Conformational Isomerism and Effective Redox Geometry in the Oxidation of Heme Proteins by Alkyl Halides, Cytochrome *c*, and Cytochrome Oxidase†

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ABSTRACT: In contrast to its lethargy at physiological pH, horse heart cytochrome *c* can be oxidized at room temperature by the axial inner sphere oxidant bromomalononitrile (BMN) at higher acidities. The following stoichiometry obtains: $2Fe^{II}$ *c* + $BrCH(CN)_2$ + H^+ → $2Fe^{III}$ *c* + $CH_2(CN)_2$ + Br^-, and the rate law is given by: rate = $k_2(Fe^{II}\ c)$(BMN). At an ionic strength of 1.0 (KCl), second-order rate constants vary from 300 l. per mol per sec (pH 2–3) to 0 (pH 9). Below pH 6 there is a noticeable increase in rate with ionic strength while there is no specific salt effect for the process. At pH 7.4 there is no influence of added salt (0.01–1.0 M) upon the slow rate of reaction. The vast changes in rate occur over a pH region (3–6) in which only very minor changes in the visible spectrum of the cytochrome are manifest. The results are interpreted in terms of a conformational isomerism of cytochrome *c* in which the effective redox geometry alters from a predominantly "short C" form (in which an axial position is available for substitution) at lower pH's to a predominantly "C" form (axial positions encumbered) in the physiological region. At 5°, pH 7.4, both hemes of beef heart cytochrome oxidase are oxidized by the addition of BMN (k_2 = 29 ± 3 l. per mol per sec). However, the reaction is inhibited by potassium cyanide and the protein containing iron(II) cyt *a* along with the cyano adduct of iron(II) or iron(III) cyt a_3 is inert. The results demonstrate cytochrome a_3 as the site of reaction and that *a* reduces a_3 in the process. Cytochrome oxidase does catalyze the oxidation of cytochrome *c* with BMN as substrate. Taken together the results provide additional support for a recent theory and they demonstrate BMN to be an efficient probe for the effective redox geometry of a hemoprotein in solution.

The conformation of the protein about its highly ordered organometallic active site has been portrayed as a dominant factor in explaining the wide variety of chemical reactivity exhibited by hemoproteins (Castro, 1971). This modulating constraint along with the influence of the axial ligands of iron upon the redox properties of its porphyrin complex allow an assignment of specific "electron transfer" characteristics. One set of predictions, the relative ease of oxidation of the iron(II) complexes of hemoglobin, myoglobin, and cytochrome *c* by alkyl halides, has been verified (Wade and Castro, 1973a). Based upon the mechanism of this reaction with hemes (Wade and Castro, 1973b), it can be concluded that the general "G" and "C" conformation ascribed to these proteins, and manifest in the solid state (Perutz *et al.*, 1968; Kendrew and Shore, 1960; Watson, 1969; Dickerson *et al.*, 1971; Dickerson and Geiss, 1969; Takano *et al.*, 1972a,b; Mathews *et al.*, 1972), are dominant in solution at physiological pH. Thus a correlation of solution geometry with redox reactivity should be possible and it should provide a basis for assessing the general conformation type of hemo proteins of unknown structure. The present work was undertaken to more critically test the theory by determining whether a given protein (cytochrome *c*) of low reactivity toward oxidation by a neutral axial inner sphere oxidant (bromomalononitrile) could be made reactive by deliberately altering its solution conformation, then, if the results were also consistent with theoretical interpretation, to apply the same substrate to a protein of unknown structure (cytochrome oxidase).

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Cytochrome *c* itself has been the subject of a recent onslaught of inorganic reagents all aimed at plumbing the mechanism of electron transfer by this protein. Thus kinetics and activation parameters for the reduction of the ferriprotein by Cr^{II} (Yandell *et al.*, 1973; Dawson *et al.*, 1972; Grimes *et al.*, 1974), $Ru^{II}(NH_3)_6$ (Ewall and Bennet, 1974), Fe^{II}(EDTA) (Hodges *et al.*, 1974), dithionite (Miller and Cusanovich, 1974; Creutz and Sutin, 1973; Lambeth and Palmer, 1973), and e^-_{aq} (Land and Swallow, 1971; Pecht and Farragi, 1972) have been ascertained, and recent summaries have been made by Sutin and Cusanovitch. In order to minimize the possible ambiguity of conformation changes that may be attendant upon the interaction of a charged metal ion with binding sites on the protein surface, and to avoid the additional ambiguity of liganding in the transition state of metal ion reactions, we have chosen neutral organic reagents to probe the electron transfer capacities of hemoproteins. Moreover, the organic products themselves can provide some mechanistic insight.

Results

Cytochrome c. STOICHIOMETRY. The overall stoichiometry for the oxidation of iron(II) cytochrome *c* by bromomalononitrile (BMN)[1] at acid pH is sketched in eq 1. As

$$2Fe^{II}\ \text{cyt}\ c + Br\text{—}C(CN)_2 + H^+ \longrightarrow 2Fe^{III}\ \text{cyt}\ c + NC\text{—}CH\text{—}CN + Br^- \quad (1)$$

noted previously, at physiological pH, the reaction is slow on a time scale for the corresponding oxidation of hemoglobin or myoglobin (Wade and Castro, 1973a). The stoichi-

[1] Abbreviation used is: BMN, bromomalononitrile.

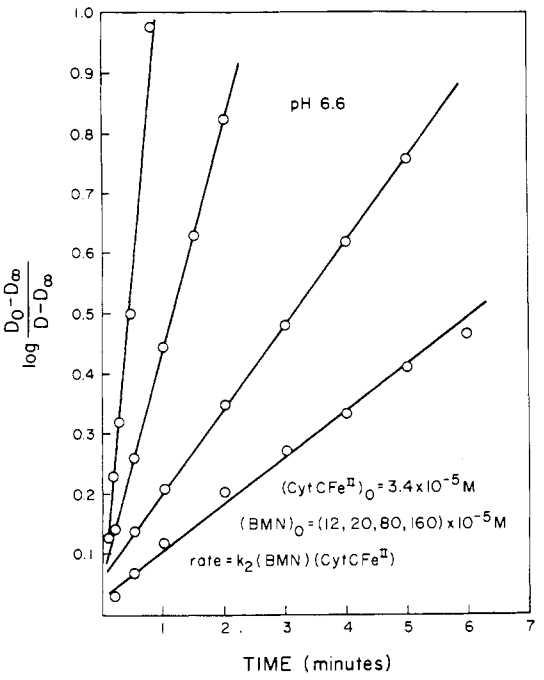


FIGURE 1: Rates of oxidation of iron(II) cytochrome *c* by bromomalonotrile, at pH 6.6, μ = 1.0 (KCl), 25°.

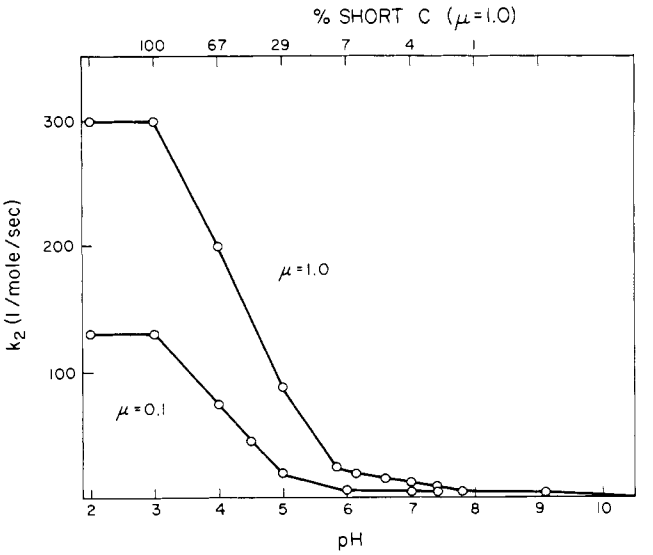


FIGURE 2: Rate constants for the oxidation of iron(II) cytochrome *c* as a function of pH and ionic strength at 25°.

ometry was established at pH 4.0 in solutions that had an ionic strength of 0.1 (KCl). Under these conditions, with initial concentrations of 3.5×10^{-5} M cyt *c* and 6×10^{-4} M BMN, the reaction was complete in ~5 min. The yield of the hydrogenolysis product, malononitrile, was quantitative. The spectrum of the ferriprotein in the product solutions corresponded to that of the starting iron(III) spectrum before dithionite reduction. At all conditions of pH and ionic strength employed herein the product spectrum matched that of stock solutions of the ferricytochrome *c* in the same millieu.

KINETICS. Rates were monitored spectrophotometrically by following the decrease in absorbance at 550 mμ. Typical pseudo-first-order plots of the data at pH 6.6, μ = 1.0 (KCl), are presented in Figure 1. The reaction is an overall second-order process under all conditions examined: rate = $k_2(Fe^{II}$ cyt $c)$(BMN). Plots like those in Figure 1 were obtained at each pH examined, and in general rate constants under any set of conditions were in agreement within 10%.

The influence of pH and ionic strength upon the process is portrayed in Figure 2. The upper curve portrays the influence of pH upon the rate at an ionic strength corresponding to 1.0 M KCl. The lower curve reflects the same measurements taken in the presence of 0.1 M electrolyte. It also represents the rate obtained in the presence of 0.01 M buffer without the addition of added salt. Each point is an average of at least four determinations and reproducibility from pH 4 to 9 was ±10%. The quite rapid reactions at pH 3 and 2 are at the limits of our methodology. Reproducibility in this range was ±30% and reflects the difficulty of maintaining the cytochrome in the reduced state at these pH's. An estimate of the per cent of conformers that possess an axial ligand available for substitution is indicated as % short C at the top of Figure 2 (*cf.* Discussion). It was calculated from the rates assuming a k_2 of 300 l. per mol per sec represents the reaction of an unencumbered heme (Wade and Castro, 1973).

In the acid range the rates diminish substantially as the ionic strength decreases. Moreover, at either high or low ionic strength the same pattern of response to pH is manifest—a marked falling off as the physiological range is approached. Indeed at pH 7.4 a variation in added salt concentration from 0 to 1.0 M causes no measurable change in the rate.

No specific salt effects were observed for this process. The same constants were obtained with either sodium or potassium cations and chloride, nitrate, acetate, sulfate, or perchlorate anions.

For a comparison with the rate data the visible absorption at bands that may be expected to change with conformation or ligand substitution of both the iron(II) and iron(III) protein was examined. Only very slight changes in the spectrum can be discerned (μ = 1.0) in either valence state over the pH range 3–7. Thus, absorption at 416, 550, or 543 nm of the iron(II) protein changes by at most ±4%. On the other hand, the 695-nm band in the iron(III) cytochrome noticeably lessens below and above this range in agreement with the observations of Shejter and George.

Cytochrome Oxidase. STOICHIOMETRY. Reactions with the reduced beef heart oxidase were conducted at 5° in 0.1 M phosphate buffer at pH 7.4 in an argon atmosphere. The overall stoichiometry is that given in eq 2.

$$[Fe^{II}\ \text{cyt}\ a + Fe^{II}\ \text{cyt}\ a_3] + Br{<}^{CN}_{CN} + H_2O \longrightarrow [Fe^{III}\ \text{cyt}\ a + Fe^{III}\ \text{cyt}\ a_3] + NC{<}^{}_{}{>}CN + HO^- + Br^- \quad (2)$$

At concentrations of 8×10^{-5} M oxidase and 100×10^{-5} M BMN the reaction was complete in ~1 min. The product spectrum corresponded to that of the starting iron(III) oxidase before reduction. Both hemes are oxidized. However, the reaction can be completely inhibited with 4×10^{-3} M potassium cyanide. Moreover, an oxidase comprised of the cyano adducts of cyt a_3 in both its iron(II) and iron(III) valence states along with the reduced form of cytochrome *a* (Lemberg, 1969; Yonetani, 1960) was unreactive. Thus a 6×10^{-5} M solution of the protein conglomerate obtained by dithionite reduction (λ_{max} 444 and 605) followed by the addition of cyanide (4×10^{-3} M) showed *no* spectral change 24 hr after exposure to a concentration of 6×10^{-4} M BMN. Similarly, at these same concentrations addition of

Table I: Rates of Oxidation of Cytochrome Oxidase by Bromomalononitrile in 0.1 M Phosphate Buffer at pH 7.4.

Temp (°C)	(Cyt Ox Fe_2^{II})	(BMN)	k_2 (l. per mol per sec)	Activity[a] (μmol of Fe^{III} cyt c per min per mg of P)
5	5.5×10^{-5}	2.75×10^{-4}	29 ± 3[b]	1.0–8.0
5	0.85×10^{-5}	1×10^{-3}	25.5 ± 2.5[c]	8.0
24	1.15×10^{-5}	0.7×10^{-3}	31 ± 3[d]	2.2
24, 1 day	1.3×10^{-5}	0.7×10^{-3}	16 ± 1.6	2.2

[a] At pH 6.5. [b] Average of ten runs, (cyt ox $Fe^{II})_0$ = (0.8–6.5) × 10^{-5} M, $(BMN)_0$ = (2.75–8.0) × 10^{-4} M. [c] In the presence of 1 M KCl. [d] Average of two runs.

cyanide to the iron(III) oxidase followed by dithionite reduction of cytochrome a resulted in an entity (λ_{max}, 430, 440, and 603) that was equally inert. These results establish that cytochrome a_3 is the site of oxidation by bromomalononitrile and that cytochrome a is inert.

KINETICS. Because of the relatively fragile nature of the oxidase it was not possible to subject it to the variety of conditions employed with cytochrome c. The activity of our best preparations toward iron(II) cytochrome c and oxygen did fade with time. The influence of temperature, aging, and 1 M KCl upon the rate of oxidation of the reduced oxidase by BMN and its aerobic enzymatic activity of iron(II) cyt c are presented in Table I. Rate constants for the BMN oxidation were calculated from an initial slopes treatment using the overall second-order rate expression: rate = k_2(reduced oxidase)(BMN). The reaction was followed spectrophotometrically at 5° by monitoring the decrease in absorbance at 444 mμ. Good reproducibility was obtained over a tenfold change in each of the reactant concentrations. It will be noted that the rate of oxidation by BMN *does not* parallel the enzymatic activity of the oxidase toward oxygen and cytochrome c. Moreover there is no salt effect at this pH.

CYTOCHROME c + CYTOCHROME OXIDASE. As a check on the integrity of the oxidase during oxidation by BMN, reconstitution experiments were undertaken. A catalytic amount of the oxidase (initial concentration 0.02 × 10^{-5} M) was added to a solution containing a 180-fold excess of Fe^{II} cytochrome c (3.6×10^{-5} M) under argon at room temperature and pH 7.4 (0.01 M phosphate buffer–1 M KCl). Subsequently BMN (80×10^{-5} M) was added to the reaction mixture in place of oxygen. The disappearance of iron(II) cytochrome c was followed at 550 nm. A typical run is presented in Figure 3.

It will be noted that upon addition of the oxidase a brief oxidation of cytochrome c ensues and ceases. We presume this is due to vestiges of oxygen in the reaction solution. Upon addition of BMN reaction commences rapidly and proceeds to the complete oxidation of cytochrome c. It should be emphasized here that in repeated runs with different oxidase preparations the apparent rate of "oxidation of cytochrome c" by BMN in the presence of oxidase (1.4–2.3 × 10^{-5} mol per l. per min) was at least six but usually ten times that for cytochrome c alone (0.23 × 10^{-5} mol per l. per min). Thus, both proteins can perform a mitochondrial function in solution while the oxidase is oxidized by the halide. The sequence then is (3) followed by (2).[2]

$$2c(Fe^{II}) + a(Fe^{III}) + a_3(Fe^{III}) \longrightarrow 2c(Fe^{III}) + a(Fe^{II}) + a_3(Fe^{II}) \quad (3)$$

Moreover, the rate of turnover of cyt Ox by cyt c is of the same order of magnitude with either O_2 or BMN as substrate.

Discussion

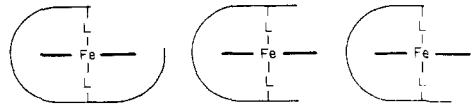
The oxidation of iron(II) porphyrins by alkyl halides proceeds in two steps (Wade and Castro, 1973) (eq 4 and 5).

$$Fe^{II} + XR \underset{}{\overset{a}{\rightleftharpoons}} Fe^{II}(XR) \overset{b}{\rightleftharpoons} \left[Fe\cdots X\cdots R \right]^{\ddagger} \rightleftharpoons Fe{-}X + R\cdot \quad (4)$$

$$Fe^{II} + R\cdot + H^+ \xrightarrow{\text{fast}} Fe^{III} + RH \quad (5)$$

The rate-determining step in the overall process is the scission of the carbon–halogen bond (eq 5). It is an "axial inner sphere" process in which a halogen atom is transferred from carbon to iron. The reversibility of (eq 4) and the very fast reduction of radicals by hemes (eq 5) are general paths of reaction of free radicals with these iron complexes (Castro *et al.*, 1974).

The dominant steric constraints of the apoprotein upon an iron porphyrin have been formulated as three broad classes of solution geometries.



G C short C

To be rapidly oxidized by an alkyl halide then, a hemoprotein must possess either the "G" or "short C" conformation in order that the oxidant can easily enter the inner coordination sphere of iron(II). Thus, cytochrome c ("C" conformation axial positions encumbered) should be inert or slow to react compared to the globins. This is the case at physiological pH (Wade and Castro, 1973a). The present work provides an additional test of the theory and an extension of our earlier findings.

Cytochrome c. The dramatic change in the rate of oxidation of the iron(II) protein by BMN with pH can be taken as a general measure of the influence of the apoprotein upon axial inner sphere oxidation mechanisms. Thus at low pH the rate of BMN oxidation approaches that of hemoglobin or myoglobin or an unencumbered heme in solution while at neutral and physiological pH it is quite slow. At pH 9 reaction does not proceed at all. (It is not possible to probe above this pH with BMN because of the increasing rate of hydrolysis of the substrate.) Thus at the low acidities an axial position is freely available for substitution and process 4b is rate limiting as it is with hemes in amide solu-

[2] The present results do not speak to the possible role of copper in these processes. Its implication is not necessary to explain the observations herein. Hence, it is eliminated from the discussion.

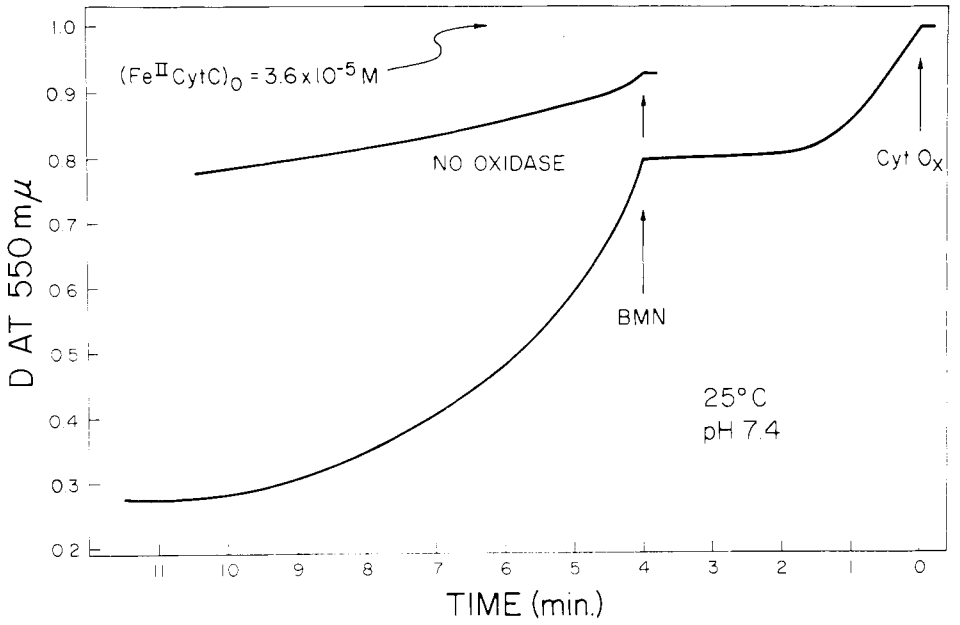
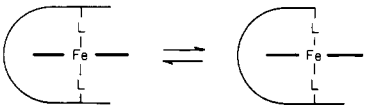


FIGURE 3: The oxidation of iron(II) cytochrome *c* catalyzed by cytochrome oxidase with oxygen and bromomalononitrile as substrates.

tions. As the pH increases, the entrance of substrate into the inner coordination sphere (eq 4a) becomes rate limiting, and reaction ceases when the substrate no longer can approach iron. It is important to note that it must be the protein and not the nature of the axial ligands on iron *per se* that control the overall rate of the reaction. Thus, the fast substitution of iron(II) hemoglobin or myoglobin by neutral molecules is of the order of 10^3–10^4 more rapid (Antonini and Brunori, 1971) than the fastest rate of oxidation of cyt *c* by BMN. Moreover, hexacoordinate iron porphyrins that contain imidazoles bonded to iron and covalently linked to the porphyrin ring have been prepared. The imidazoles are easily substituted from these iron(II) active sites in solution (Castro, 1974). The rate of substitution into the inner coordination sphere of the iron(II) protein by BMN must then be governed by the tertiary structure of the protein and not the nature of the axial ligand that is being substituted. Thus, the BMN rates allow a direct assessment of effective redox geometry of the protein in solution. Of the population of conformations of iron(II) cyt *c* in solutions at high ionic strength it can be stated that all of them have an axial position available for substitution at pH 3 but only ~1% possess such a conformation at pH 8 (*cf.* Figure 2). In our formulation the results represent a conversion in solution from the C to the short C conformation type (eq 6). This view is



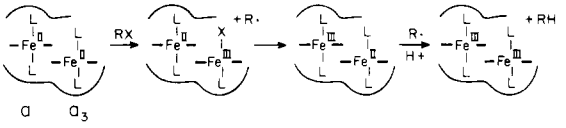
(6)

pH 7.4 pH 3

consistent with the notion of conformational isomerism in cytochrome *c* and its potential significance as discussed by Shejter and George (1964) over a decade ago. Indeed the general "C" conformation ascribed to the cytochromes and noted above can be taken as a formulation of the "closed crevice" or "two bond" crevice structure advocated by George and Lyster (1958) before X-ray analyses were available. It is to be emphasized here, however, that the vast changes in reactivity with pH noted *are not* accompanied by any significant change in the absorption spectrum of the iron(II) protein. Moreover the intensity of the 695-nm band in the ferriprotein is not altered. (Recent evidence demonstrates the 695-nm band *cannot* be the result of an iron–thioether linkage, *cf.* Castro, 1974, for a discussion.) In agreement with Shejter and George (1964) this band diminishes at the low and higher end of the pH range examined herein but absorption is essentially constant over the range for which the iron(II) complex most markedly changes its oxidizability by BMN. Thus the BMN rates form a direct measure of effective redox geometry of the protein and they are much more sensitive to solution conformation than is the visible spectrum.

The effect of changing ionic strength further underlines the dominant influence of protein geometry upon redox characteristic. For example, below pH 7, an axial ligand is more accessible at high rather than low ionic strength. At pH 2, the short C conformation dominates while at pH 7.4 the C conformation is not altered by added salt. As with pH it is to be emphasized that the salt effects that manifest in a rate difference are the result of a changing protein conformation and not an alteration of iron coordination. Thus, the reaction of an iron(II) porphyrin with BMN represents the interaction of two neutral centers and there should be no significant salt effect on this process.

Cytochrome Oxidase. Cytochrome a_3 was predicted to be oxidized by alkyl halides while cyt *a* was predicted to be inert. The present results accord with these predictions and provide additional support for the theory (Castro, 1971). The oxidation of both hemes by cytochrome but only one of them by BMN suggests a juxtaposition of hemes in the oxidase and a reaction sequence like that depicted in eq 7.

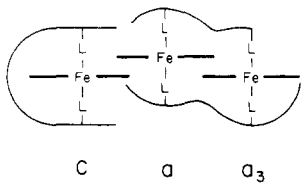


(7)

Clearly the lack of a correlation between the rate of oxidation by BMN and reduction of the oxidase by cyt *c* empha-

size the fact that minor alterations in the preparations influence interaction of this fragment with *c* but not with BMN. Thus, small changes in the surface structure of the conglomerate oxidase may greatly alter proper alignment with cyt *c* for maximal electron transfer rates in solution (*cf.* Smith *et al.*, 1974). The BMN oxidation, while a sensitive probe to gross conformation of the cytochromes, provides no information on the surface factors that may be involved for the proper orientation of these entities in their native mitochondrial environment.

The reconstitution experiment in solution, however, does suggest a collisional alignment leading to reaction that may be more efficiently operative in a fixed membrane. The similarity in rates for the cyt ox catalyzed oxidation of cyt *c* with O_2 and BMN as substrate suggests reaction 3 is the rate-limiting step in the overall process in solution. Under these conditions neither iron(II) cytochrome *c* nor cytochrome *a* is capable of rapid axial inner sphere oxidation. In accord with these findings and the determined mitochondrial sequence (Chance and Williams, 1956; Chance *et al.*, 1973; Erecinska and Chance, 1972), the reduction by cyt *c* can be formulated as a peripheral π transfer:



(8)

In addition the results do show that the oxidase can be employed to carry out a catalytic hydrogenolysis of an organic halide in aqueous solution.

Experimental Section

Cytochrome *c* was Sigma Type III and it was employed directly. The oxidase was isolated from beef heart by the Yonetani procedure (Yonetani, 1960). After the last fractionation, 27–34% ammonium sulfate, the oxidase was dissolved in 0.1 M phosphate buffer at pH 7.4 in the presence of 0.1% Tween-80. Bromomalononitrile was obtained as previously described (Wade and Castro, 1973). Oxidase preparations were discarded after 1 week. The effect of aging upon activity is noted in Table I. The enzymatic activity toward cytochrome *c* and oxygen was measured by the procedure of Smith (1955) except that a 0.1 M phosphate buffer at pH 6.0 was employed. Iron(II) cytochrome *c* for the assay was obtained as outlined by Morrison (Horie and Morrison, 1963). Protein concentration was determined with bovine serum albumin as a standard (Itzahaki and Gill, 1964). The product malononitrile was analyzed by direct flame ionization gas chromatography in the manner previously outlined (Wade and Castro, 1973).

Kinetics. In general 50 ml of fresh oxidase ($\sim 10^{-5}$ M) was reduced with a slight excess of sodium dithionite in a three-necked flask under a gentle argon sweep at 5°. The flask was immersed in an ice bath and it contained a serum cap fitted with a long hypodermic needle to which was fitted a spectrophotometric cell similarly equipped. After sweeping the solution for 12 hr, 2 ml of precooled solution was transferred *via* the hypodermic needle into the cuvet with argon pressure. Appropriate amounts of a fresh 2×10^{-2} M solution of BMN were added under argon to the cuvet by a microliter syringe. The reaction was monitored at 444 mμ at 5°.

Cytochrome *c* reactions were conducted in similar fashion and monitored at 550 mμ at 25°.

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Proteolysis of Paramyosin from *Mercenaria mercenaria* and Properties of Its Most Stable Segment†

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ABSTRACT: The helical muscle protein paramyosin appears to consist of three segments of approximately equal size that differ in stability to guanidine hydrochloride and heat. The N-terminal segment is most stable and the C-terminal segment is least stable. These differences in stability serve as a basis for design of proteolytic digestions to specifically remove segments of low and intermediate stability. Thus, at room temperature only the C-terminal region was susceptible to digestion by pepsin or trypsin. Proteolytic removal of the latter region resulted in the accumulation of the remaining ⅔ of the paramyosin molecule as a segment (PPC-1) of 140,000 daltons that was still in a stable helical conformation. Proceeding to more rigorous conditions, papain digestion of either paramyosin or PPC-1 in 4 M guanidine · HCl that would be expected to destabilize all but the N-terminal segment did result in cleavage of all except that region. The N-terminal region accumulated as a helical segment of 74,000 daltons (PPC-2) if digestion was limited to 1.5 hr or a smaller segment of 58,000 daltons (PPC-3) if digestion continued for 24 hr. Stability of the three PPC segments to guanidine · HCl and heat was measured by change in fluorescence of tyrosyl residues upon loss of the helical conformation. The stability of the segments corresponded well with the stability of those regions in the paramyosin molecule from which the segments were believed to have come. Amino acid composition of the PPC segments and of paramyosin were all very similar, and prediction of relative stability of these helical proteins from inspection of gross amino acid composition does not appear promising.

Paramyosin is the protein that constitutes the core of thick filaments in adductor muscle of the clam (Kahn and Johnson, 1960,; Szent-Györgyi *et al.*, ,1971). It is a large molecule of 200,000 daltons that is rod shaped, 1255 Å long by 20 Å diameter, and consists of two intertwined α-helical chains (Lowey *et al.*, 1963; Cohen *et al.*, 1971). About 90% of the polypeptide chain is in the α-helical conformation on the basis of optical rotatory dispersion measurements by Cohen and Szent-Györgyi (1957). Subsequent optical measurements indicated that the α-helical conformation could be disrupted in distinct stages by elevation of temperature or addition of Gdn · HCl[1] (Riddiford, 1966; Olander, 1971; Cowgill, 1972, 1974; Halsey and Harrington, 1973). The stepwise loss of helical conformation is ascribed to differences in stability of regions or segments of the molecule, and the most explicit description of these regions is given in Figure 1.

Location of the regions of different stability in paramyosin was greatly aided by the ability to partially digest the molecule with proteolytic enzymes and to isolate proteolysis-resistant segments. Cowgill (1972) exposed paramyosin to attack by five proteolytic enzymes and in each case obtained a single high molecular weight segment. Two of these, the pepsin-resistant core (PPC-1) and the trypsin-resistant core (PTC-1) of 140,000 daltons, were studied extensively and appear to be identical except for minor differences at the C-terminal region. Halsey and Harrington (1973) digested paramyosin with trypsin and obtained a segment termed light paramyosin. From a comparison of digestion procedures and reported properties of the product, PTC-1 and light paramyosin are the same segment. This segment is believed to arise from the N-terminal two-thirds of paramyosin on the basis that cysteine is N-terminal in PPC-1 and PTC-1 as well as paramyosin (Cowgill, 1972). If Figure 1 is correct and the middle segment of paramyosin is less stable than the N-terminal region, it should be possible to continue this digestive process under conditions that are believed to destabilize all except the N-terminal segment and to observe the proteolysis of all of the paramyosin or PPC-1 except for this most stable segment. This prediction has proved to be true and the digestive procedures and some properties of the smaller segments designated PPC-2 and PPC-3 are described in this paper.

Materials and Methods

Materials. The enzymes mentioned in Table I were all of the highest purity available from Sigma Chemical Co. or Worthington Biochemical Corp. Gdn · HCl was of the Ultra Pure grade supplied by Schwarz/Mann Research Lab. and was found free of any fluorescent impurities in the region of interest (270–350 nm).

Preparation of Paramyosin, PPC-1, and PTC-1. Par-

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[1] Abbreviations used are: Gdn · HCl, guanidine hydrochloride; PPC-1, paramyosin pepsin-resistant core; PTC-1, paramyosin trypsin-resistant core; PPC-2, the paramyosin papain-resistant segment of 74,000 daltons; PPC-3, the paramyosin papain-resistant segment of 58,000 daltons; Mops, morpholinopropanesulfonic acid.