Stoichiometric Aspects of Uncoupling of Oxidative Phosphorylation by a Salicylanilide Derivative*

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ABSTRACT: The amount of the uncoupler, 5-chloro-3-*tert*-butyl-2′-chloro-4′-nitrosalicylanilide, required for 100% uncoupling and maximal release of respiratory control in rat liver mitochondria was dependent on the type of substrate and the rate of oxidation.

At equivalent oxidation rates, the number of molecules of the uncoupler per molecule of endogenous cytochrome *a* was approximately 0.39 with ascorbate–*N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (third phosphorylation site), 0.81 with succinate (second and third sites), and about 1.1 with glutamate–malate (all three sites) as substrate. Thus the uncoupler titers were approximately proportional to the number of phosphorylation sites. The uncoupler titer required for maximal stimulation of azide-inhibited respiration was the same (1.2–1.4 moles/mole of cytochrome *a*) for succinate as well as glutamate–malate; but the titer varied with the concentration of tetramethylphenylenediamine. High concentrations of the dye produced uncoupling.

A wide variety of compounds uncouple mitochondrial oxidative phosphorylation. One of the more effective uncouplers is carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (Heytler, 1963). In the studies of Margolis and coworkers (1967), this uncoupler appeared to be effective at levels stoichiometric with the number of coupling sites that were actively phosphorylating during the oxidation. When the oxidation rate was limited, the uncoupler requirement also decreased proportionately. In recent preliminary communications (Kurup and Sanadi, 1968; Sanadi, 1968) it was reported that under optimal conditions for maximal oxidative rates, one molecule of the carbonyl cyanide phenylhydrazone was required for uncoupling each phosphorylation site of the respiratory chain. Thus, the ratio of uncoupler concentration for uncoupling with NADH, succinate, and ascorbate–TMPD[1] was nearly 3:2:1. The assays used for the measurement of uncoupling efficiency were the decolorization of membrane-bound bromothymol blue coupled to substrate oxidation and ATP-driven NAD reduction by succinate. Wilson (1969) and Wilson and Azzi (1968) attempted to verify these measurements using release of respiratory control and activation of latent ATPase of rat liver mitochondria as assays and a salicylanilide derivative (CBCN-salicylanilide) (Williamson and Metcalf, 1967) as the uncoupling compound. They reported that one molecule of the salicylanilide per molecule of cytochrome *a* (or per respiratory assembly) sufficed to produce maximal release of azide-inhibited respiration. They also concluded that the release of respiratory control could not be used to determine the stoichiometry of uncoupler binding sites in intact mitochondria (Wilson and Azzi, 1968). In the present experiments the relationship of uncoupler concentration to respiratory assembly has been determined in tightly coupled mitochondria using the most active available uncoupler and the most acceptable assay for energy conservation in mitochondria, namely, P:O determinations. Data obtained with other assays have been compared with above stoichiometry.

Experimental Section

Rat liver mitochondria were isolated by differential centrifugation (Hogeboom, 1955). The initial homogenizing medium was 0.25 M sucrose–10 mM Tris-chloride (pH 7.5)–0.1 mM EDTA. After three washes with the same medium, the mitochondria were finally suspended in 0.25 M sucrose. The protein concentration was measured by the biuret reaction in the presence of deoxycholate (Jacobs *et al.*, 1956). The mitochondria used for the experiment with ascorbate–TMPD were homogenized in a medium containing 0.225 M mannitol, 0.07 M sucrose, 10 mM Tris-chloride (pH 7.5), and 1 mM EDTA.

Release of respiratory control was determined by polarographic analysis of the rate of oxygen uptake using the Clark oxygen electrode (Estabrook, 1967). The reaction medium (1.5 ml) contained 0.25 M sucrose, 10 mM Tris-chloride, 10 mM KCl, 4 or 10 mM potassium phosphate, 4 mM $MgCl_2$, and 0.2 mM EDTA at a final pH of 7.4. Active respiration was determined in the presence of 0.13 mM ADP. The substrates were 5 mM ascorbate plus 0.2 mM TMPD (with 2 μg of rotenone and 1 μg of antimycin A) or 10 mM succinate (with 2 μg of rotenone) or a mixture of 5 mM glutamate and 5 mM malate. Three to four successive additions of CBCN-salicylanilide in 1 or 2 μl of ethanol were made at 1–2-min intervals to the reaction mixture, with continuous monitoring of the oxygen concentration. Solutions of salicylanilide of different concentrations were used and at least two separate experiments were

* From the Department of Cell Physiology, Boston Biomedical Research Institute, Boston, Massachusetts 02114. *Received August 1, 1969.* This is part XX in the series: Studies on Oxidative Phosphorylation. This work was supported by grants from the Public Health Service (5-R01-GM 13641), the Life Insurance Medical Research Fund (G-67-20), and the American Heart Association (67-749).

† Recipient of a Research Career Development award (HE 24,202) from the U. S. Public Health Service.

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[1] The following abbreviations will be used: TMPD, *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine; CBCN-salicylanilide, 5-chloro-3-butyl-2′-chloro-4′-nitrosalicylanilide.

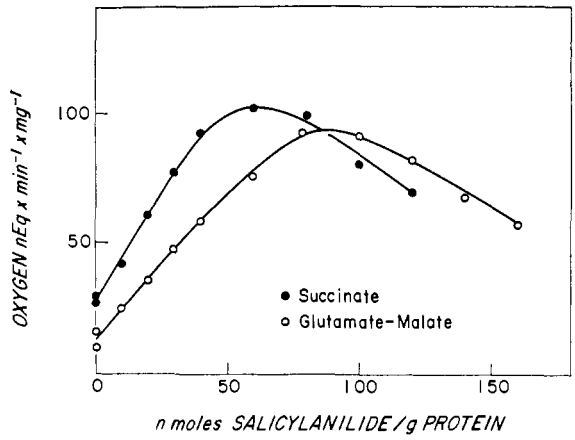

FIGURE 1: Stimulation of mitochondrial respiration by the salicylanilide derivative. Respiration was measured with a Clark oxygen electrode as described in the Experimental Section. The rate of oxygen uptake was calculated after each addition of the uncoupler.

made with overlapping concentrations of uncoupler to cover a wide range. Each determination was completed in 5–7 min. Approximately 2 mg of mitochondrial protein was used for each experiment. The respiratory control ratio using glutamate–malate was in excess of 4 with all preparations of mitochondria used in these experiments. The reaction temperature was 30°.

Oxidative phosphorylation was measured in the differential respirometer (from Gilson Medical Electronics Co.) at 30° in a medium containing 0.2 M sucrose, 20 mM Tris-sulfate buffer (pH 7.5), 10 mM potassium phosphate buffer (pH 7.5), 6.7 mM $MgCl_2$, 1 mM ATP, 20 mM glucose, 16 units of crystalline hexokinase, 2 mg of crystalline bovine serum albumin, substrate, and about 7 mg of mitochondrial protein in 3.0 ml. The substrates were 6.7 mM succinate, or 16.7 mM glutamate plus 16.7 mM malate, or 16.7 mM pyruvate plus 16.7 mM malate, or 5 mM ascorbate plus 0.25 mM TMPD. The reaction was terminated after 18–24 min by the addition of 0.1 ml of

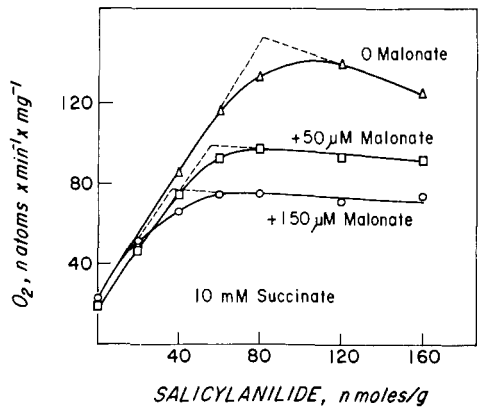

FIGURE 2: Uncoupler titers in succinate oxidation limited by malonate. The respiration was determined as described under Experimental Section. Malonate was added to the mitochondria before addition of succinate and then the uncoupler was added in increasing amounts. Each curve represents the rate of oxygen uptake obtained by the addition of increasing amounts of salicylanilide to the mitochondrial suspension.

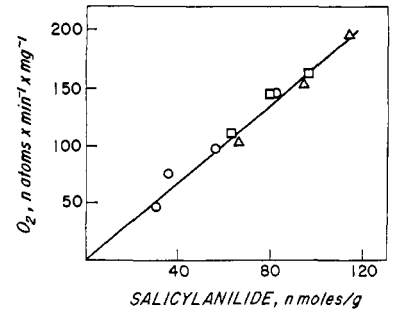

FIGURE 3: Relationship between oxidation rate and uncoupler titer. The different symbols relate to different experiments. The experiments were done as under Figure 2. The titers determined from these curves are plotted against the maximal rate of oxygen uptake obtained by extrapolation as shown by the dotted lines in Figure 2.

60% perchloric acid. Phosphate was measured by the Fiske–Subbarow (1925) method. The salicylanide was added in 0.05 ml of ethanol. For the determination of cytochrome *a*, the 605-mμ − 630-mμ absorbance difference was measured in the Aminco-Chance dual-wavelength spectrophotometer. Mitochondria (5 mg) which had been kept frozen was suspended in 3 ml of 0.2 M sucrose and 20 mM phosphate (pH 7.5). Anaerobiosis was achieved a few minutes after the addition of 3 μmoles of NADH to the reaction medium. ϵ_{mM} for cytochrome *a* was taken as 24 (Van Gelder, 1966). This value actually refers to the Δ 605 mμ, reduced minus oxidized cytochrome *a;* the ϵ_{mM} in the dual wavelength spectrophotometer for 605–630 mμ, reduced minus oxidized, is closer to 28. However, the former value has been used to allow ready comparison with recent similar work (Wilson, 1969).

Results

In preliminary experiments, the comparative effectiveness of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone and CBCN-salicylanilide in uncoupling energy-linked reactions was measured. The reactions employed were ATP-driven NAD reduction by succinate and decolorization of membrane-bound bromothymol blue in submitochondrial particles (Kurup and Sanadi, 1968; Sanadi, 1968). It was found that the salicylanilide was effective at less than one-third the concentration of the carbonyl cyanide. All the experiments presented here were carried out with the salicylanilide in view of its greater potency.

The release of mitochondrial respiratory control and stimulation of mitochondrial respiration by CBCN-salicylanilide are shown in Figure 1. In all of our experiments, excess uncoupler produced inhibition of respiration. This property seems to be common to most uncouplers (Van Dam and Slater, 1967). The level of salicylanilide necessary for maximum stimulation of respiration (referred to as uncoupler titer) was determined by extrapolating the ascending and descending parts of the curve as illustrated in Figure 2. Figure 2 also shows that the uncoupler titer varies with the oxidation rate. Succinate was the substrate in the experiment, and as oxidation rate was decreased by adding increasing levels of malonate, the uncoupler titer decreased progressively. The uncoupler titer is, in fact, directly proportional to the oxidation rate (Figure 3). The data from three experiments with differ-

TABLE I: Salicylanilide Level for Maximal Release of Respiratory Control.[a]

	Succinate		Glutamate–Malate	
	O_2 (μg-atoms × min^{-1} × g^{-1})	Salicylanilide (nmole/g)	O_2 (μg-atoms × min^{-1} × g^{-1})	Salicylanilide (nmole/g)
6[b]	128 ± 6	71 ± 9	109 ± 9	88 ± 10
	128[c]	71[c]	128[c]	104[c]
Ratio		2.0		2.9

[a] Normalized titer refers to the calculated salicylanilide concentration for an oxidation rate equivalent to that observed with the NAD-linked substrate. The titers were taken from experiments illustrated in Figure 2. The oxygen uptake refers to the maximal extrapolated value. See Experimental Section for additional details. [b] Number of experiments. [c] Normalized titers.

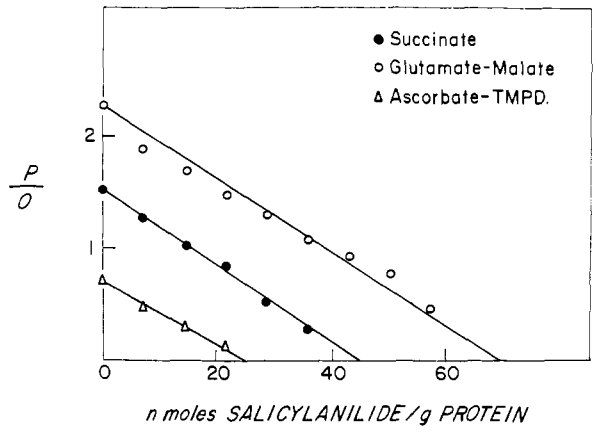

FIGURE 4: Titration of oxidative phosphorylation system by the salicylanilide derivative. The assay was carried out as described in the Experimental Section. In the case of glutamate–malate, the points appear to be sigmoidal, but this was not the case in other experiments.

ent preparations of rat liver mitochondria are plotted in this figure. Margolis and coworkers (1967) also found that the uncoupler titer for the complete uncoupling of phosphorylation varies directly with the oxidation rate. A similar relationship was observed by Kurup and Sanadi (1968) using the intramitochondrial pH change (decolorization of bromothymol blue) produced by substrate oxidation as an index of energy conservation. Table I shows the results of several experiments to determine the optimal uncoupling level with succinate and glutamate–malate as substrates. The oxidation rate with the NAD-linked substrate was slower. For valid comparison of the titers with different substrates, it is necessary to normalize the oxidation rate (see Figure 3). The uncoupler titers calculated for equivalent oxidation rates are shown in line 2 of the table. It is seen that the titers with succinate and glutamate–malate are in the ratio of 2:2.9, in close agreement with the number of phosphorylation sites involved in the oxidation of the respective substrate.

The high effectiveness of CBCN-salicylanilide in uncoupling oxidative phosphorylation is also apparent from the titration type of response in Figure 4. The experimental points are on a straight line at least up to 80% uncoupling. The extrapolated level for 100% uncoupling varies with the substrate. It is least for ascorbate–TMPD and highest for NAD-linked substrates. The mean titers for several experiments are shown in Table II. Since the rates of oxidation with the different substrates varied, the titers were normalized to correspond to the oxidation rate with the NAD-linked substrate. The ratio of the normalized titers is 1:2.1:2.8 for ascorbate–TMPD, succinate, and NAD-linked substrate oxidation, respectively. This ratio is also in close agreement with the number of phosphorylation sites involved in the oxidation of the respective substrates.

The uncoupler titers are expressed in relation to the cytochrome *a* content of the mitochondria in Table III. It is seen that when all phosphorylation sites are operating, the uncoupler titer is close to 1, but is less (between 0.7 and 0.8) with succinate and even lower (about 0.39) with ascorbate–TMPD. Wilson (1969) also found that the uncoupler titer varied with the oxidation rate. In agreement with our results, his value for the titer with succinate (from his Figure 1) was 0.6, and it was 0.87 with glutamate–malate when its oxidation rate was normalized to that of succinate oxidation rate.

In a different type of experiment where state 3 oxidation (ADP present) was inhibited by azide and the inhibition was partially released by salicylanilide, the results were different (Wilson, 1969). Maximal release of inhibition required approximately 1.3 molecules of salicylanilide/cytochrome *a* whether the substrate was glutamate and malate, succinate, or ascorbate–TMPD. Secondly, in the concentration range of 0.28–1.14 mM TMPD, the titer was not affected by azide. We have attempted to probe the reasons for the differences in titers measured by release of respiratory control or uncoupling (our studies) as against those obtained by stimulation of azide inhibited respiration. One factor to be taken into consideration in the comparison is the effect of high TMPD on oxidative phosphorylation. As seen in Figure 5, the oxidation rate increases linearly up to 400 μM TMPD and then more gradually.[2] On the other hand, the P:O increases at first, then begins to decline around 0.25 mM TMPD in a biphasic response. The peak in P:O around 0.25 mM TMPD was observed in each of the four experiments and is considered real and significant. We have also observed that the uncoupling at higher levels of TMPD is more pronounced in manometric experiments reaching near 0 at 1 mM TMPD, presumably due to the longer time for the measurement during which extensive damage to mitochondria may be expected due to uncoupling (unpublished data). Table IV gives the salicylanilide to cytochrome *a* ratios for release of azide-inhibited respiration at different TMPD concentrations. In the range from 0.125 to 0.315 mM, which would normally allow optimal phosphoryla-

[2] The autoxidation rate of TMPD was measured under identical conditions with boiled mitochondria. With 100, 250, 500, 750, and 1000 μM TMPD, the rates were 25, 38, 38, 38, and 44 ng-atoms of oxygen × min^{-1}, respectively. If correction were applied for this, the P:O would increase more at low TMPD than at higher levels, and show even sharper decline in P:O.

TABLE II: Uncoupler Titers with Different Substrates.[a]

Expt	NAD-Linked Substrates		Succinate		Ascorbate–TMPD	
	O_2 (μg-atoms $\times$ $min^{-1} \times g^{-1}$)	Salicylanilide (nmoles/g)	O_2 (μg-atoms $\times$ $min^{-1} \times g^{-1}$)	Salicylanilide (nmoles/g)	O_2 (μg-atoms $\times$ $min^{-1} \times g^{-1}$)	Salicylanilide (nmoles/g)
Mean	82 ± 8 (6)	65 ± 6 (6)	61 ± 6 (5)	36 ± 2 (5)	142 ± 2 (5)	37 ± 3 (5)
Normalized titers	82	65	82	48	82	23
Ratio		2.8		2.1		1

[a] The NAD-linked substrate was pyruvate-malate in two experiments and glutamate–malate in the others.

tion as seen from Figure 5, the titer increases 4-fold while with the oxidation rate only doubles. In the absence of azide, the titer measured by release of respiratory control is 0.39 (Table III). Further increase in TMPD to 1 mM, which is in the uncoupling range, produces little change in the titer in agreement with Wilson's data (1969).

Discussion

The chemical coupling theory for ATP synthesis in mitochondria is illustrated in eq 1–3. AH_2 stands for reduced electron carrier, B of the oxidized carrier, and C for a hypothetical energy coupling factor. The action of uncoupling agents (ϕ), according to Hemker (1964), is shown in eq 4 and

$$AH_2 + B + C \longrightarrow A\sim C + BH_2 \quad (1)$$

$$A\sim C + X \longrightarrow X\sim C + A \quad (2)$$

$$X\sim C + ADP + P \longrightarrow X + C + ATP \quad (3)$$

5. It is also possible that the uncoupler reacts with X~C as in

$$A\sim C + \phi \longrightarrow \phi\text{—}C + A \quad (4)$$

$$\phi\text{—}C \longrightarrow \phi + C \quad (5)$$

eq 6 instead of reacting with A~C.

$$X\sim C + \phi \longrightarrow \phi\text{—}C + A \quad (6)$$
$$(\text{or } \phi\text{—}X)$$

The results presented in this communication show that the uncoupling titer varies with both the substrate and the oxidation rate. At equivalent oxidation rates, the uncoupler titers are proportional to the number of phosphorylation sites with each substrate. These results would indicate the following. (a) The uncoupler binds a component only in the energized state (Margolis *et al.*, 1967). If it should bind C or X in the nonenergized state, the uncoupler requirement would be constant and independent of the substrate or the oxidation rate. (b) As shown in the scheme, interaction between uncoupler and A~C or X~C should be followed by breakdown of the product ϕ—C (or ϕ—X). If the product were stable, the uncoupler titer would be constant for any given substrate and would not change with the oxidation rate. This is because

TABLE III: The Ratio of Salicylanilide Titers to Cytochrome *a* Concentration.[a]

Assay	Salicylanilide Titer:Cytochrome *a*		
	NAD Linked	Succinate	Ascorbate–TMPD
P:O	1.11	0.81	0.39
Respiratory stimulation	1.13	0.72	

[a] The average value for cytochrome a ($\Delta A_{605-630\,m\mu}$; $\epsilon_{m\mu}$ 24) from five analyses was 92 nmoles/g of protein. The salicylanilide titers in Table II have been corrected to an oxidation rate of 128 μg-atoms of oxygen per min per g to correspond to the rates shown in Table I.

TABLE IV: Stimulation of Azide-Inhibited Respiration by Uncoupler.[a]

Substrate	O_2 (ng-atoms $\times$ $min^{-1} \times$ mg^{-1})	Salicylanilide: Cytochrome *a*
TMPD, 0.125 mM	39	0.44
TMPD, 0.150 mM	52	0.74
TMPD, 0.325 mM	80	1.9
TMPD, 1.000 mM	84	2.2
Succinate, 10 mM	45	1.42

[a] The assay medium contained 0.25 M sucrose, 10 mM Tris-chloride, 1 mM KCl, 10 mM potassium phosphate, 4 mM $MgCl_2$, and 0.2 mM EDTA at pH 7.4. With TMPD as substrate, the medium contained also 1.4 mg of rat liver mitochondria, 5 mM ascorbate, 2 μg of rotenone, 1 μg of antimycin A, 600 μM ADP, and 0.75 mM sodium azide in 1.0 ml. With succinate the medium contained 2 μg of rotenone, 0.9 mM ADP, 0.5 mM sodium azide, and 0.9 mg of mitochondrial protein. The respiration was started by adding TMPD or succinate, followed by azide to inhibit the oxygen uptake and then salicylanilide to release the inhibition. The titers were taken from the plots of ratio of uncoupler:cytochrome *a* against oxygen uptake (see Wilson, 1969, Figure 5).

ϕ—C (or ϕ—X) would accumulate from A~C or (or X~C), which could be continuously generated as long as a free C (or X) remained. (c) Our data do not allow a choice between A~C and X~C as the site of uncoupler action. The maximum titer, observed when all three sites are operative, is close to 1.0/respiratory assembly. The uncoupler could be reacting either with A~C, in which case approximately 0.3 molecule/site suffices to release respiratory control fully, or stoichiometrically with X~C. In the former case, the rate of breakdown of the ϕ—C should be faster than its rate of formation in order to account for the substoichiometry.

In the preliminary experiments, an unusually high uncoupler titer (carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone) was obtained for the first site of phosphorylation compared to the titers for the other sites (Kurup and Sanadi, 1968). The data were considered inconsistent with the chemiosmotic hypothesis (Mitchell, 1966). However, the present results, showing that the highest uncoupler titer with all sites participating is nearly one uncoupler molecule per respiratory assembly, would remove the objection. It could be argued that the increase in uncoupler titer with increasing oxidation rate may be related to the magnitude of the proton gradient. For a given rate of oxidation, the gradient would also depend on the number of phosphorylation sites producing the gradient. As discussed earlier, the data are also consistent with the chemical intermediate theory.

There is a serious discrepancy in the uncoupler titers determined by the P:O determination or stimulation of respiration in the absence of ADP compared with the titers measured by maximal release of azide inhibited respiration. The former method gave titers of approximately 0.4 (Tables II and III) for ascorbate–TMPD oxidation while the titers by the latter method varied from 0.44 to 2.2 depending on the concentration of TMPD and the oxidation rate (Table IV). Similarly, the titers with succinate using the former methods were around 0.8 (Table III) while in the presence of azide it changed to 1.4. Clearly, the titer in the presence of azide is influenced by other parameters. Wilson (1969) has pointed out that uncouplers release azide-inhibited respiration by causing a 10-fold or greater increase in the inhibitor constant. The mechanism of respiratory inhibition by azide is still in dispute. Wilson and Chance (1966) attribute the inhibition to stabilization of an intermediate common to both electron transport and energy conservation. Palmieri and Klingenberg (1967) regard the inhibition as a phenomenon related to the accumulation of azide inside the mitochondria. Finally, Nichols and Kimmelberg (1968) have concluded that uncouplers change the electron transfer pathway in the cytochrome oxidase region and that azide is a more effective inhibitor of the coupled pathway. It would appear, therefore, that release of respiratory control and P:O measurements are more reliable parameters for measuring uncoupler titer.

Based on their observations on the release of azide-inhibited respiration by uncoupler, Wilson and Azzi (1968) and Wilson (1969) have advanced the hypothesis that uncouplers act only at the third phosphorylation site even in the absence of azide. Uncoupling at the other sites is attributed to equilibration of the site-specific A~C through X~C. However, in view of the alteration in uncoupler titers produced in the presence of azide (Table IV) and the uncertainty of the mechanism of inhibition of respiration by azide, the more conventional methods of measuring uncoupler titer used in our studies and those of Margolis and coworkers (1967) seem more reliable for valid conclusions. The most effective known uncoupler, CBCN-salicylanilide, uncouples oxidative phosphorylation at a concentration of one molecule per respiratory assembly under optimal conditions. The site of action could be at the level of either A~C or X~C in the chemical theory for oxidative phosphorylation.

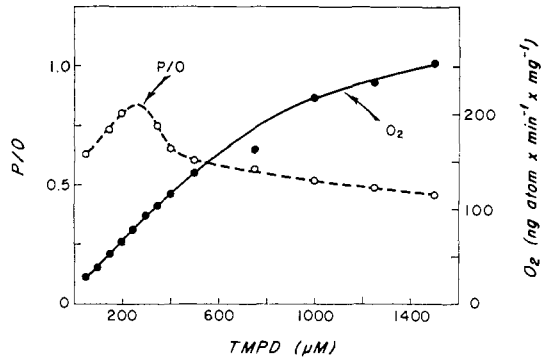

FIGURE 5: Uncoupling of oxidative phosphorylation by excess TMPD. The oxygen uptake was followed by the polarographic method. The assay medium, in a final volume of 1.0 ml, contained, besides that described under the polarographic method, 1.2 mg of rat liver mitochondria, 2 μM rotenone, 1 μg of antimycine A, 0.6 mM ADP, 5 mM ascorbate, purified [^{32}P]P_i (200,000 cpm), 20 mM glucose, and 20 units of crystalline yeast hexokinase, and different concentrations of TMPD as indicated. The reaction was started by adding TMPD followed by ADP and carried out until it became anaerobic. The reaction mixture was then taken out and precipitated with 0.1 ml of 20% trichloroacetic acid.

Acknowledgment

The technical assistance of M. Robinson is appreciated.

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Intramolecular β-Pleated-Sheet Formation by Poly-L-lysine in Solution*

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ABSTRACT: In order to learn whether the β form of poly-L-lysine is intramolecular or intermolecular, the rate of the heat-induced conformational transition of this polymer from the α-helical to the β-pleated-sheet conformation was studied polarimetrically over the concentration range 1.5×10^{-4} to 7.0×10^{-2} g per 100 ml at pH 10.8. Interpretation of the rates for polymer with degree of polymerization 240 or 1000 shows that, at concentrations of 10^{-2} or less, poly-L-lysine folds into the intramolecular β form. In contrast, at higher concentrations both inter- and intramolecular β forms are generated simultaneously. For degree of polymerization 70, however, the β form is intermolecular at all concentrations.

These observations support the assignment of the peptide circular dichroism extremum at 216 nm to the anti-parallel-chain β-pleated-sheet structure.

Poly-L-lysine, a synthetic polypeptide, is shown by unambiguous kinetic measurements, presented in this study, to be capable of folding into *intramolecular* as well as intermolecular β-pleated-sheet structures in aqueous solution. Poly-L-lysine thus exhibits in solution a versatility of conformational transformations rivaling that of proteins. The solution properties of poly-L-Lys have been extensively studied previously (Applequist and Doty, 1962; Rosenheck and Doty, 1961; Davidson and Fasman, 1967). These previous studies have shown that at neutral or acid pH, poly-L-Lys is disordered, somewhat like a protein in 6 M guanidine hydrochloride. At pH's greater than 10.5, the molecule adopts the α-helical conformation if the temperature is 30° or less. At more elevated temperatures, for example, 50°, poly-L-Lys molecules in alkaline solution adopt the β form.

However, it has been a matter of dispute whether the β form adopted involves parallel or anti-parallel chains, and whether the β form is intermolecular or intramolecular. From molecular weight measurement through sucrose gradient centrifugation on the three forms of poly-L-Lys, Sarkar and Doty (1966) suggested that the β form exists in an intramolecular H-bonded pleated sheet. By contrast, studies by Davidson and Fasman (1967) showed that the initial rate of the thermal transition of the α helix to the β structure is dependent upon concentration under appropriate conditions. These data led Davidson and Fasman to conclude that the β form of poly-L-Lys is an intermolecular pleated sheet. In addition, the effect of molecular weight on the circular dichroism of heated alkaline poly-L-Lys solutions has been reported by Li and Spector (1969), who sought accurate values of the circular dichroism of the β form in order to estimate β content in proteins. Their studies, which were carried out at pH 11.5, supported the view that at concentrations of 0.01 g/100 ml of poly-L-Lys forms large intermolecular β sheets. The detergent sodium dodecyl sulfate, shown by Sarkar and Doty to cause poly-L-Lys in neutral aqueous solutions to adopt the β conformation, was shown by Li and Spector to lead to very small, thus presumably intramolecular, β form.

It occurred to us that such opposed conclusions could have arisen from the difference in the experimental conditions used by the two groups. Sarkar and Doty worked at a relatively low concentration (0.01 g/100 ml) at pH 11; Davidson and Fasman measured kinetics over a concentration range (0.007–0.11 g/100 ml) at pH 11.6–11.7 but most of the data reported are for concentrations greater than 0.01. In the study presented here, the kinetics of the α–β thermal transition are investigated over the concentration range 1.5×10^{-4} to 7×10^{-2} g per 100 ml, *i.e.*, to concentrations 50-fold more dilute than any previously studied. Moreover, by studying the kinetics of the α–β transition for polymers with degree of polymerization 70, 240, and 1000, the effect of chain length upon the β structure is examined. It is to be expected that a competition between inter- and intramolecular processes would be reflected in dependence of the kinetics upon molecular weight.

Materials and Methods

Poly-L-Lys. Three poly-L-Lys samples having three different average molecular weights were used in these experiments. The two samples of higher molecular weight were purchased as the hydrobromide salt from Pilot Chemicals, Inc. The

* From the Department of Biophysics, The University of Chicago, Chicago, Illinois 60637. *Received May 5, 1970.* This work was supported by Grant NB-07286 from the National Institute of Neurological Diseases and Blindness of the National Institutes of Health, U. S. Public Health Service. The spectropolarimeter was purchased through a grant from the Special Research Resources Branch of the U. S. National Institutes of Health.