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Dinitrophenol-induced ATPase of rat-liver mitochondria

MYERS AND SLATER¹ found that with increasing concentrations of 2,4-dinitrophenol the optimum pH of the dinitrophenol-induced ATPase of rat-liver mitochondria moved to higher pH's. From the form of the pH-activity curves with different concentrations of 2,4-dinitrophenol, these authors drew the conclusion that three enzyme systems with pH optima at 6.3, 7.4 and 8.5, respectively, were present in the mitochondria, the 6.3 enzyme being stimulated by low concentrations of dinitrophenol (and inhibited by higher) while higher concentrations were necessary to induce activity in the 8.5 enzyme.

The alternative explanation of these results, namely that the decreasing activity of dinitrophenol with increasing pH was due to the fact that the undissociated phenol is the active form, was rejected because at pH 7.4 *p*-nitrophenol and 2,4-dinitrophenol have similar uncoupling activity, whereas the concentrations of the undissociated phenols differ by a factor of about 1,000 at this pH (*pK* of 2,4-dinitrophenol, 4.1; of *p*-nitrophenol, 7.2). In agreement with this conclusion, PARKER² reported that at pH 6.8 the most potent uncouplers of oxidative phosphorylation in rat-liver mitochondria are those phenols with the lowest *pK*'s.

GLADTKE AND LISS³, on the other hand, drew attention to cases where there was a poor correlation between *pK* and the uncoupling activity. They, and independently HÜLSMANN⁴, introduced a new factor into the consideration of the relative effectiveness of substituted phenols as uncoupling agents, namely the lipid solubility.

MYERS AND SLATER¹, PARKER² and HÜLSMANN⁴ have drawn attention to the inhibition of the dinitrophenol-induced ATPase by higher concentrations of dinitrophenol. The concentration (*C*_{opt}) giving maximum ATPase has been used in the present

TABLE I

COMPARISON OF OPTIMAL CONCENTRATIONS OF DIFFERENT NITROPHENOLS AT VARIOUS pH's FOR INDUCTION OF ATPase ACTIVITY OF RAT-LIVER MITOCHONDRIA

ATPase activity was determined as described by MYERS AND SLATER¹. The *pK* and *pQ* values were determined spectrophotometrically.

Compound	<i>pK</i>	<i>pQ</i> *	<i>pC</i> _{opt} ** at pH's				
			5	6	7	8	9
<i>p</i> -nitrophenol	7.2	+0.45	3.30	3.37	3.51	3.39	—
2,6-dinitrophenol	3.7	—2.35	4.77	4.22	3.68	3.12	—
2,6-dinitro-3,4-dimethylphenol	***	***	4.92	4.51	4.36	3.95	3.60
2,6-dinitro-4-isobutylphenol	4.3	—3.54	5.16	4.88	4.71	4.48	4.26
2,6-dinitro-4-isoamylphenol	4.1	—4.15	5.55	5.42	5.18	4.92	4.71
2,6-dinitro-4-iso-octylphenol	4.05	—4.75	6.08	5.89	5.64	5.41	5.24

$$* pQ = -\log_{10} \frac{\text{concentration of undissociated phenol in xylene}}{\text{concentration of undissociated phenol in water}}$$

For the dinitrophenols at pH's > 5,

$$pQ = pK - pH - \log_{10} \frac{\text{concentration of undissociated phenol in xylene}}{\text{concentration of total phenol in water}}$$

$$** pC_{opt} = -\log_{10} C_{opt}$$

$$*** pK - pQ = 7.44$$

Abbreviation: ATP, adenosine triphosphate.

study for a quantitative study of the relationship between the lipid solubility and the uncoupling activity of alkyl-substituted nitrophenol compounds.

The maximum ATPase was found to be the same for 2,6-dinitrophenol and a series of alkyl-substituted 2,6-dinitrophenols. At a fixed pH, C_{opt} was found to decrease with increasing lipophilic character of the compound. For each of the nitrophenols listed in Table I, except *p*-nitrophenol, C_{opt} increased with increasing pH, there being a linear relationship between pH and pC_{opt} . In the case of *p*-nitrophenol, which differs from the dinitrophenols studied by virtue of its much higher pK and lower lipid solubility, pC_{opt} was largely independent of pH between 5 and 7. Above pH 8, it followed the same relationship with pH as the dinitrophenols.

These results can be explained on the assumption that the undissociated phenol must be taken up into the mitochondria before it induces ATPase activity. The concentration of nitrophenol in the lipid phase (C_L) will be governed by the concentration in the aqueous phase (C_{aq}), the pH of the reaction medium and the pK and pQ (see Table I) of the nitrophenol. When pK is much less than the pH, as is the case with all the dinitrophenols investigated,

$$pC_L = pC_{aq} - pK + pQ + pH$$

In Table II are given the values of pC_L when pC_{aq} equals pC_{opt} given in Table I (it is assumed that the partition coefficient between water and mitochondrial lipid is the same as that between water and xylene). From this table it can be concluded that

(i), when at a particular pH the ATPase is maximally stimulated, the concentration of the phenol in the lipid phase (C_L) is independent of the degree of lipophilicity (pQ) of the compound;

(ii), $pC_{L,opt}$ increased with increasing pH, according to the equation $pC_{L,opt} = -1.49 + 0.76 \text{ pH}$, *i.e.* C_L necessary for maximal ATPase is less the higher the pH.

Although these results do not prove that it is the undissociated phenol which reacts in the mitochondria, it is clear that the concentration of the undissociated

TABLE II
CALCULATION FROM DATA IN TABLE I OF CONCENTRATION IN MITOCHONDRIAL LIPID (C_L)
OF DIFFERENT NITROPHENOLS AT VARIOUS pH'S

$$pC_L = pC_{opt} - pK + pQ + pH$$

Compound	pK	pQ	pC_L at pH's				
			5	6	7	8	9
<i>p</i> -nitrophenol*	7.2	+ 0.45	3.55	3.62	4.06	4.62	—
2,4-dinitrophenol**	4.1	— 2.06	3.61	4.06	4.52	4.96	—
2,6-dinitrophenol	3.7	— 2.35	3.72	4.17	4.63	5.07	—
2,6-dinitro-3,4-dimethylphenol	***	***	2.48	3.07	3.82	4.51	5.16
2,6-dinitro-4-isobutylphenol	4.3	— 3.54	2.33	3.05	3.88	4.65	5.43
2,6-dinitro-4-isoamylphenol	4.1	— 4.15	2.30	3.04	3.93	4.67	5.56
2,6-dinitro-4-iso-octylphenol	4.05	— 4.75	2.28	3.09	3.84	4.61	5.44

* In this case, the simple expression is not applicable, since pH is not greater than pK . pC_L was calculated from the expression $pC_L = pC_{opt} + pQ + pH + \log(K + [H^+])$.

** This compound was not so extensively investigated as 2,6-dinitrophenol, but no significant difference was found between the values of pC_{opt} for the two compounds. For purposes of calculation, it has been assumed that they were identical.

*** $pK - pQ = 7.44$.

phenol in the medium determines the degree of uncoupling by a particular dinitrophenol and that this is the explanation of the decreasing degree of uncoupling with increasing pH. In comparing different nitrophenols, two factors have to be considered—the pK and the lipid solubility. In a series of dinitrophenols which all have about the same pK , it is the lipid solubility which determines the relative degree of uncoupling. When comparing *p*-nitrophenol with 2,4-dinitrophenol the difference in pK (3.1) is largely compensated in the other direction by the difference in pQ (2.55), so that the fact that around neutral pH the two compounds uncouple to about the same extent is purely coincidental.

From the above results, it follows that the pH-activity curve of the dinitrophenol-induced ATPase at a fixed dinitrophenol concentration does not describe the effect of pH on the enzyme system. It is necessary to determine the relationship between pH and the maximum activity measured at the C_{opt} corresponding to each pH. In this way, a single maximum at pH 6.9 was found for 2,4-dinitrophenol and 2,6-dinitro-4-isoamylphenol.

Thus, pH-activity curves do not give any evidence of the presence in rat-liver mitochondria of more than one dinitrophenol-induced ATPase.

Preliminary studies suggest that at pH 7.2 the optimum found in the activity-concentration curve with 2,4-dinitrophenol in reality consists of two peaks close to one another. PENNIAL⁵ has recently reported that soluble extracts of mitochondrial acetone powders exhibit multiphase curves of ATPase response to dinitrophenol at pH 8.5, when tested in the absence of added salts.

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