KINETICS OF SUCCINATE UPTAKE BY RAT-LIVER MITOCHONDRIA

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1. Introduction

The permeation of anions into mitochondria has been kinetically studied so far only in a qualitative manner. No quantitative values on the rates and related kinetic parameters are available, although such data are of main importance for an understanding of these carrier-catalyzed transport processes. Only for the adenine nucleotide translocation a detailed kinetic study is available by applying a number of special techniques. Particularly short times of permeation have been measured by stopping the permeation on addition of inhibitor, such as atractyloside for the adenine nucleotide translocation [1,2]. By applying the same principle, the kinetics of succinate permeation are followed as described in the present communication with a resolution of less than 1 sec. Recently, Kraayenhof et al. [3] also studied the kinetics of succinate uptake into the mitochondria with a technique based on centrifugal filtration (cf. also [3a]). Their method, however, has a dead time of 4 sec, which is too long to resolve kinetics above 5°.

The uptake of succinate into mitochondria has been the subject of previous studies of this laboratory and of other groups [4–13]. It was known that the rate of respiration with succinate is controlled by the uptake of succinate to the mitochondria. Furthermore, it was revealed that the degree of accumulation of succinate is dependent primarily on the pH difference across the mitochondrial membrane [12].

2. Methods

Rat-liver mitochondria were prepared as previously described [14]. The third wash and resuspension were carried out in 0.25 M sucrose. The kinetic experiments are performed as follows: Mitochondria are incubated under the conditions specified in the legends to the figures for 1 min in "Eppendorf" cups. The uptake is started by addition of ^{14}C-succinate and rapid mixing with the mitochondrial suspension. After time t the uptake is terminated by rapid addition of 20 mM 2-phenyl-succinate. Phenyl-succinate, like 2*n*-butylmalonate, was shown by Chappell and Robinson [15] to be an inhibitor of dicarboxylic acid uptake. The permeated succinate is trapped in the mitochondria, since phenylsuccinate appears to block the succinate carrier, but does not penetrate into the mitochondria. The shortest time resolved under the present conditions of substrate uptake is about 1 sec. The mitochondria with the ^{14}C-succinate trapped were separated from the incubation mixture by rapid centrifugation in a microcentrifuge (Misco). The interval between the addition of the inhibitor and separation of the mitochondria is kept as low as possible (about 7 sec). Control experiments were carried out, in which phenylsuccinate was added before ^{14}C-succinate to the incubation mixture containing the mitochondria. In these controls the time of contact of the mitochondria with the labelled substrate was the same as that after the addition of inhibitor in the parallel experiments (7 sec).

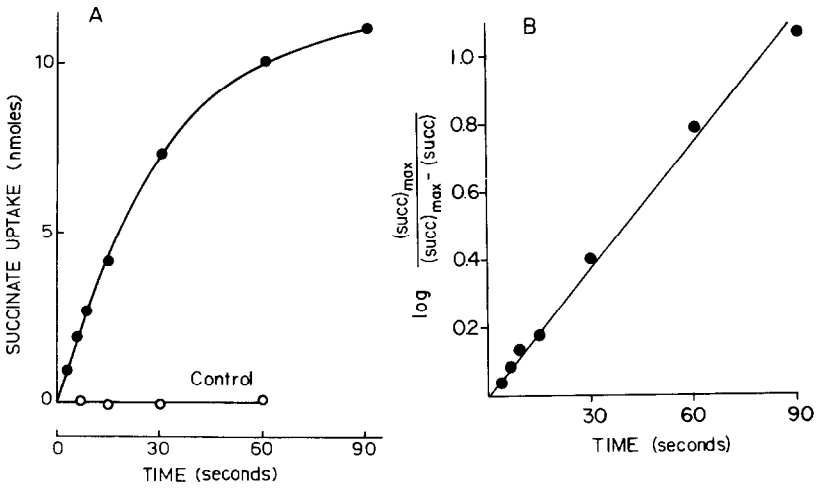

Fig. 1a. Time course of succinate uptake by rat-liver mitochondria. The reaction mixture contained 80 mM KCl, 60 mM sucrose, 20 mM imidazol buffer pH 7.2, 1 μg rotenone, 1 μg antimycin, 2.95 mg protein and (added at time 0) 0.5 mM ^{14}C-succinate. Temperature 1°C. Stop of succinate uptake by addition of 20 mM phenylsuccinate at time t indicated, followed by the centrifugal separation of the mitochondria after 7 sec. Control: 20 mM phenylsuccinate was present before ^{14}C-succinate addition, followed by the separation of the mitochondria. Other conditions as indicated in Methods. b. Logarithmic plot of succinate uptake by liver mitochondria, demonstrating first order type kinetics:

$$\log \frac{succ_{max}}{succ_{max}\text{-}succ} = k\,t$$

The slope gives $k = 1.25^{-1}$ and the rate = $\frac{1.25 \cdot 11.8}{2.95}$ = 5.2 μmoles/min/g protein. $Succ_{max}$ = succinate accumulated after equilibration is reached, measured after 5 min. $Succ_{max}$ = 11.8 μmoles.

The radioactivity in the extracts of the sediments and in the supernatants was measured in a scintillation counter (Tri-Carb). For measuring the specific permeable space in the pellet, ^{3}H-H_2O and ^{14}C-sucrose were added in parallel experiments to determine the total water of the pellet and the sucrose-permeable space, in order to account for the external succinate in the pellet. This was subtracted from the total succinate in order to obtain the internal succinate (cf. [12]).

3. Results

Fig. 1a shows the time course of succinate uptake at 1°C, obtained by addition of phenylsuccinate at the time t indicated. The conditions, such as pH, addition of respiratory inhibitor, were chosen on the basis of previous experiments [12], in order that succinate is accumulated several-fold without being rate-limited by simultaneous energy supply from substrate oxidation. The control curve proves that the inhibition of succinate uptake by phenylsuccinate is complete, because virtually no succinate is taken up when phenylsuccinate is added before.

The succinate uptake appears to follow a first order type of reaction (fig. 1b), similarly as shown in detail for the adenine nucleotide translocation [1]. The first order constant is $k = 1.25$ min^{-1} and the corresponding rate of succinate uptake is 5.2 μmoles/min/g prot. (at 0.5 mM succinate). In the present studies, rates are mostly evaluated from the initial, approximately linear range, since at increased temperature the kinetics are limited to the range of a few sec and even less than 1 sec at 21.5°C. The rate of succinate uptake increases about 15 times from 2° to 21°C (fig. 2a). This corresponds to a Q_{10} = 3.4. A straight line is obtained in an Arrhenius plot over this

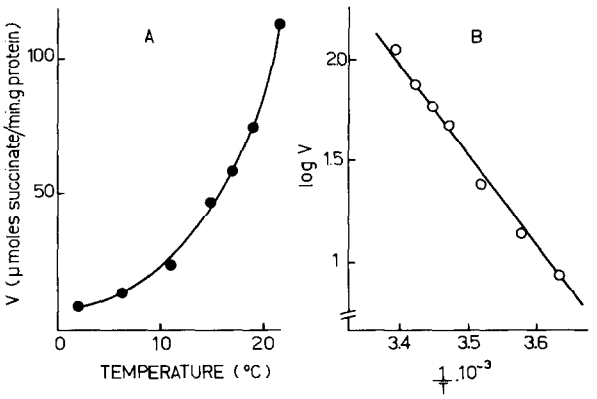

Fig. 2a. The temperature dependence of the rate of succinate uptake. The reaction mixture contained 80 mM KCl, 60 mM sucrose, 20 mM Tris-HCl, 1 mM EDTA, 1 µg rotenone, 1 µg antimycin, 1.87 mg protein and (added at time 0) 0.5 mM ^{14}C-succinate, pH 6.35. The rates of uptake were calculated from the amount of succinate taken up within the initial time period during which succinate uptake was approximately linear. Other conditions as indicated in Methods. b. Arrhenius plot of the temperature dependence of succinate uptake. Data from the experiment in fig. 2a.

range which shows an activation energy E = 22 kcal (fig. 2b).

The dependence on substrate concentration of the rate of succinate uptake reveals hyperbolic saturation characteristics (fig. 3). The K_m and V_{max} values for the rate of succinate uptake were 0.83 mM and 51 µmoles succinate/min/g protein respectively at 10°C.

Fig. 4a illustrates the influence of the pH of the medium on the rate of succinate uptake. It can be seen that as the pH decreased, the rate of succinate uptake increased. Fig. 4b shows that at different pH the uptake converges with increased concentration of succinate. It can be concluded that the inhibition by higher pH is competitive with succinate.

4. Conclusions

The results obtained on the kinetics of succinate uptake demonstrate that "the inhibitor stop method" [1] for measuring short times of uptake can be extended from the adenine nucleotide translocation also to other transport reactions. The great advantage of

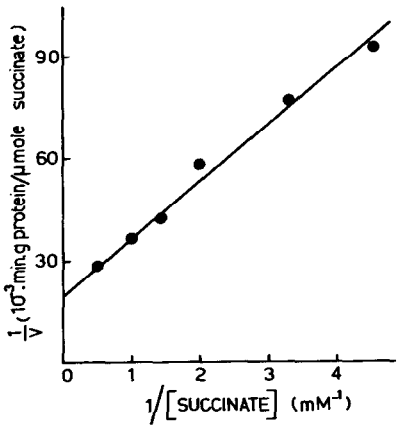

Fig. 3. The dependence of the rate of succinate uptake on succinate concentration. Experimental conditions as in fig. 2 except that ^{14}C-succinate was added at the concentrations indicated. Mitochondrial protein was 2.0 mg. Temperature 10°C.

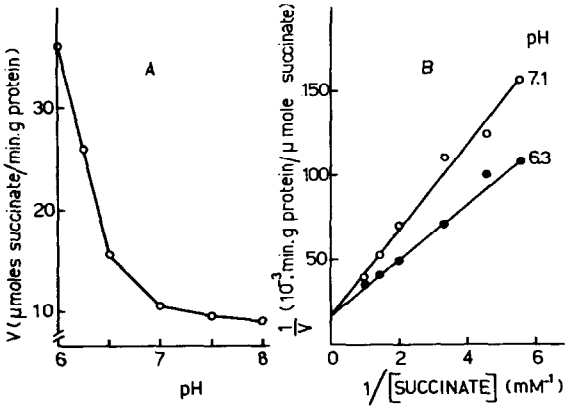

Fig. 4a. pH dependence of the rate of succinate uptake. Experimental conditions as in fig. 2. Final pH as indicated in the figure. Mitochondrial protein was 1.96 mg. Temperature 10°C. b. Competitive nature of the inhibition of the rate of succinate uptake by increasing the pH. Experimental conditions as in fig. 2 except that 50 mM Tris-HCl (instead of 20 mM Tris-HCl and 60 mM sucrose) 10 µg oligomycin, 2.5 mg protein and ^{14}C-succinate at the concentrations indicated were present. Final pH as indicated in the figure. Temperature 11°.

this method is that it is limited mainly by the mixing time and therefore is superior in time resolution to separating methods by centrifugation or by the membrane-filter techniques.

The reported studies on the kinetics of succinate uptake in mitochondria support the existence of a carrier system of substrates, which has been originally proposed based on qualitative studies of the specificity [16]. The existence of saturation kinetics and high temperature dependence can be expected from a carrier system.

The comparison with the adenine nucleotide translocation system [1,2,17,18] shows that the succinate uptake also has a relatively high temperature dependence. The K_m for succinate, however, is two orders of magnitude higher than for ADP. The rates of succinate uptake and of adenine nucleotide translocation are in the same range. A difference to the adenine nucleotide translocation system is the strong pH difference of the succinate uptake. The pH dependence appears to affect mainly the K_m of succinate, so that at high concentrations of succinate the pH dependence disappears. This can be explained in context with the finding [12] that the ΔpH across the membrane determines the gradient of accumulated succinate. Therefore, at higher external pH a higher succinate concentration is required to reach the same endogenous concentration as at lower external pH. It must be concluded, therefore, that the rate of succinate uptake is a function of the final endogenous concentration.

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References

[1] E.Pfaff, H.W.Heldt and M.Klingenberg, European J. Biochem., in press.
[2] E.Pfaff and M.Klingenberg, European J. Biochem. 6 (1968) 66.
[3] R.Kraaijenhof, C.S.Tsou and K.Van Dam, Biochim. Biophys. Acta 172 (1969) 580.
[3a] M.Klingenberg, E.Pfaff and A.Kröger, in: Rapid Mixing and Sampling Techniques in Biochemistry, IUB symposium (Academic Press, New York, 1964) p. 333.
[4] E.Quagliariello and F.Palmieri, European J. Biochem. 4 (1968) 20.
[5] E.J.Harris, K.Van Dam and B.C.Pressman, Nature 213 (1967) 1126.
[6] K.Van Dam, Biochim. Biophys. Acta 131 (1967) 407.
[7] E.J.Harris, M.P.Hofer and B.C.Pressman, Biochemistry 6 (1967) 1348.
[8] F.Palmieri, M.Cisternino and E.Quagliariello, Biochim. Biophys. Acta 143 (1967) 625.
[9] E.Quagliariello and F.Palmieri, in: Atti del Seminario di Studi Biologici, ed. E.Quagliariello, vol. 3 (Adriatica Editrice, Bari, 1967) p. 253.
[10] K.Van Dam and C.S.Tsou, Biochim. Biophys. Acta 162 (1968) 301.
[11] E.J.Harris and J.R.Manger, Biochem. J. 109 (1968) 239.
[12] F.Palmieri and E.Quagliariello, European J. Biochem. 8 (1969) 473.
[13] K.Van Dam, in: The Energy Level and Metabolic Control in Mitochondria, eds. S.Papa, J.M.Tager, E.Quagliariello and E.C.Slater (Adriatica Editrice, Bari, 1969) p. 319, 326.
[14] M.Klingenberg and W.Slenczka, Biochem. Z. 331 (1959) 486.
[15] J.B.Chappell and B.H.Robinson, in: Metabolic Roles of Citrate, ed. T.W.Goodwin (Academic Press, New York, 1968) p. 123.
[16] J.B.Chappell and K.N.Haarhoff, in: Biochemistry of Mitochondria, eds. E.C.Slater, Z.Kaniuga and L.Wojtczak (Academic Press, New York, 1966) p. 75.
[17] H.W.Heldt and M.Klingenberg, European J. Biochem. 4. (1968) 1.
[18] M.Klingenberg and E.Pfaff, in: Metabolic Roles of Citrate, ed. T.W.Goodwin (Academic Press, New York, 1968) p. 105.