INTERACTION BETWEEN THE BINDING OF ^{35}S-ATRACTYLOSIDE AND BONGKREKIC ACID AT MITOCHONDRIAL MEMBRANES

M. KLINGENBERG, K. GREBE and G. FALKNER
Institut für Physiologische Chemie der Universität, München, Germany

Received 26 June 1971

1. Introduction

Bongkrekic acid (BA) has been shown [1, 2] to fix adenine nucleotide (ANP) to mitochondrial membranes to an extent that the ANP cannot be removed by atractyloside (ATR) in contrast to the removability by ATR of ANP bound in the absence of BA [3]. In accordance, ANP being unable to bind to the membranes in the presence of ATR can be bound by addition of a sufficient excess of BA.

It is of great interest to determine whether ^{35}S-ATR is unable to bind in the presence of BA or bound ^{35}S-ATR is removed by BA from the mitochondria. Furthermore, since the binding of BA and ADP to the membranes has been shown to be reciprocal [4], it was of interest to determine whether BA alone or only together with ANP can remove ^{35}S-ATR. This relates to the question whether the binding sites of ATR, BA and ADP on the carrier are identical or whether there are effector sites which are different from the ANP binding sites.

In the previous publication [5] ^{35}S-ATR has been shown to bind to mitochondria in a ratio of 1 to 2 to the specific ANP binding. Because of the high affinity of ATR it was not possible to remove ATR by ADP and thus to discriminate a specific from a possibly unspecific ATR binding. A removal of ATR under the influence of BA directly, or indirectly by the fixation of ADP would give the possibility to differentiate and characterize further the binding of ATR.

Abbreviations:
ATR: atractyloside
ANP: adenine nucleotide
BA : bongkrekic acid

2. Results

In fig. 1 the removal of ^{35}S-ATR bound to membranes from beef heart mitochondria by increasing concentrations of BA is shown. The binding sites for ATR are saturated with a sufficient amount of ^{35}S-ATR and an excess of ADP is added in order to increase the binding affinity of BA. Furthermore, the experiments are performed at 30° in order to remove the kinetic inhibition of the BA effect at low temperatures. Nearly all ATR can be removed by BA when added at about 5 μmole/g protein. From the initial slope a minimum stoichiometric requirement for BA is calculated giving a ratio $\Delta ATR/\Delta BA = 0.41$.

These results permit to conclude that all ^{35}S-ATR is specifically or carrier bound since it is fully sensitive to BA. The homogeneous decrease of the ATR binding by increasing amounts of BA indicates that all ATR is bound at a single type of binding sites sensitive to BA.

It is questionable whether the stoichiometry means binding of two molecules BA per molecule ATR at the carrier sites. Usually a molar excess of BA can be expected because of the kinetic inhibition of the BA effect [4]. Therefore it is improbable that under these conditions all added BA is bound although the high concentration of ADP (100 μM) favours maximum binding of BA.

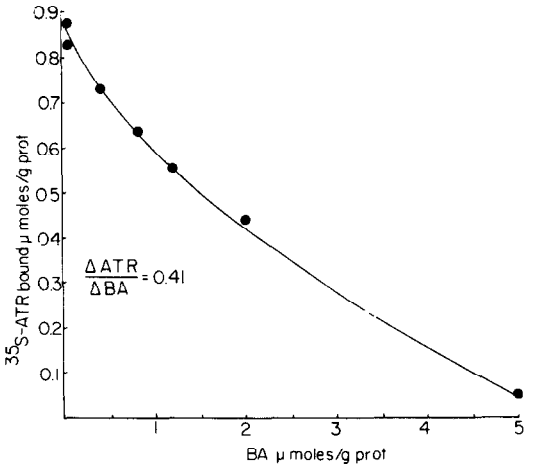

Fig. 1. Removal of bound ^{35}S-ATR by BA from mitochondrial membranes. Depleted beef heart mitochondria (BHM) incubated in 0.25 M sucrose, 20 μM Tris-maleate, pH 7.0 at 30°. To each sample was first added ADP and ^{35}S-ATR (4 μM), then mitochondria (1.03 mg protein/0.5 ml) and after 2 min BA at the concentration indicated. After 2 min centrifugation and extraction of the sediments for ^{35}S-ATR. ^{35}S-ATR was obtained according to the procedures described in the preceding paper [5].

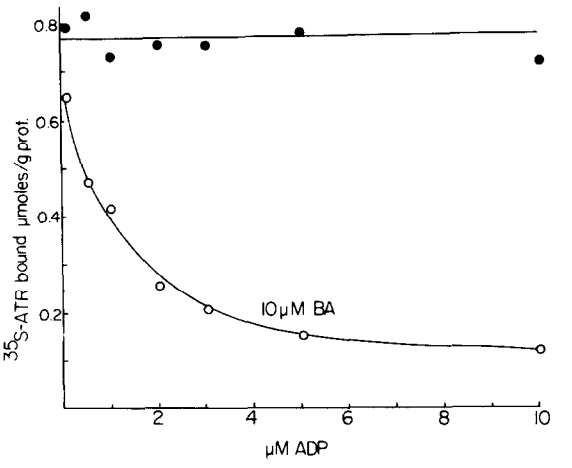

Fig. 2. Influence of ADP on the removal of ^{35}S-ATR by BA. Binding of ^{35}S-ATR in the absence (•) and presence of 10 μM BA (○). Depleted BHM (2 mg protein/ml) incubated as described for fig. 1 with 5 μM ^{35}S-ATR.

The dependence of the removal of ATR by BA on the concentration of ADP is illustrated in fig. 2. Here again sufficient ATR is added in order to saturate the binding sites. The addition of BA alone decreases the binding of ATR only by about 15%. At rather low concentrations of ADP the binding decreases with an initial slope of Δ^{35}S-ATR/ADP = 0.35. The removal appears to be saturated at 10 μM ADP in this case. The results demonstrate that the removability of ATR by BA is strongly dependent on the presence of ADP in agreement with the finding that the affinity increase for BA and for ADP is reciprocal.

Because of the reciprocity in the fixation of BA and ADP on the ANP carrier it cannot be clearly decided whether ATR is removed by competition with BA or with ANP for the binding site. From the viewpoint of the chemical structures and the results of the previous paper [5] a direct competition between ANP and ATR for the binding site is the preferred interpretation.

In fig. 3. the question is investigated whether the inhibition by BA of ATR binding is competitive. For

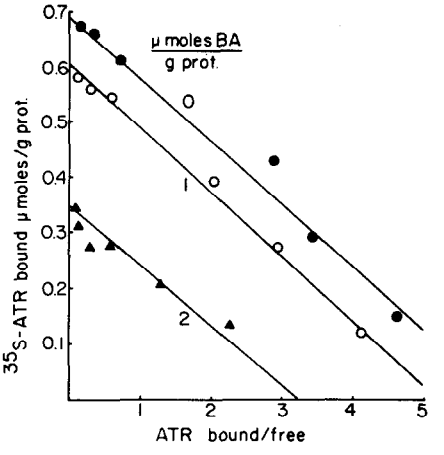

Fig. 3. ATR removal by BA. Influence of the ATR and BA concentration. Scatchard plots of the binding data. Depleted BHM (2.5 mg protein/0.5 ml) incubated at 25° in 0.25 M sucrose, 20 μM Tris-maleate. First BA and 100 μM ADP are added, the suspension cooled to 0° and then after 5 min ^{35}S-ATR is added. After 2 min centrifugation and subsequent extraction of the sediment.

this purpose the ATR binding is measured in dependence on the concentration of ATR in the presence of 1 and 2 μM BA and an excess of ADP.

BA causes a parallel shift of lines in the Scatchard

plot indicating that the affinity is not influenced but that the maximum number of binding sites is diminished. This corresponds to a non-competitive effect of BA on ATR for the present range of BA and ATR concentrations. However, it does not exclude a competition at a higher concentration range of ATR where ATR has a lower affinity. The dissociation constant evaluated from the 3 curves is $K_D = 4.6 \times 10^{-7}$ M, in agreement with the results of the preceding paper [5] for the high affinity binding range at lower ATR concentration. A second type, low affinity binding of ATR, was demonstrated [5] in the binding curves.

These results could be interpreted to show that BA or ADP remove ATR not by direct competition from the binding sites if one implies a free interaction between the ligands and binding sites at the carrier protein. It must be considered that, however, only parts of the binding sites at a carrier can be freely accessible, for example when there are inner and outer localized sites as proposed for the ANP carrier. Non-competitive effects could then result from fixation of BA and ADP at the inner surface of the membrane, opposite to the ATR binding site.

Acknowledgements

The authors gratefully acknowledge the receipt of bongkrekic acid from Prof. W. Berends, Delft, and seeds of *Atractylis gummifera* from Prof. F. Ranzoli, Catania. This work was supported by grants from the Deutsche Forschungsgemeinschaft (SFB 51).

References

[1] M.J. Weidemann, H. Erdelt and M. Klingenberg, Biochem. Biophys. Res. Commun. 39 (1970) 363.
[2] H. Erdelt, M.J. Weidemann and M. Klingenberg, European J. Biochem., in press.
[3] M.J. Weidemann, H. Erdelt and M. Klingenberg, European J. Biochem. 16 (1970) 313.
[4] M. Klingenberg, H. Erdelt, M. Buchholz and M.J. Weidemann, in preparation.
[5] M. Klingenberg, G. Falkner, H. Erdelt and K. Grebe, FEBS Letters 16 (1971) 296.