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Inhibitory Effects of Lower Aliphatic Alcohols on Electron Transport Phosphorylation Systems

2. Secondary, Tertiary, and Di-alcohols

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A number of aliphatic alcohols with 4 and 5 carbon atoms have been tested on photophosphorylation in isolated spinach chloroplasts and chromatophores from the photosynthetic bacterium *Rhodospirillum rubrum* as well as on respiration and phosphorylation in rat liver mitochondria.

Among the 4-carbon alcohols tested, namely 1-butanol, 2-butanol, isobutanol, tertiary butanol, and 1,4-butanediol, a strong inhibition of respiration and phosphorylation in mitochondria and of cyclic phosphorylation in chromatophores and chloroplasts was obtained with the primary and secondary alcohols. The tertiary alcohol and the di-ol had only slight effects in the same concentration range.

The following 5-carbon alcohols were tested on the three phosphorylating systems: 1-pentanol, 2-pentanol, 3-pentanol, and 1,5-pentanediol. Of these 1-pentanol and 1,5-pentanediol were the most potent inhibitors of phosphorylation in all three systems, 2-, and 3-pentanol were less effective. In the mitochondria 1,5-pentanediol was found to act as an uncoupling agent with both succinate and the NAD*-linked substrates β-hydroxybutyrate and glutamate. In contrast to other alcohols tested it had a weaker inhibitory effect on NAD-linked than on succinate-linked electron transport.

To ascertain whether the alcohol group was essential for inhibitory effects of the alcohols on mitochondrial oxidative phosphorylation, the hydrocarbon pentane was tested. With this compound no significant effect on mitochondrial respiration and phosphorylation was obtained in the concentration range employed.

It has previously been shown that aliphatic unbranched, primary alcohols inhibit photophosphorylation in chromatophores [1,2] and chloroplasts [2] and respiration and phosphorylation in mitochondria.[1] The inhibitory effects of the

* Abbreviations: NAD, nicotinamide-adenine dinucleotide; PMS, phenazine methosulfate; DOC, sodium desoxycholate.

alcohols in the homologous series increased with the size of the aliphatic carbon skeleton. It has been suggested [1-3] that inhibitors such as alcohols act upon biological functions on surface structures in a general physical rather than a specific chemical manner.

In this investigation the effects of a number of primary, secondary, and tertiary alcohols with 4 or 5 carbon atoms have been tested on photophosphorylation in chloroplasts and chromatophores and on respiration and phosphorylation in mitochondria. Two di-alcohols have also been tested, namely 1,4-butanediol and 1,5-pentanediol.

MATERIALS AND METHODS

All alcohols were of analytical grade except the 1-pentanol and the 1,5-pentanediol, which were of KEBO purum grade. The pentane was of analytical grade.

The methods used in this investigation have been described in a preceding paper.

The alcohols were assayed by heating with potassium bichromate in 3 M sulfuric acid for 20 min at 70 – 80°C, followed by determination of the decrease in absorbancy at 445 mμ.

RESULTS

In Fig. 1 are shown the effects of some aliphatic alcohols with 4 carbon atoms on phosphorylation and respiration in mitochondria with succinate as substrate. A strong inhibitory effect on phosphorylation and respiration was obtained with 1-butanol, 2-butanol, and isobutanol, while 1,4-butanediol and tertiary butanol had no or only a weak effect. Two extra washings of the

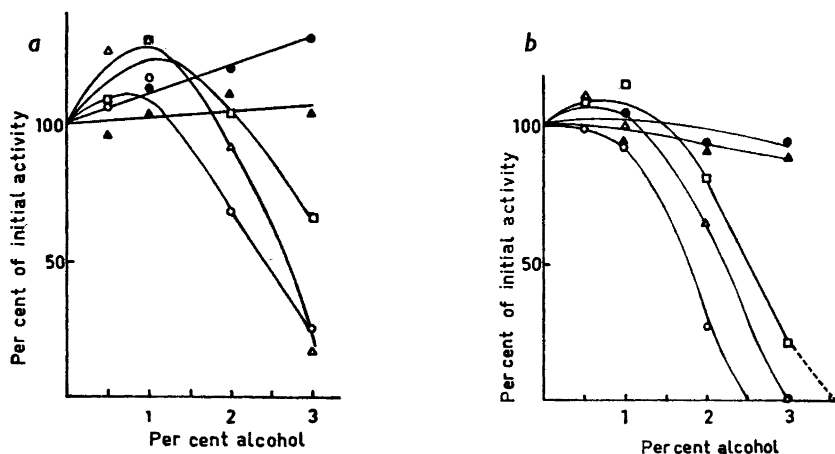

Fig. 1. Effects of various butanols on oxygen uptake (Fig. 1a), and phosphate esterification (Fig. 1b) in mitochondria. Substrate: succinate. 1 mg mitochondrial protein/ml incubation mixture, incubation time 25 min. Other conditions are given in Ref. 1. 100 % activity was approximately 8 μmoles oxygen uptake and 10 μmoles phosphate esterified/ mg mitochondrial protein/h. △: 1-butanol; O: 2-butanol; □: isobutanol; ▲: tert. butanol; ●: 1,4-butanediol.

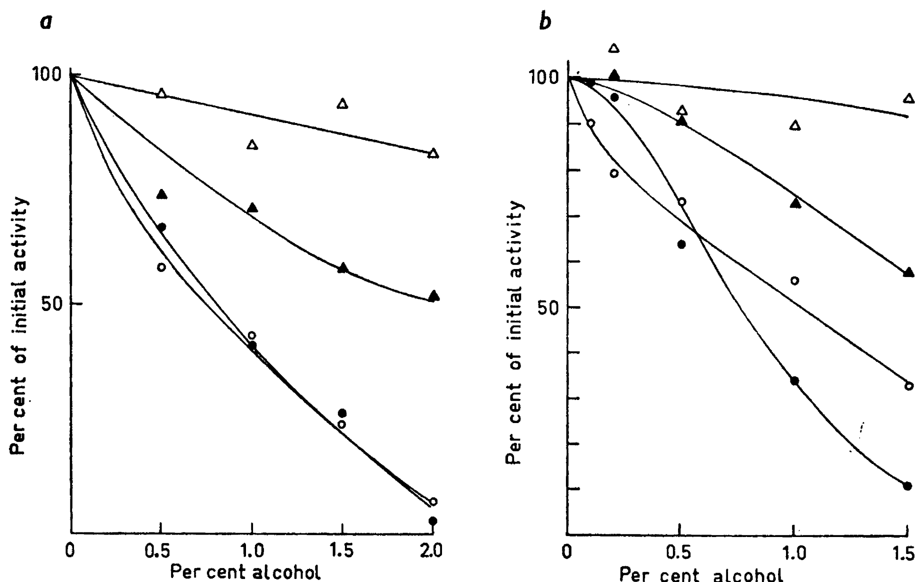

Fig. 2. Effects of various butanols on cyclic photophosphorylation in a) chromatophore "fragments" with no external electron carriers added, and b) "whole" spinach chloroplasts with PMS added as external electron carrier. The chlorophyll content in the chloroplast experiment was 0.05 mg/3 ml incubation mixture. Other conditions are given in Ref. 1. 100 % activity was 356 and 240 μmoles phosphate esterified/mg chlorophyll/h in systems a) and b), respectively. △ : 1-butanol; ○ : 2-butanol; ▲ : tert.butanol; ● : 1,4-butanediol.

mitochondria abolished the stimulatory effects of low concentrations of alcohol on phosphorylation and respiration.

Similar differences between the activities of the various alcohols were obtained in cyclic photophosphorylation in chromatophores from *Rhodospirillum rubrum* and in "whole" spinach chloroplasts, as shown in Fig. 2 a and b.

In Fig. 3 is shown the influence of 1-pentanol and 1,5-pentanediol on phosphorylation and respiration in mitochondria with succinate as substrate. The alcohols had equally strong inhibitory effect on phosphorylation. Only the 1,5-pentanediol, however, acted as an uncoupling agent. For example, at a concentration of 1.5 % no uptake of phosphate occurred, whereas 67 % of the respiration was still remaining. Table 1 shows the influence of 1,5-pentanediol on respiration and phosphorylation in mitochondria with succinate, β-hydroxybutyrate or glutamate as substrates. It should be noted that in contrast to other alcohols tested (*cf.* Fig. 4 in Ref. 1 where the effect of 1-butanol is shown; similar results have been obtained with 1-propanol and 1-pentanol), 1,5-pentanediol uncouples electron transport from phosphorylation with both the NAD-linked substrates and succinate. It should also be noted that the NAD-linked electron transport is less strongly inhibited than the succinate-linked, in contrast to what has been demonstrated with other alcohols tested.

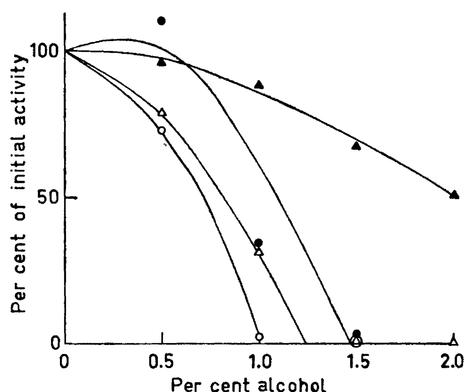

Fig. 3. Effects of 1-pentanol and 1,5-pentanediol on oxygen uptake and phosphate esterification in mitochondria. Substrate: succinate. 1.5 mg mitochondrial protein/ml incubation mixture, incubation time 24 min. Other conditions are given in Ref. 1. Filled symbols represent oxygen uptake, open symbols represent phosphate esterification. 100 % activity was 5.6 μmoles oxygen uptake and 8.0 μmoles phosphate esterified/mg mitochondrial protein/h. ▲, △ : 1-pentanol; ●, ○ : 1,5-pentanediol.

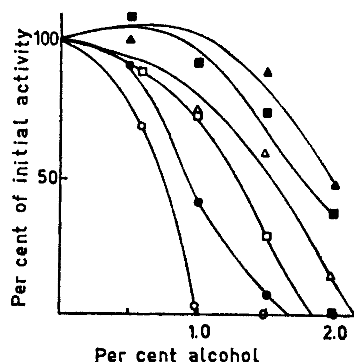

Fig. 4. Effects of various pentanols on oxygen uptake and phosphate esterification in mitochondria. Substrate: succinate. 1.5 mg mitochondrial protein/ml incubation mixture, incubation time 24 min. Other conditions are given in Ref. 1. Filled symbols represent oxygen utpake, open symbols represent phosphate esterification. 100 % activity was 6.6 μmoles oxygen uptake, and 14.8 μmoles phosphate esterified/mg mitochondrial protein/h. ● , ○ : 1-pentanol; ■ , □ : 2-pentanol; ▲ , △ : 3-pentanol.

Table 1. Effects of 1,5-pentanediol on oxygen uptake and phosphate esterification in mitochondria with succinate, β-hydroxybutyrate and glutamate as substrates. 2 mg mitochondrial protein/ml incubation mixture, incubation time 24 min. Other conditions are given in Ref. 1.

Inhibitor	Substrate	μmoles oxygen uptake/mg protein/h	% inhibition with alcohol	μmoles phosphate uptake/mg protein/h	% inhibition with alcohol
None	succinate	6.1		9.6	
2 % 1,5-Pentanediol	succinate	3.4	44	0.9	91
None	β-hydroxybutyrate	2.2		7.2	
2 % 1,5-Pentanediol	β-hydroxybutyrate	2.0	9	0.5	93
None	glutamate	4.0		9.6	
2 % 1,5-Pentanediol	glutamate	3.0	25	1.0	90

Table 2. Lack of effect of pentane on oxygen uptake and phosphate esterification in mitochondria with succinate as substrate. 1.5 mg mitochondrial protein/ml incubation mixture, incubation time 24 min. Other conditions are given in Ref. 1.

Inhibitor	μmoles P_i esterified/mg protein/h	% of initial activity
—	12.4	100
5 % ethanol	11.9	96
5 % ethanol + 2 % pentane	11.1	89
10 % ethanol	7.3	59
10 % ethanol + 3 % pentane	6.9	56

In Fig. 4 is shown the inhibition of mitochondrial phosphorylation and respiration by 1-pentanol, 2-pentanol, and 3-pentanol, of which 1-pentanol was the most potent inhibitor. For comparison the hydrocarbon pentane was tested for possible effects on phosphorylation and respiration. The experiment had to be performed in the presence of rather high concentrations of ethanol to keep the pentane in solution (ethanol was more suitable for this

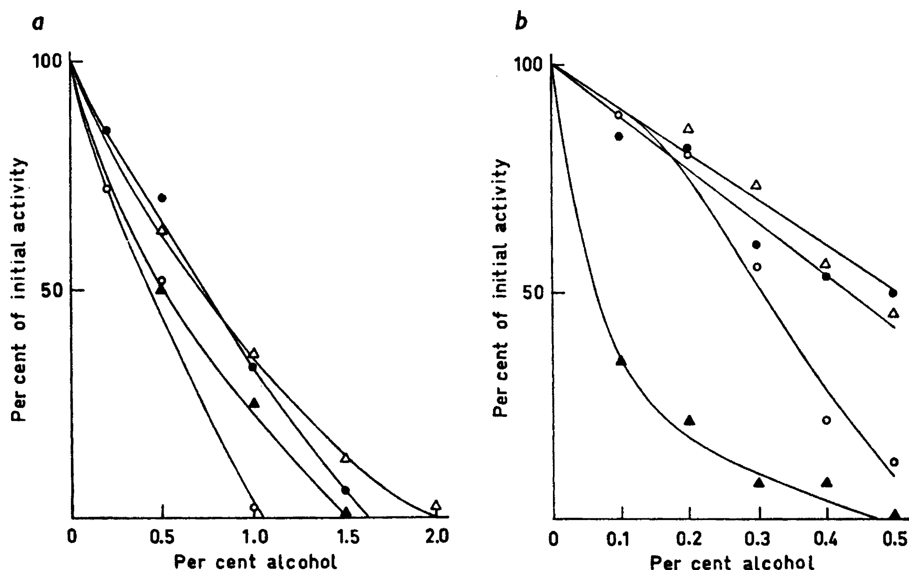

Fig. 5. Effects of various pentanols on cyclic photophosphorylation in a) chromatophore "fragments" with no external electron carriers added, and b) "whole" spinach chloroplasts with PMS added as external electron carrier. The chlorophyll content in the chloroplast experiment was 0.08 mg chlorophyll/3 ml incubation mixture. Other conditions are given in Ref. 1. 100 % activity was 305 and 94 μmoles phosphate esterified/mg chlorophyll/h in systems a) and b), respectively. O : 1-pentanol; ● : 2-pentanol; △ : 3-pentanol; ▲ : 1,5-pentanediol.

purpose than methanol). In Table 2 it is shown that no significant effects except those of the ethanol were obtained with pentane.

The cyclic photophosphorylation in the chromatophores and chloroplasts was strongly inhibited by 1-pentanol and 1,5-pentanediol. Also in these structures 2-pentanol and 3-pentanol had a somewhat weaker effect, as is shown in Fig. 5 a and b. The effect of 1,5-pentanediol has also been tested on non-cyclic electron transport in chloroplasts from H_2O to ferricyanide. An uncoupling effect, similar to that found in mitochondria, was obtained.

DISCUSSION

As has been shown, the inhibitory power of an alcohol will be influenced by the position of the -OH-group, by introduction of an additional -OH-group in the alcohol, and by branching of the aliphatic carbon chain. The variations obtained with respect to inhibitor efficiency of different 4-C and 5-C compounds are not easily explained on the sole basis of deductions from their molecular or three-dimensional structures. On the other hand, an examination of their physical properties appears to give also in this case a clue to an understanding of the effects obtained.

Lipid solubility as a factor influencing the activity of pharmacologically active compounds was early recognized by Meyer.[4] He showed that increased solubility in lipids gave stronger pharmacological effects of narcotic agents, tested *in vivo*. A similar correlation has been demonstated in mitochondria with various substances, *e.g.* 2-hydroxy-3-alkyl-1,4-naphtoquinones,[5,6] and alkylguanidines.[7]

In a quantitative investigation about the effects of lipid solubility on the action of uncoupling phenols Hemker [8] showed that the activity of an uncoupling phenol is determined by its concentration in a lipid phase within the mitochondrion, and not by its concentration in the surrounding medium. He also made calculations about these intramitochondrial concentrations.[8]

In an attempt to find an explanation for the differing effects of the alcohols, their distribution in a rather arbitrarily chosen two-phase system, namely xylene/water, was investigated. The results are shown in Table 3. With all alcohols except 1,5-pentanediol there is a correlation between the degree of enrichment in the lipid phase and the inhibitory power of the alcohol. Lipid solubility as such, is clearly not solely responsible for the inhibition caused by an added compound, since the hydrocarbon pentane, which is very soluble in lipids, was shown to have no effect on mitochondrial function. On the other hand, it appears clear that degree of enrichment in the lipid phase is of primary importance for the potency of an alcohol to interfere with electron transport phosphorylation systems.

The OH-groups of the active alcohols cause dipolarity and should confer to them a tendency to become enriched in the phase boundaries between lipid and protein phases in the membranes of the electron transport phosphorylation systems. This is the region where the electron transport and energy transfer reactions have been suggested to take place. The enrichment of alcohol may be assumed to change the threedimensional structure of the membranes, thereby interfering with the reactions which are occurring in that region.[2,9]

Table 3. Approximate partition coefficients for aliphatic alcohols in the two phase system xylene/water. The concentrations of alcohol in the water phase were determined as described in Methods before and after shaking aqueous solutions of alcohols with an equal volume of xylene for 5 min.

Alcohol	Moles per liter in water	Moles per liter in xylene	$K_c = \frac{c_{xylene}}{c_{water}}$
Methanol	0.020	0.000	0
Ethanol	0.014	0.000	0
Propanol	0.011	0.001	0.09
1-Butanol	0.022	0.010	0.46
2-Butanol	0.024	0.009	0.38
Isobutanol	0.022	0.009	0.41
1,4-Butanediol	0.016	0.000	0
1-Pentanol	0.010	0.020	2.0
2-Pentanol	0.020	0.033	1.7
3-Pentanol	0.024	0.028	1.2
1,5-Pentanediol	0.014	0.000	0

The lack of correlation between lipid solubility and activity of 1,5-pentanediol constitutes an exception to the rule as stated above. This, taken together with the facts that, a) unlike other alcohols tested,[1] it uncouples electron transport from phosphorylation in the mitochondria, both with β-hydroxybutyrate and glutamate as substrates, as well as in chloroplasts in the system where electrons are transported from water to ferricyanide, and b) the NAD-linked electron transport is more stable towards 1,5-pentanediol than the succinate-linked electron transport, shows that the inhibition caused by this alcohol is indeed of a different kind than that caused by the other alcohols tested. Unusual characteristics of this alcohol also with respect to effect on mitochondrial structure will be demonstrated in a forthcoming paper.[10]

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