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Inhibition of energy transfer reaction in mitochondria by the photosensitizing dye "NK19"–brief note*

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Abstract

Among various photosensitizing dyes, 4, 4'-dimethyl 3, 3'-di-n-heptyl-8- {2-(4-methyl-3-n-heptylthiazole) }-2, 2'-dicarbocyanin diiodide (abb. NK19), even in an extremely low concentration, is known to inhibit the proliferation of bacteria and tissue culture cells (1, 2, 3). With respect to the mechanism of such inhibitory action no other property of this NKl9 is known except that it has a marked adsorptive property to protein (4). As a step toward the elucidation of the mode of biological effect, the author studied the effect of NK19 on the energy transfer reaction of Irat liver mitochondria, followed by comparison with the mode of actions of various other inhibitors of the oxidative phosphorylation (5). NK19. NKl9 can be prepared by letting 2, 4-dimethylthiazole heptyliodide react with ethylorthoformate in anhydrous acetic acid. We used NKI9, a product of Nihon Kanko Shikiso Research Laboratories. The molecular structure is as in the following and in its MeOH state it has maximum absorbancy at 590 m,a. For the use in experiment it was made into 1 mg/ml of MeOH and was stored in the dark until used.

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INHIBITION OF ENERGY TRANSFER REACTION IN MITOCHONDRIA BY THE PHOTOSENSITIZING DYE "NK19" —— BRIEF NOTE

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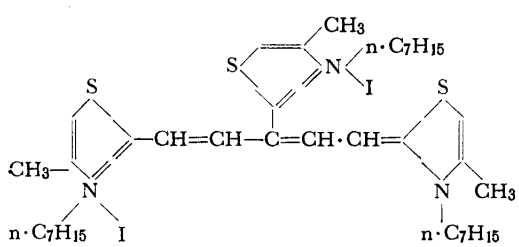
Among various photosensitizing dyes, 4, 4′-dimethyl 3, 3′-di-*n*-heptyl-8-{2-(4-methyl-3-*n*-heptylthiazole)}-2, 2′-dicarbocyanin diiodide (abb. NK19), even in an extremely low concentration, is known to inhibit the proliferation of bacteria and tissue culture cells (1, 2, 3). With respect to the mechanism of such inhibitory action no other property of this NK19 is known except that it has a marked adsorptive property to protein (4). As a step toward the elucidation of the mode of biological effect, the author studied the effect of NK19 on the energy transfer reaction of rat liver mitochondria, followed by comparison with the mode of actions of various other inhibitors of the oxidative phosphorylation (5).

NK19. NK19 can be prepared by letting 2, 4-dimethylthiazole heptyl-iodide react with ethylorthoformate in anhydrous acetic acid. We used NK19, a product of Nihon Kankō Shikiso Research Laboratories. The molecular structure is as in the following and in its MeOH state it has



maximum absorbancy at 590 mμ. For the use in experiment it was made into 1 mg/ml of MeOH and was stored in the dark until used.

Preparation of rat liver mitochondria. Rats were sacrificed by decapitation and their livers were immediately removed and placed in a 0.33 M sucrose solution containing 1 mM Tris-HCl and 1 mM EDTA at pH 7.4. Rat liver mitochondria were prepared according to the procedure of PACKER,

UTSUMI and MUSTAFA (6) after homogenation with Potter-Elbejam homogenizer and kept at 0°C soon after preparation until used. The amount of mitochondrial protein was determined by the LOERY *et al.* procedure (7),

Experimentals. The test was carried out at 25°C in general. The specific details of the experiments are provided with the respective data in the text. Oxygen utilization was recorded polarographically by oximeter (HAGIHARA's semiclosed type using rotary platinum electrods (8)). ATP-^{32}Pi exchange reaction was assayed by using ^{32}Pi and unlabeled ATP, and the formation of (^{32}P) ATP was determined according to the procedure of HAGIHARA and LARDY (9). ATPase activity was determined by measuring the amount of Pi liberated from ATP by the method of TAKAHASHI (10).

Effects of NK19 on the respiration and oxidative phosphorylation of mitochondria. Fig. 1 demonstrates a typical experiment showing the effect of NK19 on the phosphorylating respiration of the rat liver mitochondria with succinate as substrate. Namely, looking at the polargraphic trace of the

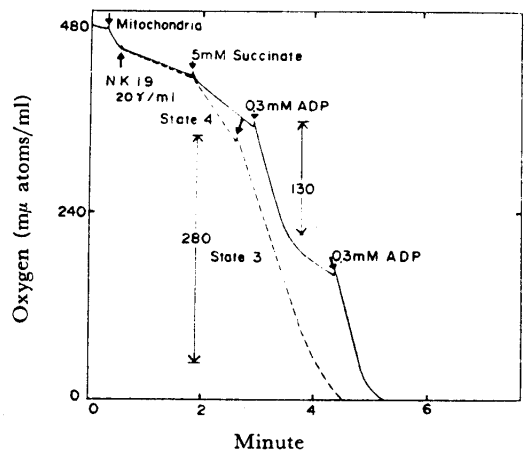


Fig. 1 The polarographic trace of oxygen consumption in the oxidative phosphorylation of rat liver mitochondria. The reaction mixture (2.5 ml) contained: 100 mM sucrose, 3 mM $MgCl_2$, 20 mM KCl, 10 mM potassium phosphate and mitoehondria (2 mg protein/ml) at pH 7.4. Where indicated, 5 mM succinate, 300 μM ADP and 20 γ/ml NK 19 in final concentration were added. The incubation was carried out at 25°C. The numbers on the graphs represented the amount of oxygen uptake (mμ atoms) in the state 3.

control experiment as represented by a solid line, the oxygen uptake of state 3 respiration (11) was 100.3 mμ atoms/mg protein/min as against 21.8 mμ atoms/mg protein/min of state 4 respiration (11). Consequently, respiratory control index (RCI) was 4.7. As the oxygen consumption during

state 3 with addition of 300 μM ADP was 130 mμ atmos, the ADP/O ratio was calculated to be 2.30. However, in the case that NK19 was added to the reaction mixture as 20 γ/ml in final concentration as represented by a dotted line, state 4 respiration was already higher than that of the control (oxygen uptake being 48.5 mμ atoms/mg protein/min) and state 3 respiration was slightly lower than that of the control (oxygen uptake being 82.5 mμ atoms/mg protein/min). Therefore, RCI was 1.7 and the ADP/O ratio was 1.37. In order to obtain decisive evidences for the characterization of action of NK19, it was necessary to study the progressive effect by increasing the concentration of NK19 added. As shown in Fig. 2, state 4 respiration rose gradually by the increase of NK19, and the state 3 respiration with succinate was diminished slightly at higher concentration of NK19. Consequently, as shown in Fig. 3, the respiratory control was

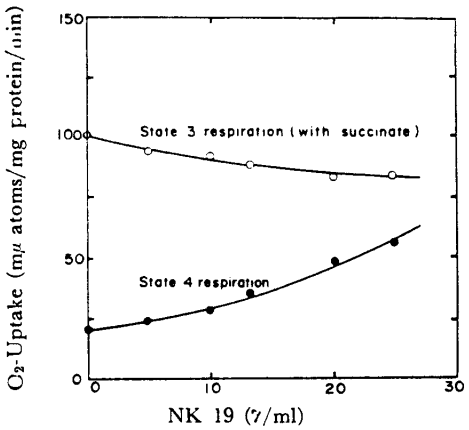


Fig. 2 Effects of NK19 on the states 3 and 4 respiration of rat liver mitochondria. The reaction mixture (2.5 ml) .contained: 100 mM sucrose, 3 mM $MgCl_2$, 20 mM KCl, 10mM potassium phosphate, 5 mM succinate, 300 μM ADP (just for the state 3 respiration) and mitochondria (2 mg protein/ml). NK19 was added to the reaction mixture as the final concentration indicated in the abscissa of the figure.

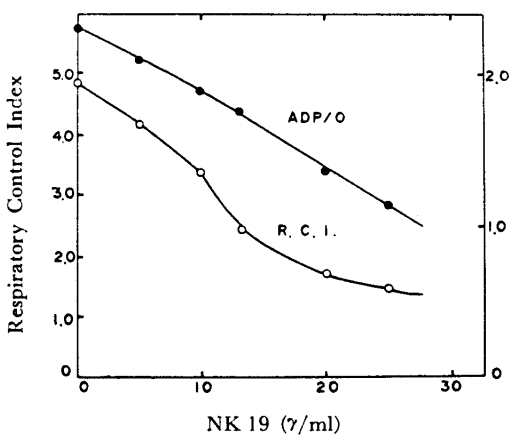


Fig. 3. Evidence for inhibitions of NK19 on the respiratory control and ADP/O ratio of the rat liver mitochondria. Both ratios were calculated from the values obtained in series of the experiments of Figs. 1 and 2.

progressively inhibited along with increase in the concentration of NK19 in the reaction mixture. Furthermore, ADP/O ratio was also dramatically diminished by the increase of NK19.

Effects of NK19 on the ATP-^{32}Pi exchange reaction. Supposing that NK19 is an uncoupler of phosphorylating respiration just as 2, 4-dinitrophenol (DNP) from the results described above, it would also inhibit the ATP-^{32}Pi exchange reaction (12). Actually as shown in Fig. 4, NK19 did inhibit this reaction as anticipated and quite intensely.

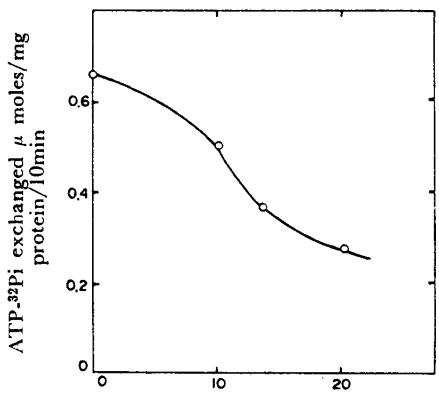


Fig. 4 Effect of NK19 on the ATP-^{32}Pi exchange reaction of rat liver mitochondria. Mitochondria (2.1 mg protein/ml) were incubated in the medium (total volume was 2.5 ml) containing 250 mM sucrose, 20 mM KCl, 3 mM $MgCl_2$, 3 mM potassium phosphate (2 μcurie of ^{32}Pi) 3 mM sodium-ATP and 5 mM Tris-HCl pH 7.4. Incubation was carried out at 25°C for 10 minutes.

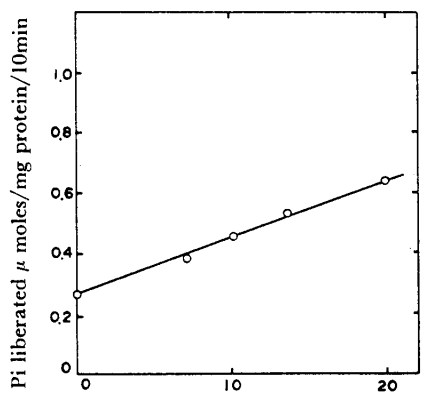


Fig. 5 Effect of NK19 on the ATPase activity of rat liver mitochondria. Mitocohndria (2.7 mg protein/ml) were incubated in the medium (total volume was 2 ml) containing 200 mM sucrose, 20 mM KCl, 3 mM $MgCl_2$, 3 mM sodium-ATP and 10 mM Tris-HCl at pH 7.4. Incubation was carried out at 25°C for 10 minutes.

Effects of NK19 on the ATPase. The activity of ATPase is hardly elicited in the state where mitochondria are intact, but when mitochondria are damaged or when DNP is added, ATPase activity is at once stimulated (13). The observations of effects of NK19 on the ATPase revealed that the increasing of NK19 concentration markedly stimulated the ATPase as illustrated in Fig. 5.

As the principal inhibitors that affect the oxidative phosphorylation of mitochondria, uncouplers such as DNP (14), and inhibitors of phosphorylating respiration as oligomycin may be pointed out. As a result it has been demonstrated that NK19 accelerated state 4 respiration of rat

liver mitochondria but it had no effect on state 3 respiration or rather inhibited it at higher concentration, resulting in a marked inhibition of respiratory control. The oxygen consumption required for the conversion of ADP into ATP was markedly increased and the ADP/O ratio was dramatically decreased by addition of NK19. These effects resembled quite closely those of the uncoupler, DNP. In addition, the ATP-^{32}Pi exchange reaction was inhibited and ATPase was enhanced. Stemming from these observation, it was presumed that NK19 acted on the initial step of the phosphorylating system just as did DNP.

In summary we may conclude that a part of the inhibitory action of NK19 on the growth of bacteria and tissue culture cells is due to the uncoupling action on the oxidative phosphorylation.

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