Integration of an ISFET with a porous microelectrode for application to protein characterization

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Abstract

This paper describes a way of dynamic buffer capacity measurement to characterize proteins, using a coulometric sensor-actuator system. The sensor-actuator is made by integrating an ISFET (ion-sensitive field effect transistor) with a porous microelectrode. Lysozyme and ribonuclease were studied. The results show that the buffer capacity of proteins is both a function of pH and their concentrations. The characters of studied proteins can be distinguished each other from measured curves.

Introduction

Integration of an ISFET (ion-sensitive field effect transistor) with a microelectrode as a chemical sensor-actuator system has found applications in microliter acid-base titration [1] and construction of a pH-static enzyme sensor [2] by a feedback-control operation. A major advantage of the integration is that it enables dynamic operation of ISFET. In this way, drift, which is often found to be a problem of ISFET, is no longer critical to the applications. Recently, we also reported the employment of a porous noble metal electrode integrated with ISFET as sensor-actuator system for directly sensing the buffer capacity of analyte [3]. In comparison with the planar electrode, a highly porous electrode closely covering the gate of ISFET forms in itself a small reaction chamber, which limits the diffusion of species to a certain degree. pH modulation can be achieved inside such a closed chamber by applying a small perturbation of titrant, which can easily be generated coulometrically by applying an alternating current to the porous electrode. The associated pH change will be a function of the buffer capacity of analyte and can be rapidly detected by ISFET. Both the theoretical description and the measurement have been presented in our early paper [3]. In practice, this approach can further be applied to characterize proteins, of which the specific primary structures and conformations will give them different buffering properties [4,5]. Such buffering properties are due to the prototropic groups in side-chains and at the ends of peptide chains [4-6]. Different intrinsic pKs of the groups as well as the specific interactions of the groups, mainly due to the conformation of a protein, will show a specific 'finger print' in its titration curve or the derivative titration curve, which makes the characterization possible. Moreover, the interaction of prototropic groups with other specific binding groups in a protein [6-9] may expand the study to the binding of other substances such as metal cations or immunological complexes, which may lead to more applications.

In this paper, we investigate a way to characterize proteins using dynamic buffer capacity measurement. Lysozyme and ribonuclease were studied as model proteins.

Principle of operation

The device of an ISFET integrated with a porous electrode is shown in Fig.1.

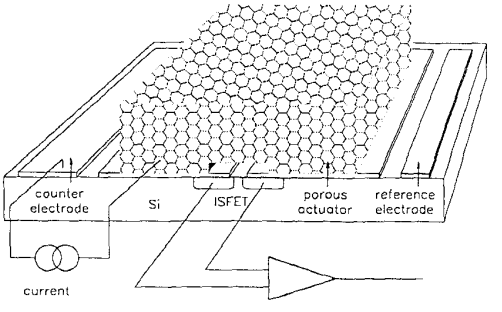

Fig.1 *ISFET with a porous electrode covering the gate.*

If a current is applied to the electrode, titrant coulometrically generated at the electrode by electrolysis of water can be either protons or hydroxyl ions, depending on the direction of applied current. The typical reactions at the electrodes are:

Anode: $2H_2O \longrightarrow 4H^+ + 4e + O_2$
Cathode: $2H_2O + 2e \longrightarrow 2OH^- + H_2$

If a sinusoidal current at a given frequency is applied to the porous electrode, it will result in an alternatively generated titrant of protons and hydroxyl ions. The associated

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concentration change of the species at the gate of ISFET will also be sinusoidal provided the perturbation of generated titrant is small [3]. The buffer capacity dependent pH changes can then be measured by an ISFET. This is illustrated in Fig.2.

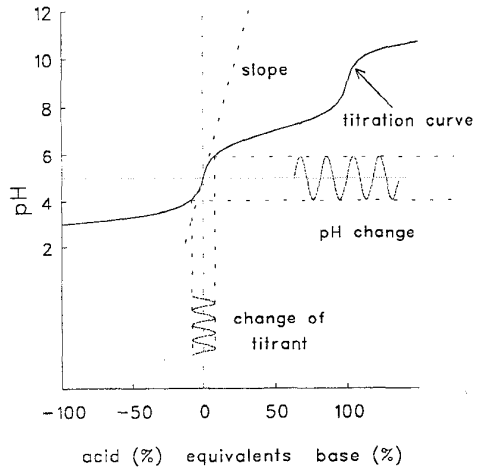

Fig.2 *Illustration of buffer capacity measurement by means of an applied sinusoidal perturbation of titrant.*

A solution with dissolved proteins functions as a polyelectrolyte, due to a great number of proton binding sites on a protein. Traditional analysis of proton binding equilibria assumes that all identical groups are chemically equivalent so that the hydrogen ion titration curve of a protein can be interpreted [5]. Accordingly, the same principle can also be applied to interpret the proton binding of a protein, measured in a dynamic way. It should be stated here that due to the difference in mobility of protons and hydroxyl ions, the dynamically measured buffer capacity will deviate from that measured in a static way, for instance by a volumetric titration. Therefore, a correction of the deviation resulting from this dynamic measurement is necessary if precision is of critical importance.

Experimental

The fabrication procedure of the device consists of integration of an ISFET with a porous gold electrode covering the gate, as has been described in the previous paper [10]. Typical thickness of the electrode is ca. 100 μm. The measurement set-up is shown in Fig.3.

Before dissolution of proteins the sample solution is initially bubbled with nitrogen for 15 minutes. Then, protein is added and the solution is stirred for several minutes until complete dissolution. The measurement vessel was purged with nitrogen throughout the measurement. As the supporting

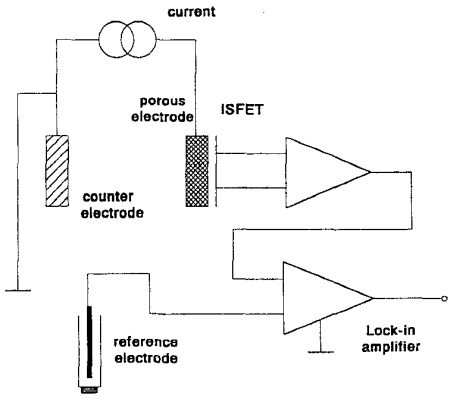

Fig.3 *Measurement set-up.*

electrolyte, 0.1 M potassium nitrate was used. The pH was changed by adding 1.0 M nitric acid or 1.0 M potassium hydroxide to the sample solution and was monitored using a conventional glass electrode (Radiometer). For all measurement pH was changed from a high to a low value. The measurements for correction were carried out with a blank solution without proteins (but with supporting electrolyte). In this way, the buffering from the protons and hydroxyls is subtracted.

Results and discussions

Buffer capacity measurement of lysozyme (Sigma L-2879) in two concentrations without correction by a blank measurement is presented in Fig.4. The output voltage of the ISFET amplifier is presented reciprocally, which corresponds to the measured buffer capacity [3]. Although the results of the dynamic measurement will be different from those of the static measurement due to different mobility of

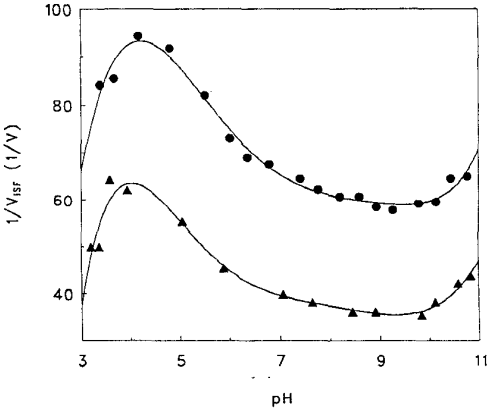

Fig.4 *Buffer capacity measurement of lysozyme in 0.1 M KNO_3.* ▲*-2g/l,* ●*-5g/l.*

protons and hydroxyl ions, some qualitative evaluation from this figure can still be significant. With increasing concentration of the protein solution the measured buffer capacity curve shifts vertically along the Y axis, indicating that the buffer capacity of a protein is positively proportional to its concentration. For the two concentrations measured, the lowest buffer capacity lies between pH 9 and pH 10. It appears that the minimum is around the isoelectric point of lysozyme, which is reported to be about pH 11 [11]. The highest buffer capacity is found at about pH 4. These measurement data suggest that the buffer capacity of lysozyme is both a function of pH and protein concentration.

Another protein, ribonuclease (Sigma R-4875), has also been investigated. The results are presented as a function of pH in Fig.5. The buffer capacity curve of the ribonuclease differs from that of the lysozyme. The lowest peak appears at about pH 8, while two maxima appear at about pH 4 to pH 5, and at pH 10 to pH 11. Besides the buffering difference at different pH values, the buffer capacity of ribonuclease over the pH range of concern is also found to be smaller than that of lysozyme. Considering that the molecular weight of ribonuclease is slightly lower than that of lysozyme, the molar concentration of ribonuclease is therefore slightly higher in our experiment. This suggests that ribonuclease has less binding sites for protons or that the static interaction that rejects further binding between the groups is stronger than in the case of lysozyme.

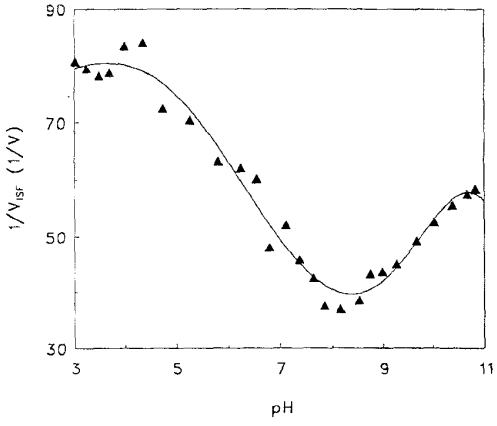

Fig.5 *Buffer capacity measurement of 5 g/l ribonuclease in 0.1 M KNO_3.*

In these measurements the buffer effect of protons and hydroxyl ions has not been excluded, which also function themselves as a strong buffer beyond intermediate pH range. In addition, the diffusion of the protons and hydroxyl ions will also exert an extra buffer effect during dynamic measurement [3]. Protons that have high mobility will show higher buffer effect than that of hydroxyl ions. That is the reason why dynamically measured buffer capacity always shows a higher value in acidic region than in basic region. Therefore, the measurements with blank solution (without protein) were performed for correction purpose in order to subtract the buffer effect from protons and hydroxyl ions. A problem of these measurements in the intermediate pH range is that the observed output from the ISFET amplifier is not stable. This is caused by the fact that blank solution has hardly any buffer action in the intermediate pH.

The corrected results of Fig.4 and 5 for a protein concentration of 5 g/l are presented in Fig.6. The difference between the buffer capacities of two proteins is distinct. The peaks in the curves after correction are more evident. Lysozyme shows a high buffer capacity in the acidic region around pH 5 and has almost no buffer function above pH 10. Ribonuclease has a good buffer function in the acidic region around pH 5 as well as in the basic region around pH 10, but shows a low buffer capacity in the neutral solution around pH 8.

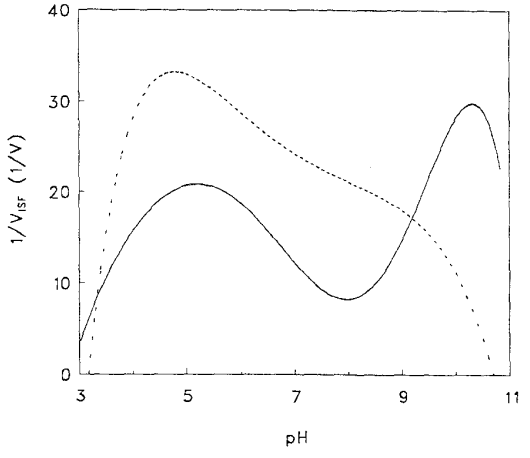

Fig.6 *Buffer capacity measurement of 5 g/l ribonuclease (solid line) and lysozyme (dashed line) in 0.1 M KNO_3 after correction.*

Finally, some remarks about the experimental results must be mentioned here. The measured buffer capacity at a pH value higher than 11 or lower than 3 has a relatively large error, because at these extremes protons or hydroxyl ions will manifest themselves as a strong buffer. Furthermore, the interpretation of the experimental results have not taken into account the kinetic effect, which might also be of importance in the case of dynamic measurement. The results obtained in this paper are therefore not exactly comparable to those carried out by a normal potentiometric titration [11-13].

Conclusions

Dynamic measurements of buffer capacity using a coulometric sensor-actuator system provide a rapid and continuous way of characterizing proteins. Results have shown that the measured buffer capacity of a protein is a function of pH as well as its concentration. Different proteins differ in their buffer capacity as has been observed from the measurement results of the lysozyme and ribonuclease. As this difference in protein buffer capacity results from the specific proton binding sites in the peptide chains as well as the static interactions among binding sites, it reflects the specific primary structure and the spatial conformations of proteins. Therefore, the buffer capacity can be considered as a kind of 'finger print' for the characterization of proteins. Attention should be paid when this approach is applied to the characterization of proteins. The measured results will deviate from those obtained by the static measurement due to the different mobility of protons and hydroxyl ions. Therefore, a more precise study is necessary. This suggests the need for a more rigorous modeling of the system operated at a sinusoidal or other programmed current.

References

[1] W. Olthuis, B.H. v.d. Schoot, F. Chavez and P. Bergveld, A dipstick sensor for coulometric acid-base titration, Sensors and Actuators, 17 (1989) 279-283.

[2] B.H. v.d. Schoot, P. Bergveld, The pH-static enzyme sensors, an ISFET-based enzyme sensors, insensitive to the buffer capacity of the sample, Anal. Chim. Acta. 199 (1987) 157-160.

[3] J. Luo, W. Olthuis, P. Bergveld, M. Bos, W.E. v.d. Linden, Determination of buffer capacity by means of an ISFET-based coulometric sensors-actuator system with a gate-covering porous actuator, Sensors and Actuators, B20, (1994)7-16.

[4] J. Steinhardt and J.A. Reynolds, Multiple equilibria in proteins, in Molecular biology series, Academic Press New York and London, 1969.

[5] C. Tanford, The interpretation of hydrogen ion titration curves of proteins, Advances in Protein Chemistry, Vol.17 (1962) 70-165.

[6] J.S. Fruton and S.Simmonds, General biochemistry, John Wiley & Sons, New York, 1963.

[7] A.S. Brill and J.H. Venable, Jr., Effect of site symmetry and sequential metal binding upon protein titration, J. Am. Chem. Soc., Vol.89 (1967) 3622-3626.

[8] C. Tanford and J. Epstein, The physical chemistry of insulin. II Hydrogen ion titration curve of zinc-free insulin, J. Am. Chem. Soc., Vol.76 (1954) 2163-2169.

[9] C. Tanford and J. Epstein, The physical chemistry of insulin. II Hydrogen ion titration curve of crystalline zinc insulin. The nature of its combination with zinc, J. Am. Chem. Soc., Vol.76 (1954) 2170-2176.

[10] J. Luo, W. Olthuis, P. Bergveld, M. Bos, W.E. v.d. Linden, A porous gold actuator for the ISFET-based coulometric sensors-actuator systems, Proceedings of Transducers'91, The Sixth International Conference on Solid-state Sensors and Actuators, San Francisco, September, 1991, p. 229-232.

[11] C. Tanford and M. Wagner, Hydrogen ion equilibria of lysozyme, J. Am. Chem. Soc., Vol.76 (1954) 3331-3336.

[12] H.B. Bull, An introduction to physical biochemistry, F.A. Davis Company, Philadelphia, 1964.

[13] C. Tanford, The physical chemistry of macromolecules, John Wiley & Sons, Inc., New York, 1961.