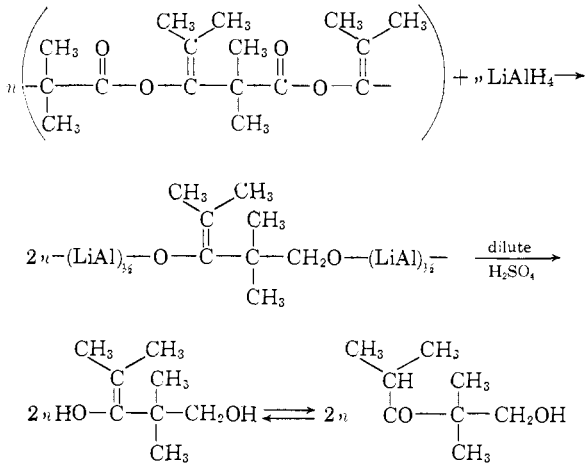
When polymer II dissolved in tetrahydrofuran was treated with $LiAlH_4$, we obtained 2,2,4-trimethyl-1-pentanol-3-one (80% yield). This result agrees with a structure of the polyester type:



Furthermore, ozonolysis of II gave a good yield of acetone. The chemical behavior of polymer II enables us to conclude that it consists of macromolecules derived from the regular, alternate polymerization of the two monomeric units (α) and (β).

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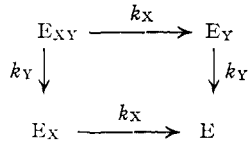
EVIDENCE FOR INVOLVEMENT OF A METHIONINE RESIDUE IN THE ENZYMATIC ACTION OF PHOSPHOGLUCOMUTASE AND CHYMOTRYPSIN

Sir:

In studies to clarify the role of histidine in phosphoglucomutase action it was found[1] that loss of enzyme activity on photoöxidation could be explained on the basis of the model shown in equation 1. Here the various enzyme species are indi-



(1)

cated by subscripts which identify intact amino acid residues. Thus, E_{XY} refers to original enzyme with X and Y unchanged, E_X refers to enzyme in which X is intact but Y has been oxidized, etc. E_X has an activity equal to about 8% of the original activity, while E_Y and E are inactive in the present assay systems.[1] Since Y has been identified as histidine, it was clearly of interest to identify X which is apparently even more important to enzyme activity.

To allow such an identification, the rate constants for oxidation of the susceptible residues of the protein were obtained for comparison with the value of 0.38 min.$^{-1}$ attributed to X by both rate

(1) W. J. Ray, Jr., J. J. Ruscica and D. E. Koshland, Jr., THIS JOURNAL, **82**, 4739 (1960).

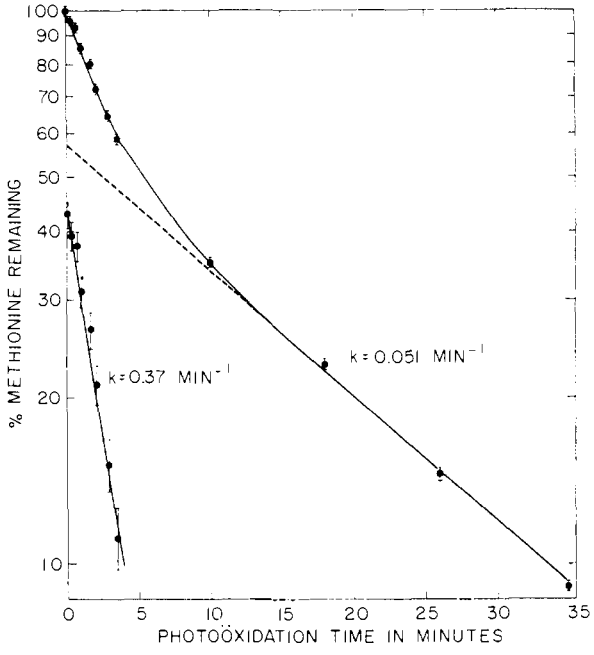


Fig. 1.—Loss of methionine on photoöxidation of phosphoglucomutase.

and "all-or-none" assays. Aliquots of the enzyme were removed at various time intervals during photoöxidation, hydrolyzed and assayed on the automatic amino acid analyzer of Spackmann, Stein and Moore.[2] Since acid hydrolysis is known to destroy tryptophan and was found to cause regeneration of methionine from methionine sulfoxide, these residues were determined after barium hydroxide hydrolysis of the photoöxidized protein. Other residues were determined after acid hydrolysis except for cysteine which was determined by the *p*-chloromercuribenzoate reaction.

The fraction of methionine remaining as a function of photoöxidation time is shown in Fig. 1. This biphasic curve can be analyzed as described previously[3] giving oxidation constants of 0.37 min.$^{-1}$ for the accessible methionines and 0.051 min.$^{-1}$ for the inaccessible residues. The constants obtained for other amino acids were cysteine, 0.07 min.$^{-1}$, tryptophan, about 0.01 min.$^{-1}$, and tyrosine, about 0.01 min.$^{-1}$. No other residues were detectably affected. X can therefore be identified as an accessible methionine residue. Since photoöxidation of this residue produces enzyme which is nert in the "all-or-none" assay, methionine oxidation decreases enzyme activity by a factor of more than 200. It is of interest in this regard that Gundlach, Stein and Moore have found that carboxymethylation of methionine also reduces ribonuclease activity.[4]

Since a similarity in the bond-breaking or "catalytic" residues at the active sites of phosphoglucomutase and chymotrypsin had been indicated in previous work,[3] it was of interest to per-

(2) D. H. Spackmann, W. H. Stein and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).

(3) D. E. Koshland, Jr., W. J. Ray, Jr., and M. J. Erwin, *Federation Proc.*, **17**, 1145 (1958).

(4) H. G. Gundlach, W. H. Stein and S. Moore, *J. Biol. Chem.*, **234**, 1754 (1959).

form equivalent experiments with chymotrypsin. The results of these experiments are shown in Table I. Again the sum of the oxidation constants

TABLE I

RATE CONSTANTS FOR PHOTOOXIDATION OF CHYMOTRYPSIN

Property assayed	k (min.$^{-1}$)
Enzyme activity[a]	0.67
Accessible histidine	.32
Accessible methionine	.36
Inaccessible histidine	.022
Inaccessible methionine	.037
Tyrosine	.01
Tryptophan	.013

[a] Assayed by rate of hydrolysis of acetyltyrosine ethyl ester.

for the accessible histidine and accessible methionine is approximately equal to the observed rate constant of 0.67 min.$^{-1}$ for loss of enzyme activity. These data therefore provide presumptive evidence for analogous involvement of both the histidine and methionine in these two enzymes and hence support the hypothesis[3] that the bond changing or "catalytic" amino acids may be similar in enzymes of widely diverse specificity.

The inference that the thioether group of a methionyl residue is involved in enzyme catalysis is of particular interest since this group has no significant acidic or basic properties in aqueous solutions, but it is a powerful neighboring group as indicated by the reactivity of mustard gas.[5]

(5) *Cf.* J. Hine, "Physical Organic Chemistry," McGraw-Hill Book Co., New York, N. Y., 1956.

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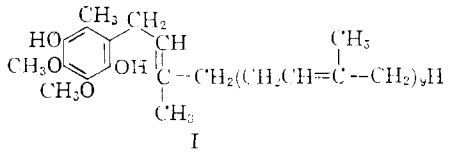
RECEIVED JULY 8, 1960

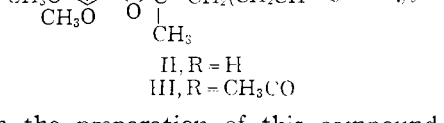
COENZYME Q. XXI. CONVERSION OF COENZYME Q_{10} INTO THE CORRESPONDING CHROMANOL

Sir:

The reduction of coenzyme Q_{10} to the hydroquinone (I) with subsequent refluxing in acetic acid containing potassium bisulfate has yielded a product which has the properties of the corresponding chromanol II or 7,8-dimethoxy-2,5-dimethyl-2-[3′-methyl-2′-butenyl-oktakis-(3′-methyl-2′-butenylene)methyl]-6-chromanol.



I



II, R = H
III, R = CH_3CO

Studies on the preparation of this compound, also called ubichromanol, have been summarized,[1,2] but the solutions were examined only by paper chromatography and ultraviolet spectral measurements; we repeated these experiments,[1,3] but were unable to obtain the chromanol. Other reaction conditions successfully converted hexahydrocoenzyme Q_4 to 7,8-dimethoxy-2,5-dimethyl-2-(4′,8′,12′-trimethyltridecyl)-6-chromanol[4]; such conditions with coenzyme Q_{10} apparently gave chromanol formation with concurrent cyclizations of the 10-unit isoprenoid side chain. The product[4] lacked detectable unsaturated isoprenoid units by n.m.r. data. Others[5] attempting to prepare ubichromanol reported materials with unexpected properties, possibly due to side chain cyclization.

A solution of coenzyme Q_{10} hydroquinone in acetic acid containing potassium bisulfate was refluxed one hour and then concentrated. A solution of the residue in Skellysolve B was water-washed and chromatographed on Florisil. Fractions eluted with ether–isoöctane yielded the chromanol II which could also be purified by distillation *in vacuo*. The 60 megacycle n.m.r. spectrum of the oily product in carbon tetrachloride is the most critical criterion of its identity; tau (τ) 4.84 (-OH); 4.96 (HC=); 6.12 and 6.25 (CH_3O—); 7.50 triplet (—CH_2C=); 7.98 (CH_3C= ring); 8.02 (=$C(CH_2)_2C$=); 8.40 (CH_3C=); 8.73 (CH_3—C—O). This spectrum excluded a coumaran nucleus.[6] Ultraviolet spectrum: λ_{max} 292 mμ, $E^{1\%}_{1\ cm.}$ = 38; infrared spectrum, band at 2.85 μ (—OH), no band for C=O in the 6 μ region and a band at 9 μ.

Anal. Calcd. for $C_{59}H_{92}O_4$: C, 81.89; H, 10.72. Found: C, 82.11; H, 10.50.

The Q_{10} hydroquinone (I) and the chromanol II are not clearly distinguishable by ultraviolet maxima alone in mixtures, and by infrared data, but are differentiated by R_f and n.m.r. comparisons.

The chromanol II in pyridine was treated with acetic anhydride. Subsequent purification by adsorption on silica gel from isoöctane solution and elution by ether in isoöctane gave a chromatographically homogeneous band; λ_{max} 282 mμ, $E^{1\%}_{1\ cm.}$ = 26 in isoöctane. The n.m.r. spectrum is consistent with the chromane acetate structure III.

Anal. Calcd. for $C_{61}H_{94}O_5$: C, 80.75; H, 10.44. Found: C, 80.79; H, 10.24.

Drs. David Green and Robert Lester[7] have tested the chromanol II and reported it to have no coenzymatic activity *in vitro* like that of Q_{10} for electron transfer.

This chromanol II is of current interest[8-10]

(1) J. Bouman, E. C. Slater, H. Rudney and J. Links, *Biochim. Biophys. Acta*, **29**, 456 (1958).
(2) J. Links, *ibid.*, **38**, 193 (1960).
(3) J. Bouman and E. C. Slater, *ibid.*, **26**, 624 (1957).
(4) C. H. Shunk, N. R. Trenner, C. H. Hoffman, D. E. Wolf and K. Folkers, *Biochem. Biophys. Res. Com.*, **2**, 427 (1960).
(5) D. A. Laidman, R. A. Morton, J. Y. F. Paterson and J. F. Pennock, *Biochem. J.*, **74**, 541 (1960).
(6) I. Cmielewska, *Biochim. Biophys. Acta*, **39**, 170 (1960).
(7) Enzyme Institute, University of Wisconsin.
(8) R. D. Dallam and J. F. Taylor, *Fed. Proc.*, **18**, 210 (1959).
(9) P. J. Russell and A. F. Brodie, *ibid.*, **19**, 38 (1960).
(10) E. E. Jacobs and F. L. Crane, *Biochem. Biophys. Res. Com.*, **2**, 218 (1960).