nitrogen with the π-electrons of the aromatic system. By contrast, in the γ-amino propyl case, the hydrogens on the aliphatic amine appear from models to be in close proximity to the carbonyl groups without rotation of the 1-nitrogen to 1-carbon bond.





In agreement with our explanation we find that chemical reactivity of the aliphatic amine in the β-position toward acetylation or alkylation reagents is greatly reduced. 1,4-Bis(β-acetamidoethyl)-anthraquinone could not be made by direct acetylation of I, X = NH_2. Refluxing with ethyl acetate allowed recovery of the diamine, while acetic anhydride–sodium acetate treatment led to a mixture of products with acetylation apparently taking place initially on the α-nitrogen. We are continuing the study of chemical and other physical properties of these dyes.

Experimental

The reaction between leucoquinizarin and aliphatic amines has been reviewed.[6] Numerous recent patents[7] give useful methods, and of particular value where the quantity of amine is limited is the method of McSheehy.[8]

Unless otherwise stated, reactions were carried out in the presence of air and care was taken to exclude carbon dioxide. Analytical and spectral data are summarized in Tables I and II. Microanalyses were by A. Bernhardt, Mulheim, Germany, or C. Fitz, Needham, Mass.

1,4-Bis-(*sec*-butylamino)-anthraquinone.—To a solution of 2.4 g. (0.01 mole) of leucoquinizarin in 30 ml. of pyridine was added 7.0 ml. of *sec*-butylamine. The originally brown solution became blue on refluxing overnight. The product was isolated by pouring the reaction mixture into 100 ml. of 10% HCl, filtering, and recrystallizing from Methyl Cellosolve to yield 1.4 g., m.p. 151–154°, and 1.1 g. melting below 150°. Total crude yield was 71.5%. Recrystallization from 2-methoxyethanol raised the melting point to 153–155.5°.

One gram of material melting below 150° was dissolved in ethyl acetate and passed through an alumina column, the eluate was evaporated to dryness, dissolved in benzene and rechromatographed. Recrystallization from hexane (with a small amount of benzene) yielded 0.41 g., m.p. 155–158°.

(6) J. Houben and W. Fischer, "Das Anthracen und die Anthrachinone," G. Thieme, Leipzig, 1929, pp. 427–428.

(7) (a) J. G. McNally and J. B. Dickey, U. S. Patents 2,188,369 (Jan. 30, 1940) and 2,191,029–30 (Feb. 20, 1940); (b) E. Gutzwiller, U. S. Patent 2,448,094 (Aug. 31, 1948).

(8) J. A. McSheehy, U. S. Patent 2,727,045 (Dec. 13, 1955).

1,4-Bis-(isopropylamino)-anthraquinone.—A commercial sample was similarly purified to give material, m.p. 179–180°.

1,4-Bis-(β-hydroxyethylamino)-anthraquinone.—Leucoquinizarin in excess ethanolamine was heated at 100° for 3–4 hours. The product, which had separated out as fine needles, was washed with water, dissolved in pyridine and precipitated into 2% HCl, then recrystallized from 2-methoxyethanol to yield needles, m.p. 235–236°. A second crystallization gave material, m.p. 242.5–244.0°.[9]

1,4-Bis-(β-acetamidoethylamino)-anthraquinone.—A solution of 5.4 g. (0.052 mole) of β-acetamidoethylamine[10] and 6.25 g. (0.026 mole) of leucoquinizarin in 40 ml. of 1-butanol was refluxed under nitrogen for 5 hours. The red-brown solution was oxidized by pouring into a solution of 2 g. of sodium persulfate in 100 ml. of water and 25 ml. of ethanol and letting stand under a current of air overnight. The semisolid residue which remained after evaporation was washed with water and crystallized from methanol to give 1.9 g., m.p. 213–217°, and 2.9 g., m.p. 210–213°; total crude yield 4.8 g., 45%. Recrystallization from methanol raised the melting point to 218–219°, small dark blue needles.

1,4-Bis-(γ-aminopropylamino)-anthraquinone.—A solution of 2.4 g. of leucoquinizarin in 10 ml. of 1,3-diaminopropane was heated for 30 minutes at 100°, then air was bubbled through for another 10 minutes; 30 ml. of water was added and the mixture allowed to cool. The resulting crystalline mass was filtered, then washed thoroughly with water. Recrystallization from petroleum solvent (b.p. 90–120°) yielded the 1,4-bis-(γ-aminopropylamino)-anthraquinone, m.p. 135.5–137.0°, which was converted into the dihydrochloride, m.p. 251–253°, for analysis.

1,4-Bis-(β-aminoethylamino)-anthraquinone.—A solution of 9.6 g. (0.04 mole) of leucoquinizarin in 50 ml. of ethylenediamine was heated 1 hour at 100°, then air was bubbled through the hot solution for 3 minutes and the mixture let stand overnight at room temperature. The crystalline product which had separated was filtered, washed with a small amount of ether and hot methanol to yield 3.6 g. of copper-colored crystals, m.p. 204–204.5°. Another 0.4 g., m.p. 199–201°, was isolated from the methanol wash solution to give a total of 4.0 g., 41%, crude product. Recrystallization from butanol gave 80% recovery of black crystals with a copper reflex, m.p. 207–208°.

1,4-Bis-(β-N-ethylaminoethylamino)-anthraquinone.—A mixture of 2.4 g. (0.01 mole) of quinizarin, 0.5 g. of sodium carbonate 3.4 g. (0.036 mole) of phenol and 8 ml. of water was swept out with nitrogen, and 3.1 g. (0.035 mole) of β-(N-ethylamino)-ethylamine in 12 ml. of ethanol was added. The mixture was heated under nitrogen at 100° overnight, then precipitated into a saturated sodium chloride solution. The oily product was dissolved in acetone and reprecipitated into saturated sodium chloride solution. The product, still oily, was dissolved in a small amount of ethyl acetate, diluted with benzene, and chromatographed on alumina. Elution with ethyl acetate yielded 0.5 g. of dark needles, m.p. 115–120°. Crystallization from hexane raised the melting point to 120.5–122°.

1,4-Bis-(β-N-diethylaminoethylamino)-anthraquinone.—A mixture of 2.4 g. (0.01 mole) of quinizarin, 4.1 g. (0.035 mole) of β-N-diethylaminoethylamine, 0.5 g. of sodium carbonate, 3.4 g. (0.036 mole) of phenol, 12 ml. of ethanol and 8 ml. of water was refluxed in a nitrogen atmosphere for 6 hours. Spectra of the reaction solution were taken every hour, and the reaction was stopped when the characteristic spectrum remained constant for 2 hours.

The product was isolated by precipitation into saturated sodium chloride solution, reprecipitating from acetone solution into sodium chloride solution two times, from Methyl Cellosolve solution by addition of a small amount of water, and crystallization from hexane to yield 2.1 g., 48.0%, m.p. 109–110°, glistening purple platelets with a red reflex. Chromatography from hexane on alumina followed by elution with chloroform gave a 70% recovery of dark blue radiating needles with a violet reflex, m.p. 111.5–112°. Two more crystallizations from hexane gave dark needles with a purple reflex, m.p. 112–112.5°.

Acknowledgment.—We wish to thank Mr. J. F. Downey and Dr. A. D. Jarrett for the samples of 5,8-dihydroxy-1,4-bis-(β-aminoethylamino)-anthraquinone, Dr. S. M. Bloom for the samples of 1,5-bis-(β-aminoethylamino)-anthraquinone and 1,5-bis-(β-diethylaminoethylamino)-quinone, Mrs. R. W. Fajer for the sample of 1,5-(γ-aminopropylamino)-anthraquinone, and Dr. S. Dershowitz for the sample of 1,5-bis-(β-amino-α,β-dimethylethylamino)-anthraquinone. We also thank Dr. S. G. Cohen for informative discussions.

(9) Y. Bansho, *J. Chem. Soc. Japan, Ind. Chem. Sect.*, **55**, 666 (1952); *Chem. Abstr.*, **48**, 6701*d* (1954), reports m.p. 239°.

(10) S. R. Aspinall, *J. Am. Chem. Soc.*, **63**, 853 (1941).

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL SCIENCES, PURDUE UNIVERSITY, LAFAYETTE, IND., AND THE BIOLOGY DEPARTMENT, BROOKHAVEN NATIONAL LABORATORY, UPTON, N. Y.]

An All-or-None Assay for Assessing the Role of Amino Acid Residues in Enzyme Action—Application to Phosphoglucomutase[1,2]

BY W. J. RAY, JR., AND D. E. KOSHLAND, JR.

RECEIVED DECEMBER 12, 1962

An "all-or-none" assay, which distinguishes fully active and partially active enzymes from inert enzymes in a mixture of all of these, is described. Unlike the usual "efficiency" assay, the "all-or-none" assay gives equal weight to active and partially active enzymes. The difference in the two assays is exploited in interpreting the effect on phosphoglucomutase of the methylene blue-catalyzed photooxidation reaction, and provides evidence for a formation of partially active enzyme intermediates, probably *via* oxidation of a single "surface" histidine residue. A correlation of the activity loss as determined by the "all-or-none" assay with previous data on amino acid modification during photooxidation indicates that production of an inert enzyme having an efficiency less than $1/_{200}$ of the native enzyme parallels the modification of a single surface methionine residue. The general applicability of such "all-or-none" assays to enzymes which form a measurable intermediate of reasonable stability is discussed.

Introduction

Methods utilizing chemical modification as a means of identifying amino acid residues involved in enzyme action have many pitfalls, not the least of which arises from the possibility of producing a partially active enzyme by virtue of the modification reaction under study. For example, if the observed activity of an enzyme has been reduced 10-fold by treatment with a given reagent, the remaining activity might be the result of (a) a small amount (10%) of fully active enzyme in the presence of inert enzyme (90%), (b) the reduced efficiency of a modified enzyme, 10% as active as the native enzyme and (c) complex mixtures of active, partially active and inert enzyme. To aid in the detection and analysis of such situations, an "all-or-none" assay has been devised which, in conjunction with the conventional "efficiency" assay, may also be used to determine whether modification of a given residue produces partially active or inert enzyme.

The conventional "efficiency" assay for assessing enzymic activity measures the amount of substrate converted, or product produced per unit time, under standard conditions. It therefore gives a weighted average of the catalytic efficiencies of the various enzymic species present in the assay. For example, an enzyme preparation which, by virtue of chemical modification, contained fully active enzyme (15%),

(1) A preliminary account of some of this work has been published.[2]

(2) W. J. Ray, Jr., J. J. Ruscica and D. E. Koshland, Jr., *J. Am. Chem. Soc.*, **82**, 4739 (1960).