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Effect of papain digestion on redox-linked proton translocation in b-c_1 complex from beef heart reconstituted into liposomes

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Papain treatment of the cytochrome b-c_1 complex from beef heart results in partial proteolysis of core protein II, the iron-sulphur protein and the 15-kDa subunit. Under these conditions a significant inhibition of electron flow and complete suppression of proton translocation in the complex reconstituted into liposomes are observed. Kinetic experiments indicate a correlation between the digestion of core protein II and 15-kDa subunit and the suppression of proton translocation. The results suggest an active involvement of polypeptides of the complex in stabilizing the semiquinone species and/or providing pathways to exchange protons between bound quinone systems and aqueous phases.

Cytochrome b-c_1 *complex* *Proton translocation* *Papain digestion*

1. INTRODUCTION

The mitochondrial ubiquinol-cytochrome c reductase (b-c_1 complex) catalyzes the transfer of reducing equivalents from ubiquinol to cytochrome c. As revealed by conventional SDS-PAGE, the protein complex is composed of 8 major bands. The two high molecular mass bands represent core proteins, bands 3–5 are apoproteins holding specifically the metal redox centres, namely b and c_1 cytochromes and the Rieske iron-sulphur protein, respectively [1,2]. Electron flow in the reductase is compulsorily linked to proton translocation in both native [3–5] and reconstituted systems [6–10].

Two types of models have been developed to account for the mechanism of redox-linked proton translocation in cytochrome c reductase: (i) direct ligand mechanism [4] and (ii) indirect proton pump mechanism [3,8–10]. The latter envisages a specific role of apoproteins of the complex in the vectorial proton transfer through a process conformationally linked to electron transfer reaction at the metal centre. A mechanism based on combination of anisotropic protonmotive catalysis by protein-stabilized semiquinone/quinone and cooperative proton transfer by apoproteins has also been proposed [10].

Attempts to elucidate the mechanism of proton translocation, and in particular the involvement of proteins, have been made by using chemical modifiers of amino acid residues. Among these, DCCD, the well known inhibitor of the H^+-ATPase complex [11] has been extensively used in many laboratories [12–17].

Work carried out in this and other laboratories [12,13] has shown that the 8-kDa subunit of the b-c_1 complex is predominantly labelled by [^{14}C]DCCD (cf. however [15,16]). At certain concentrations DCCD strongly inhibited the energy-linked proton translocation process without inhibiting the rate of electron transfer [12,13] or

Abbreviations: DCCD, N,N'-dicyclohexylcarbodiimide; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; DQH_2, durohydroquinone; CCCP, carbonyl cyanide m-chlorophenylhydrazone

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enhancing the passive proton permeability of the b-c_1 reconstituted into phospholipid vesicles. This effect of DCCD, that is referred to as a decoupling effect, has also been demonstrated in intact rat liver mitochondria [13,17]. It indicates involvement of proteins in proton translocation.

Here, results are presented on the effect of partial proteolysis of the b-c_1 complex with papain on electron flow and proton translocation. The results obtained provide further evidence for the involvement of polypeptides in redox-linked proton translocation in the complex.

2. MATERIALS AND METHODS

The b-c_1 complex was isolated according to Rieske [18] and characterized as described in [10].

2.1. *Papain treatment*

b-c_1 complex (20–30 mg protein/ml), dissolved in a medium containing 0.05 M Tris-Cl (pH 8.0)/0.67 M sucrose/0.001 M histidine (TSH buffer), 5 mM cysteine and 2 mM EDTA, was incubated for various intervals with papain at a concentration of 1 mg papain/20 mg complex protein at 25°C. After incubation, the mixture was diluted 50 times with cold TSH buffer and the redox activity measured. For reconstitution into phospholipid vesicles and SDS-PAGE, aliquots of the diluted enzyme solution were added to sonicated phospholipid suspension or to 10% trichloroacetic acid, respectively.

Separate samples in which papain was added after the incubation at room temperature directly to phospholipid-protein suspension or to the complex protein in the presence of trichloroacetic acid showed that papain did not have any detectable effect during subsequent operations.

2.2. *Reconstitution into liposomes*

Reconstitution of the b-c_1 complex into phospholipid vesicles was performed by the cholate dialysis method as described by Leung and Hinkle ([19], see also [10]).

The gel chromatography elution pattern of b-c_1 vesicles through an AcA-34 column showed that the control as well as the papain-treated enzyme were strictly associated to the phospholipids. Furthermore, oxidation by ferricyanide of the b-c_1 complex reduced by duroquinol showed that vesicles, containing both the control or papain-treated enzyme, were around 90% right-side oriented.

Passive proton conductance in b-c_1 vesicles was measured as described in [12].

2.3. *Electrophoretic analysis*

SDS-PAGE was performed on slab gels (17 cm long, 0.1 cm thick) using a discontinuous acrylamide gradient as in [20] with the addition of 6 M urea in both the separating and stacking gels. The stacking gel was 8% acrylamide and the separating gel was a 12–20% acrylamide gradient.

Enzyme samples precipitated with 10% trichloroacetic acid were dissolved in 4% SDS, 6 M urea, 0.0625 M Tris-Cl (pH 8.3), 1% 2-mercaptoethanol and 0.01% bromophenol blue.

2.4. *Measurement of redox and proton translocation activities*

Cytochrome c reductase activity was measured as described in [10] with duroquinol as substrate. The activity of both the control and the papain-treated b-c_1 complex was 95% sensitive to antimycin. Measurement of the K_m of the b-c_1 complex for duroquinol showed that the value (80–100 μM) did not vary with the papain treatment, whereas the V_{max} was significantly lowered. Measurement of redox-linked proton translocation was carried out as described in [10].

Papain and horse heart cytochrome c were from Sigma, USA. Durohydroquinone was from K & K Laboratories. All other reagents were of the highest purity grade commercially available.

3. RESULTS AND DISCUSSION

Fig.1 shows the effect of papain treatment on the polypeptides of the b-c_1 complex. The first lane represents the control enzyme, and the second refers to the reductase in the presence of papain, whose proteolytic activity was blocked at zero time by trichloroacetic acid. The band corresponding to papain can be clearly seen. The other lanes show the electrophoretic profile of the reductase after various intervals of treatment with papain. This resulted in a partial digestion of core protein II as shown by its enhanced migration. This was complete after 5 min incubation and corresponded to the loss of a 3-kDa fragment. Papain also caused

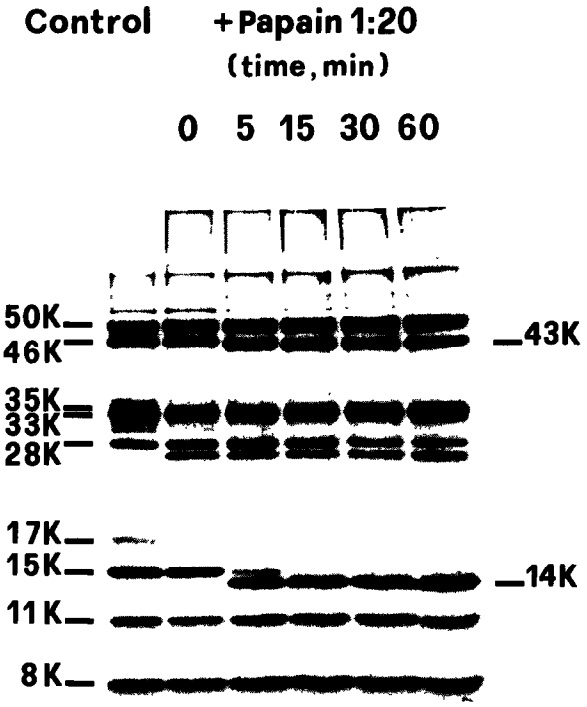

Fig.1. Coomassie-blue stained gels of b-c_1 complex; effect of papain digestion on electrophoretic mobility. b-c_1 complex was treated with papain as described in section 2. Samples of the enzyme were taken at the times indicated and subjected to electrophoresis. The molecular masses of polypeptides were determined by using molecular mass standards [12]. K, kDa.

enhanced migration of the 15-kDa fragment. Furthermore progressive digestion of the Fe-S protein occurred.

Fig.2 shows that treatment of soluble b-c_1 complex with papain resulted in marked depression of the initial rate and the extent of proton release elicited by the addition of duroquinol to cytochrome c supplemented b-c_1 vesicles and in a smaller inhibition of the rate of cytochrome c reduction. The H^+/e^- ratio was lowered from 2 in the control to around 1. This residual H^+ release derives from the scalar oxidation of hydrogenated quinol by cytochrome c. The measured H^+/e^- ratio did not vary whether the b-c_1 vesicles were used as prepared in [10] or after gel chromatography elution.

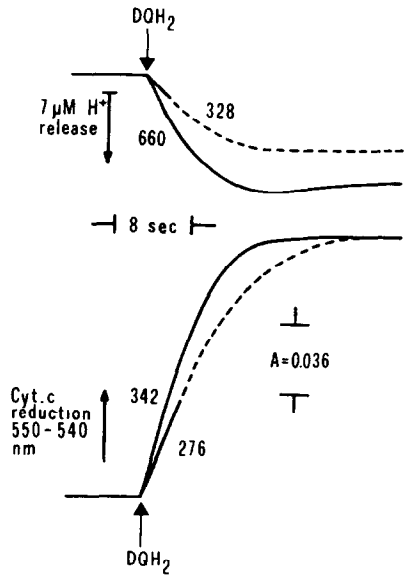

Fig.2. Effect of papain digestion on redox and protonmotive activity of b-c_1 vesicles. Where indicated the soluble b-c_1 complex was digested for 30 min with papain as described in section 2. b-c_1 vesicles were suspended at 0.17 mg protein/ml in 150 mM KCl, 5 mM $MgCl_2$, 7 μM ferricytochrome c, 1 μg/ml valinomycin and 1 mM NaN_3. Final pH, 7.2. (——) Control, (– – –) papain-treated b-c_1 complex. The figures on the traces represent initial rates of H^+ release and cytochrome c reduction as nequiv. H^+ and $e^- \cdot min^{-1} \cdot mg^{-1}$ protein.

In fig.3 the time course of the effect of papain digestion on electron transfer and proton translocation activities in b-c_1 vesicles is shown. Papain digestion resulted in a progressive inhibition of electron flow from quinol to cytochrome c and in an even more marked depression of proton release with a consequent depression of the H^+/e^- ratio. This depression cannot be accounted for by the inhibition of the rate of electron flow. It has, in fact, been found that by lowering up to 90% the rate of electron flow by treatment of the b-c_1 complex with the amino acid reagents ethoxyformic anhydride or tetranitromethane, the H^+/e^- ratio remains unaffected [12]. It thus appears that papain digestion exerts two independent effects: inhibition of electron transfer within the complex and suppression of the proton pumping activity.

The possibility that these two effects result from digestion of different subunits is supported by the results in fig.4. These show that whilst the inhibi-

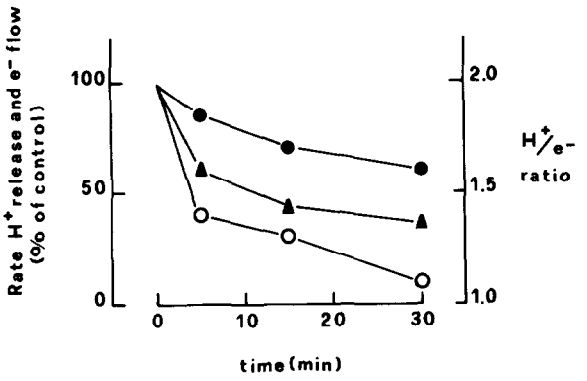

Fig.3. Time course of the effect of papain digestion on electron transfer and proton translocation activities in *b*-c_1 vesicles. The experimental conditions are those described in the legend to fig.2 and section 2. The initial rates of electron flow (●—●) and proton translocation (▲—▲) are expressed as percentage of the rates measured in the control; (○—○) H^+/e^- ratio.

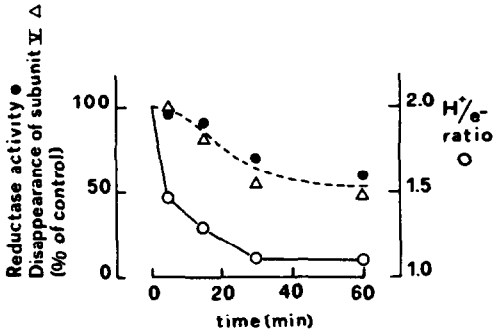

Fig.4. Time course of the effect of papain digestion on polypeptides and enzymatic activity of *b*-c_1 complex. Incubation with papain of soluble *b*-c_1 complex was carried out as described in section 2. Measurement of cytochrome *c* reductase activity and H^+/e^- ratio and SDS-PAGE electrophoreses were carried out as described in section 2, and in the legend to figs 1 and 2. The band corresponding to subunit V (△—△) was integrated and the integrals are reported as percentage of the control band.

tion of the reductase activity paralleled strictly the digestion of the Fe-S protein, proton pumping was depressed by 60% after 5 min incubation when the Fe-S protein digestion and inhibition of electron flow were negligible. As previously pointed out, the partial digestion of core protein II and the 15-kDa band were almost complete at 5 min.

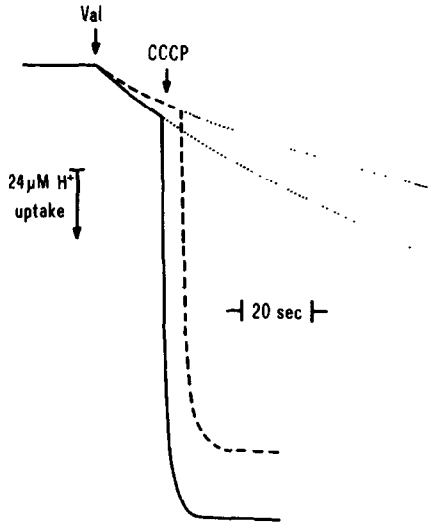

Fig.5. Effect of papain digestion on passive proton conductance in *b*-c_1 vesicles. Vesicles with the control or papain treated *b*-c_1 complex were suspended at 0.2 mg protein/ml in a mixture containing 220 mM sucrose and 1 mM K-Hepes (pH 7.2). H^+ influx, measured potentiometrically, was initiated by the addition of 1 μg/ml valinomycin. Where indicated 2 μM CCCP was added. (——) Control, (– – –) papain-treated *b*-c_1 complex.

Fig.5 shows that valinomycin-mediated proton diffusion across the phospholipid membrane in the vesicles containing papain-treated *b*-c_1 complex was lower with respect to that of the control vesicles. Thus the depression of redox-linked proton translocation by papain treatment of the *b*-c_1 complex was not due to enhancement of the rate of passive proton back-diffusion.

In conclusion, our data show that partial proteolysis of *b*-c_1 complex with papain leads to suppression of respiration-linked proton translocation by the complex reconstituted into phospholipid vesicles. The gel filtration controls showed that this was not due to lack of incorporation of the enzyme into vesicles. Kinetic experiments indicate a correlation between the digestion of the Fe-S protein and the inhibition of the electron flow, whereas the suppression of vectorial H^+ translocation seems to be correlated with the digestion of core protein II and the 15-kDa subunits. Thus these proteins, in addition to the 8- and 12-kDa proteins modified by DCCD [12], appear to be involved in the proton pumping activity of the *b*-c_1 complex.

It seems possible to conclude that coupling between electron flow and proton translocation in the reductase depends critically on the ordered arrangement and interaction of various polypeptide subunits. Both the Q cycle [11] as well as the Q-gated proton pump model favoured by Papa et al. [10] involve specific semiquinone/quinol couples [21]. Polypeptides of the complex could bind and stabilize the semiquinone species, thus preventing uncoupled dismutation processes. Polypeptides could also constitute pathways for transmembrane proton conduction from one aqueous phase to the bound quinone system and from this to the opposite aqueous phase [22].

REFERENCES

[1] Marres, C.A.M. and Slater, E.C. (1977) Biochim. Biophys. Acta 462, 531–548.

[2] Capaldi, R.A. (1982) Biochim. Biophys. Acta 694, 291–306.

[3] Papa, S. (1976) Biochim. Biophys. Acta 456, 39–84.

[4] Mitchell, P. (1976) J. Theor. Biol. 62, 327–367.

[5] Papa, S., Guerrieri, F., Lorusso, M., Izzo, G., Boffoli, D., Capuano, F., Capitanio, N. and Altamura, N. (1980) Biochem. J. 192, 203–218.

[6] Leung, K.H. and Hinkle, P. (1975) J. Biol. Chem. 250, 8467–8471.

[7] Guerrieri, F. and Nelson, B.D. (1975) J. Biol. Chem. 250, 8467–8471.

[8] Von Jagow, G., Engel, W.D., Shägger, H., Machleidt, W. and Machleidt, I. (1981) in: Vectorial Reactions in Electron and Ion Transport in Mitochondria and Bacteria (Palmieri, F. et al. eds) pp.149–161, Elsevier/North-Holland, Amsterdam.

[9] Wikström, M.K.F., Krab, K. and Saraste, M. (1981) Annu. Rev. Biochem. 50, 623–655.

[10] Papa, S., Lorusso, M., Boffoli, D. and Bellomo, E. (1983) Eur. J. Biochem. 137, 405–412.

[11] Beechey, R.B., Robertson, A.M., Halloway, C.T. and Knight, I.G. (1967) Biochemistry 6, 3867–3879.

[12] Lorusso, M., Gatti D., Boffoli, D., Bellomo, E. and Papa, S. (1983) Eur. J. Biochem. 137, 413–420.

[13] Degli Esposti, M., Saus, J., Timoneda, J., Bertoli, E. and Lenaz, G. (1982) FEBS Lett. 147, 101–105.

[14] Beattie, D.S. and Clejan, L. (1982) FEBS Lett. 149, 245–249.

[15] Nalecz, M.J., Casey, R.P. and Azzi, A. (1983) Biochim. Biophys. Acta 724, 75–82.

[16] Clejan, L., Bosch, L.G. and Beattie, D.S. (1984) J. Biol. Chem. 259, 11169–11172.

[17] Price, B.D. and Brand, M.D. (1983) Eur. J. Biochem. 132, 595–601.

[18] Rieske, J.S. (1967) Methods Enzymol. 10, 239–245.

[19] Leung, K.H. and Hinkle, P. (1975) J. Biol. Chem. 250, 8467–8471.

[20] Laemmli, U.K. (1970) Nature 227, 680–685.

[21] Yu, C.A., Nagahota, S., Yu, L. and King, T.E. (1978) Biochem. Biophys. Res. Commun. 82, 1076–1078.

[22] Papa, S. (1982) J. Bioenerg. Biomembranes 14, 69–86.