The high resolved structural data now available for rubredoxin and desulforedoxin allows an accurate description of the iron center with a good definition of the S-Fe distances and coordination angles. These parameters are required for further analysis of the relationship between structure, function and reactivity, and are important for the control of electron transfer mechanisms and the control of the redox potential of the center. They are also essential for the establishment of correlations between these properties and the spectral characterization of both Dx and Rd.

The electrochemical determination of the redox potentials of rubredoxin type proteins enables a rapid method to access the value of the redox potential of the metal center. Also, a variety of proteins from different origins (native, overexpressed and chemically synthesized) were study. The work will be now extended to the association of these centers with other type of redox centers, as the indicated cases of Rr and Dfx. The chemical modification of these centers, obtained by replacing the native iron site in Rd and Dx by other metals (i.e. cobalt and nickel) will be also considered.

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REFERENCES

[1] Masaki, T., Tanabe, M., Nakamura, K. and Soejima, M. (1981) *Biochim. Biophys. Acta* **660**, 44-50
[2] Bruschi, M. and LeGall, J. (1972) *Biochim. Biophys. Acta* **271**, 279-282
[3] Moura, I., Bruschi, M., LeGall, J. Moura, J.J.G. and Xavier, A.V. (1977) *Biochem. Biophys. Res Commun.* **75**, 1037- 1044
[4] Czaja, C., Litwiller, R., Tomlinson, A.J., Naylor, S., Tavares, P., LeGall, J., Moura, J.J.G., Moura, I. and Rusnak, F. (1995) *J. Biol. Chem.* **270**, 20273-20277
[5] Tavares, P., Wunderlich, J.K., Steven, G.L., LeGall, J., Moura J.J.G. e Moura, I. (1995) *Biochem. Biophys. Res Commun.* **208**, 680-687
[6] Archer, M., Huber, R., Tavares, P. Moura, I., Moura J.J.G., Carrondo, M.A., Sieker, L.C., LeGall, J. e Romão, M.J. (1995) *J. Mol.Biol.* **251**, 690-702

ELECTROCHEMICAL STUDIES OF *Desulfovibrio desulfuricans* ATCC 27774 ALDEHYDE OXIDO-REDUCTASE (AOR)

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ABSTRACT - *Electrochemical studies on* Desulfovibrio desulfuricans *ATCC 27774 Aldehyde Oxido-Reductase (AOR) were carried out in order to optimize the conditions for the development of an enzymatic sensor for aldehydes. In presence of both aliphatic and aromatic aldehydes, AOR develops a catalytic activity with a characteristic Michaelis-Menten type behavior.*

KEY WORDS - Electrochemistry, Biosensors, Aldehydes, Aldehyde Oxido-Reductase.

INTRODUCTION

The sensitivity and the specificity of biological activities make enzymes a target for the development of enzyme electrodes. Biosensors (a special group of bioelectrodes) combine a sensitive layer based on biological components capable of detecting or responding to surrounding chemicals. The biological component translates the specific molecular recognition of the analyte into a signal that can be readily chemically or physically measured. In addition, enzyme immobilization is frequently used in the development of biosensors.

Aliphatic aldehydes are found in wastes waters which have a damage effect for the environment. On the other hand, aromatic aldehydes are present in several compounds of biological importance.

From nitrate-grown cells of *Desulfovibrio desulfuricans* ATCC 27774 (a sulfate reducing organism), a molybdenum-[iron-sulfur] containing aldehyde oxido-reductase (converting aldehydes into carboxylic acids) was isolated [1,2].

This enzyme has a significant potential for the use in the construction of biosensors, as it carries out a specific and well defined chemical transformation, is available in large amounts, can be purified by well established procedures and is quite stable in solution.

For AOR, we have now adapted a previously reported study on a biosensor for nitrite detection based on the nitrite reductase system [3,4].

We demonstrate here an electrochemical response of an enzyme on a glassy carbon electrode and compare the enzymatic activity and amperometric aldehyde response of AOR in solution and in a polyacrylamide entrapped phase; this latter phase was chemisorbed onto the surface of a glassy carbon electrode. Application to the analytical determination of the substrates is potentiated.

EXPERIMENTAL

Growth of organism and purification of Aldehyde Oxido-Reductase (AOR) *Desulfovibrio desulfuricans* ATCC 27774 cells were grown as indicated (5) using a lactate-sulfate medium. The molybdenum enzyme (AOR) was purified from cell-free crude extract using identical chromatographic methods, as proviously described (6). UV/Visible absorption spectra were recorded with a Shimadzu model 265 spectrophotometer. Protein concentration were calculated using the extinction coefficient of 20,000 ($l.mol^{-1}.cm^{-1}$) at 462 nm.

Electrochemical measurements A two-compartment cell with a three-electrode system was used. The working electrode was a Metrohm disc glassy carbon electrode, whereas the reference electrode was a Radiometer saturated calomel electrode (SCE). The counter electrode was a platinum wire. All potentials are given against the normal hydrogen electrode (NHE) ($E^{0'}$ SCE = 242 mV vs. NHE).

All reaction mixtures contained 10 mM Tris/HCl buffer at pH=7.6 in 0.1 M KNO_3 as supporting electrolyte. Solutions were prepared with distilled water. Enzyme concentratrions used were those indicated in the Figure legends. Experiments were conduced at room temperature. All sample were deoxygenated for 10 min before electrochemical experiments and a continuos flow of higth purified argon was led over the solution during the measurements. The working electrode was polished before each experiment using an aluminium oxide (0.075 mm)/water slurry on cotton wool. The standards solutions of aldehydes were prepared diluting the correspondent amount of aldehydes in distilled water. The addition of aldehydes into the electrochemical cell were made by a 10 µl Hamilton serynge. Before aldehyde addition, the containing enzyme solutions were clicled betweem 0 and -0.9V (SCE) until a stable voltammogram was obtained.

Cyclic voltammograms were obtained with AUTOLAB 10 potenciostat. Both acquisition and manipulation of data were made by GEPES 3.2 software from Eco Chemie.

RESULTS AND DISCUSSION

The results of the cyclic voltammetry experiments indicated that electrochemical response of AOR were significantly increased by the presence of both aliphatic and

aromatic aldehyde. The dispropotional increased of the current as response to the increasing amount of added aldehyde show that a catalytic mechanism (EC') (7) is operative in this system.

Upon plotting the measured electrochemical current against benzaldehyde concentrations (Figure 1 **A)** a typical Michaelis-Menten curve was observed. The data fitting using the formalism of enzyme kinetic theory gives a K_m value of 2.0 µM. Figure 1 **B** shows the linear relation existing between limiting current and the benzaldehyde concentration lower than the K_m value. Thus relation can be used as calibration curve for benzaldehyde determination in solution.

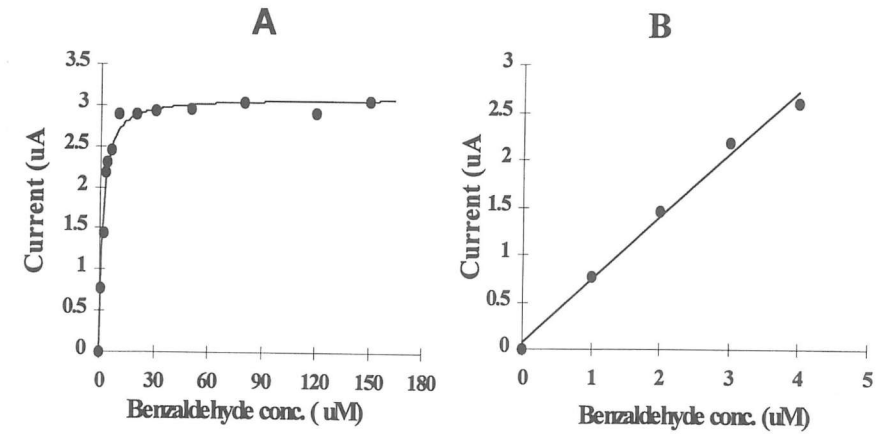

Figure 1. **A)** Michaelis-Menten type curve for electrochemical response of AOR (30 µM) in the presence of the benzaldehyde ; **B)** Calibration curve for benzaldehyde determination (conditions: see experimental part).

A similar electrochemical response was obtained for AOR when in the presence of the acetaldehyde and propionaldehyde. The values of the K_m were 9 and 7 µM, respectively. Figure 2 **A** and **B** show the Michaelis-Menten curve for these substrates. The kinetics parameters (K_m) determinated for aldehydes by cyclic voltammetry were in concordance with the values found in the literature (8,9).

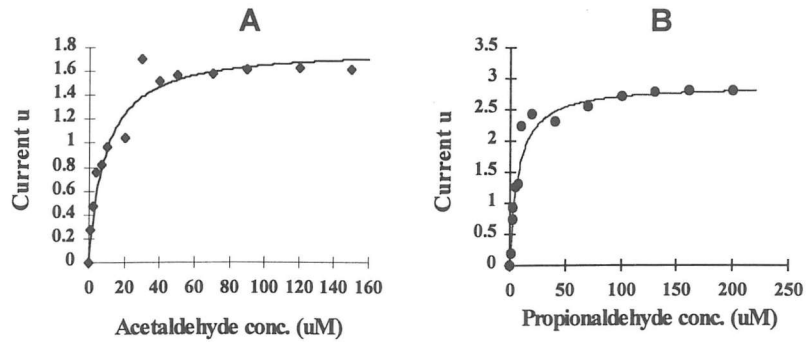

Figura 2. **A)** and **B)** Electrochemical response of AOR in the presence of acetaldehyde and propionaldehyde, respectively (conditions: see experimental part).

The results clearly show that AOR is a interesting system which can be used for aldehyde determination in solution. So, future developments of this work will focus in the construction of a biosensor device. The simplest way to make a biosensor is to immobilize the enzyme in a matrix, and strategies are currently being developed. In paralell, studies regarding the stability and reproducibility of this system are now under progress.

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REFERENCES

1. Costa, C., Moura, J.J.G., Moura, I., M.C.Liu, M. C., Peck,Jr., H.D., Le Gall, J., Wang, Y., Huynh, B.H. (1990) *J. Biol. Chem.* **265**, 14382-14387
2. Barata, B.A.B., LeGall, J., Moura, J.J.G (1993) *Biochemistry* **32**, 11559-11568
3. Moreno, C., Costa, C., Moura, I., LeGall, J., Liu, M.Y., Payne, W.J., VanDijk, C. E., Moura, J.J.G. (1992) *Eur. J. Biochem.* **212**, 79-86.

4. Scharf, M., Moreno, C., Costa, C., VanDijk, C. E., Payne, W.J., LeGall, J., Moura, I. and Moura, J. J. G. (1995) *Biochem. Biophys. Res. Comm.* **209**, 1018-1025.
5. LeGall, J., Mazza, G. and Dragoni, N. (1965) *Biochim. Biophys. Acta* **99**, 385-387.
6. Moura, J. J. G., Xavier, A. V., Cammack, R., Hall, D. O., Bruschi, M. and LeGall, J. (1978) *Biochem. J.* **173**, 419-425.
7. Bard, A. J. and Faulkner, L. R. (1980) in "Electrochemical Methods. Fundamental and Applications", Wiley Pub., page 197.
8. Barata, A.S., LeGall, J. and Moura, J. J. G. (1993) *Biochemistry* **32**, 11559-11568.
9. Huber, C., Skopan, H., Feicht, R., White, H. and Simon, H. (1995) *Arch. Microbiol.* **164**, 110-118.