Sensors and Materials, Vol. 17, No. 5 (2005) 259–268
MYU Tokyo

S & M 0604

MOSFET-Type Biosensor for Detection of Streptavidin-Biotin Protein Complexes

Dong-Sun Kim, Hey-Jung Park[1], Jee-Eun Park[1], Jang-Kyoo Shin[*], Shin-Won Kang, Hwa-Il Seo[2] and Geunbae Lim[3]

School of Electronic & Electrical Eng., Kyungpook National University, Daegu 702-701, Korea
[1]Dept. of Sensor Eng., Kyungpook National University, Daegu 702-701, Korea
[2]School of Information Technology, Korea University of Technology & Education, Cheonan 330-708, Korea
[3]Dept. of Mechanical Eng., Pohang University of Science and Technology, Pohang 790-784, Korea

(Received August 22, 2004; accepted December 5, 2004)

Key words: FET-type sensor, biosensor, protein detection, self-assembled monolayer (SAM)

A p-channel metal oxide semiconductor field-effect transistor (PMOSFET)-type biosensor for detecting streptavidin-biotin protein complexes has been fabricated. Au, which has a chemical affinity with thiol, was used as the gate metal in order to form a self-assembled monolayer (SAM). A SAM was used to immobilize streptavidin. The hydroxyl group of SAM was bound with the amine group of streptavidin. Biotin was then injected into the solution to form streptavidin-biotin protein complexes in the solution. Streptavidin and biotin were bound by a high affinity (Ka~10^{15} Mol^{-1}). Measurements were conducted in a phosphate buffer saline (PBS; pH 6.4, 20 μM) solution and a Pt electrode was used as the reference electrode. The bindings of SAM, streptavidin, and biotin caused a variation in the drain current of the PMOSFET-type biosensor. To verify interactions among SAM, streptaividn, and biotin, a quartz crystal microbalance (QCM) measurement was performed.

1. Introduction

Recently, interest in the genome project has increased, and the need to investigate biomolecules (*e.g.*, antibodies, peptides and nucleotides) has reached a critical level. Biochip technology is being developed to analyze biomolecules effectively. The biosensor, which is a result of studies combining biology and engineering, has drawn considerable attention due to its fast analysis and convenient measurements. Examples of the biochip include a deoxyribonucleic acid (DNA) chip and a protein chip.[1,2]

*Corresponding author, e-mail address: jkshin@ee.knu.ac.kr

However, while DNA information basically controls life activity, the occurrence of disease has appeared in the protein level. Therefore, we need to identify the protein itself. Typical methods used for protein analysis are very similar to those for DNA sequences, for example, mass spectrometry, optical measurements, and electrochemical measurements.[3–5] The most common method is optical measurement. This method, however, has a disadvantage in that it requires the use of fluorescent materials and expensive equipment. Moreover, a portable diagnostic application is not easy. To overcome these problems, various methods and devices have been investigated, such as capacitance and impedance measurements, piezoelectric devices, and field-effect transistors (FETs).[6–9] A FET-type sensor, which is fabricated by semiconductor integrated circuit technology, has recently attracted considerable attention due to its numerous advantages in terms of miniaturization, standardization, and mass production.[10–13]

In the current study, we have fabricated a p-channel metal oxide semiconductor (PMOS)FET-type biosensor in order to detect protein molecules. The electrical characteristics of a PMOSFET-type biosensor were investigated. A quartz crystal microbalance (QCM) measurement was performed under the same conditions to verify interactions among SAM, streptaividn and biotin.

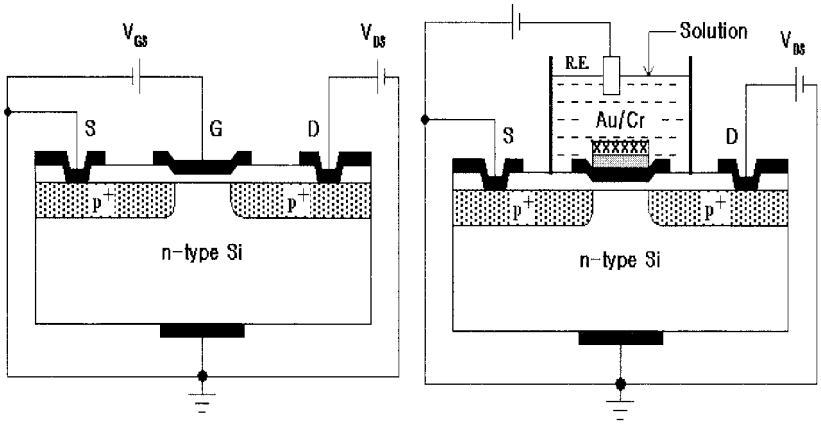
2. Theory

2.1 *Operating principle*

The physical structure of a FET-type biosensor for detecting protein is very similar to that of a MOSFET and the operating principle can be explained on the basis of self-assembled monolayer (SAM) and MOSFET operation theory. It is based on the interaction of thiol on gold. Figure 1 shows the structures of a conventional MOSFET and a MOSFET–type biosensor. The normal metal or polysilicon gate electrode of the MOSFET is replaced by the reference electrode in an electrolyte solution and Au, which has a chemical affinity with thiol on the top of the gate insulator. A gate voltage is applied to the gate insulator via the reference electrode and the electrolyte solution for the proper operation of the sensor. An electrochemical potential is then developed at the surface of Au with the binding of SAM and streptavidin. In addition, the variation of the capacitance is generated by the binding of streptavidin and biotin. These results can be measured as a shift in drain current or threshold voltage. The threshold voltage V_T and the current to voltage characteristics of MOSFET are

$$V_T = \Phi_{MS} - \frac{Q_O + Q_d}{C_O} + 2\Phi_F, \tag{1}$$

$$I_{DS} = \frac{\mu C_o W}{2L}\left(V_{GS} - V_T\right)^2, \tag{2}$$



(a) (b)

Fig. 1. Structures of (a) conventional MOSFET and (b) MOSFET-type biosensor.

where Φ_{MS} is the metal-semiconductor work function difference, C_o is the gate oxide capacitance per unit area, Φ_F is the Fermi potential of the semiconductor bulk, Q_o is the charge per unit area present at the interface and bulk of the oxide, Q_d is the space charge per unit area within the depletion region of the semiconductor, I_{DS} is the drain to source current, μ is the surface mobility of minority carriers in the semiconductor, W and L are the width and length of the gate, respectively, V_{GS} is the gate to source voltage, and V_{DS} is the drain to source voltage.

Since in a MOSFET-type biosensor the metal gate is replaced with a reference electrode and an Au layer, the expressions for the threshold voltage V_{T1} and the current to voltage characteristics should be altered from those of a MOSFET in the following manner:

$$V_{T1} = \Phi_{MS} + \Phi_{RL} - \frac{Q_O + Q_d}{C_{geff}} + 2\Phi_F - \phi(\text{protein}), \tag{3}$$

$$I_{DS} = \frac{\mu C_{geff} W}{2L}\left(V_{GS} - V_{T1}\right)^2, \tag{4}$$

where Φ_{RL} is the interface potential between the reference electrode and the liquid, φ (protein) is the interface potential between proteins and the gate electrode, and C_{geff} is the capacitance per unit area of the gate insulator including the proteins and gate oxide.

2.2 *SAM, streptavidin and biotin*

Protein is an essential part of living organisms. To analyze protein, a technique for immobilizing protein on the surface of the sensor is essential.[14] One solution is by SAM formation.[15] In particular, thiols that are attached to Au, which is used as the gate metal, are used to form a SAM. A monolayer of thiols on the Au surface is a well-documented example of SAM. The head and tail groups of SAM are composed of thiols and a hydroxyl group, respectively. In this experiment, streptavidin was used as the protein to be bound with biotin. Streptavidin is built from four subunits and has a molecular weight of about 60 kilodaltons (kD).[16] Streptavidin is frequently investigated due to its specific binding with biotin. Streptavidin-biotin complexes are useful in a wide range of biotechnological applications such as tagging or the delivery of molecules. Streptavidin can be attached to a SAM because it has an amine group. After binding with streptavidin, biotin was injected in order to investigate the interaction between streptavidin and biotin. The high-affinity streptavidin-biotin complexes are characterized by an extensive hydrogen-bonding network.[17] After binding with biotin, a multilayer was formed on the gate of the biosensor. The multilayer is composed of a SAM, streptavidin, and biotin.

3. Fabrication

Figure 2 shows a cross–sectional diagram of a PMOSFET-type biosensor. The starting material was a p-type <100> Si wafer. The width and length of the gate were 200 μm and 10 μm, respectively. A PMOSFET-type biosensor for detecting streptavidin and biotin was fabricated using a PMOS process. It was fabricated as a PMOSFET-type biosensor because the thiol (-SH) radicals of SAM have a negative charge. Au was used as the gate metal to form a SAM with thiols. The fabrication process of the biosensor can be divided into two parts, a standard PMOS process and a Cr/Au formation. After a standard PMOS process, SiO_2/Si_3N_4 plasma etching for gate opening was performed. In this last process, a Cr/Au layer was deposited by a lift–off process. The biosensor was attached to the metalized alimina substrate. Then, the biosensor was electrically connected to the electrode on the substrate by bonding wire. Silicone rubber was coated onto the bonding wire

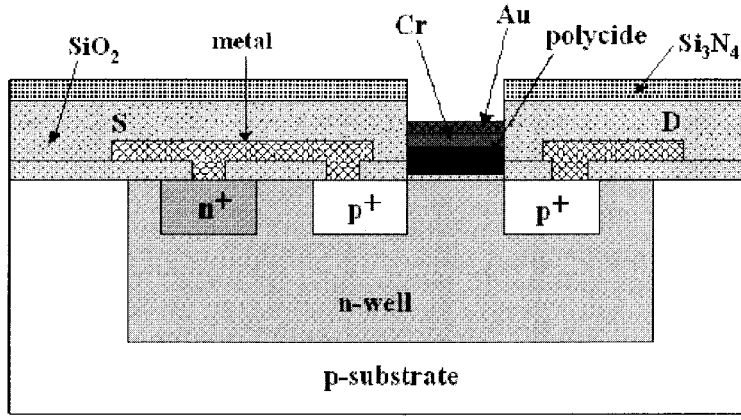


Fig. 2. Cross-sectional diagram of PMOSFET-type biosensor.

and a metal line for electrical isolation in order to allow them to be dipped into the solution. Figure 3 shows a photograph of the fabricated header with the sensor. Figure 4 shows the measurement diagram. A semiconductor parameter analyzer was used as the measuring equipment.

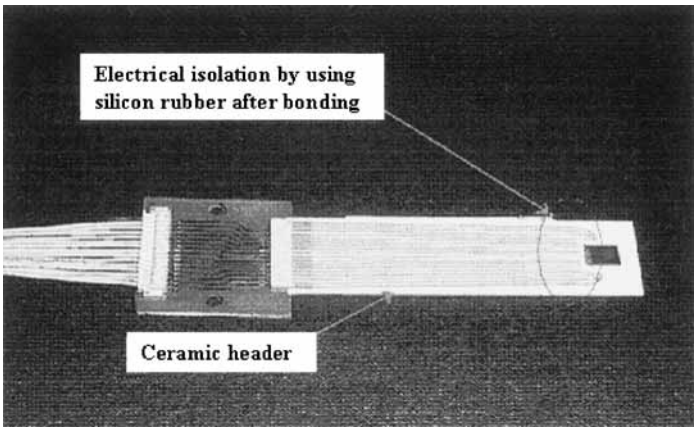


Fig.3. Photograph of fabricated header.

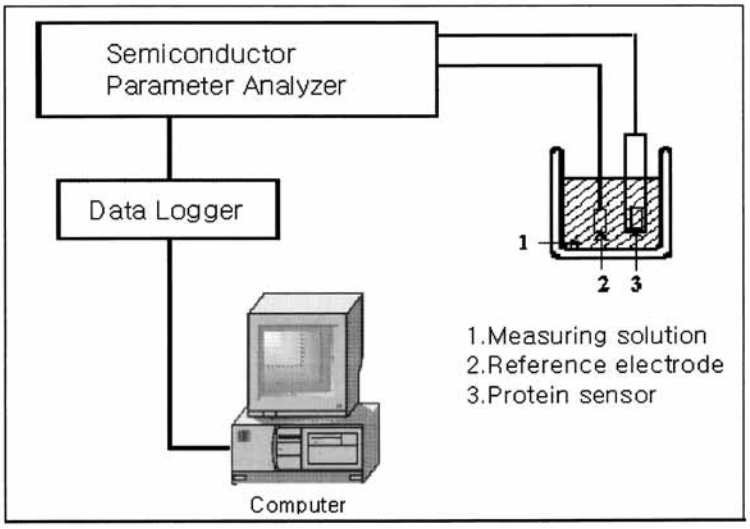


Fig.4. Measurement diagram.

4. Measurement

To fix the activation conditions of the sample, potassium phosphate buffer saline (K^+ PBS) was used as the solution. The PBS solution has a pH of 6.4 and a potassium concentration of 20 mM, which are the activation conditions for streptavidin and biotin. A Pt reference electrode was used to apply the gate bias of the biosensor. Before the measurements were taken, the biosensor and the Pt reference electrode were cleaned by acetone, methanol, and D.I. water in that order. The dark current was then measured in the PBS solution. A gate voltage of –2 V was applied. After that, 10 μl 6-mercaptohexanol (MCH) was diluted in 50 ml of D.I. water and the diluted solution was injected into the 10 ml PBS solution. Thiols of MCH were attached to the Au surface. After rinsing the biosensor in the PBS solution, the biosensor was dipped into the solution. When a SAM was formed, the drain current was measured, and the biosensor was exposed overnight to 1 μg/ml streptavidin in the PBS solution. After rinsing the biosensor in the new PBS solution, the drain current was measured. Finally, biotin was injected and the drain current was measured after 24 h.

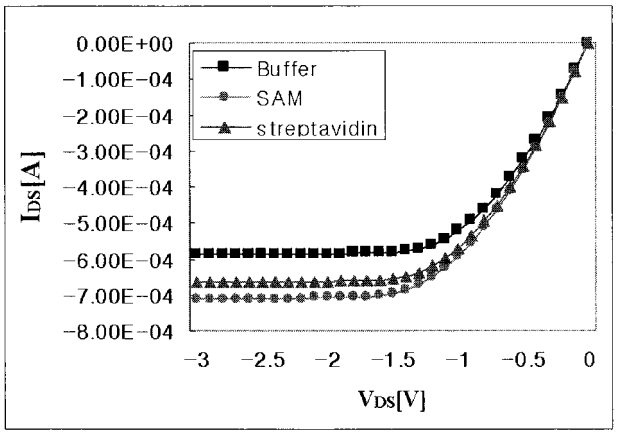
A quartz crystal microbalance (QCM) measurement was performed to verify the electrical characteristics of the PMOSFET-type biosensor. The QCM method is a well-established technique for the measuring of small nanoscale mass changes; it is based on the relationship between changes in mass of materials attached to the crystal and oscillation frequency of the crystal.[18,19] The experiment using QCM was performed under the same measurement conditions as those used for the PMOSFET-type biosensor.

5. Results and Discussion

The electrical characteristics of the SAM, streptavidin, and biotin were detected by a PMOSFET-type biosensor. The drain current increased due to the negative charge of thiols when the SAM was formed on the Au. The variation in the drain current was on the average 164 μA. The drain current decreased due to the positive charge of the amine group when the SAM and streptavidin were bound together. The charges of the SAM and streptavidin changed the drain current of the PMOSFET-type biosensor since they were functioning as the gate bias. The drain current further decreased to an average of 71 μA. The drain current decreased to an average of 50 μA due to the decrease in the capacitance when the streptavidin and biotin were bound. The variation in the capacitance can be explained in terms of the Debye length. It is well known that only potential changes that occur within the order of the Debye length can be detected.[20] However, the dimensions of macromolecules such as biotin are much longer (about 10 nm) than those of the double layer at the electrolyte-insulator interface. Therefore, the measurable effect of potential due to the biotin charge is very small in a solution with a high ionic strength, (>0.1 M) because biomolecule charges will be at a greater distance from the surface than the Debye length. In this case, the capacitance effect of biotin was more dominant than their charge effect. Therefore, the C-V measurement can also be used to detect the reaction characteristics of streptavidin-biotin protein complexes by employing the semiconductor's flat band potential shift. The drain current of the biosensor decreased because the increase in the

distance between capacitor plates lowered the capacitance. Figure 5 shows the measured drain current as a function of interactions among SAM, streptaividn and biotin. Consequently, as the variation of the drain current of a PMOSFET-type biosensor, the existence of streptavidin, as well as the specific binding between streptavidin and biotin could be defined.

(a)



(b)

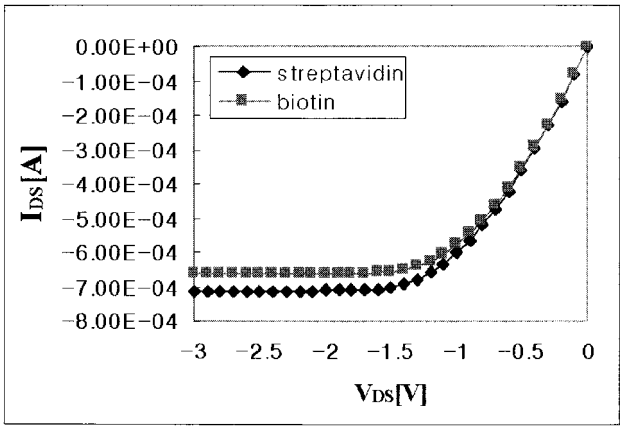


Fig. 5. Measured drain current as a function of interactions among SAM, streptavidin, and biotin. (a) I_{DS}–V_{DS} characteristics for SAM and streptavidin. (b) I_{DS}–V_{DS} for streptavidin and biotin.

Interactions among SAM, streptavidin, and biotin on the Au glass were detected by the QCM measurement. Since the QCM is a continuous, real-time detector, it is possible to assess the kinetics of interaction. Figure 6 shows the real-time detection of SAM, streptavidin, and biotin according to the variation in resonance frequency on the surface of the QCM. The variations in resonance frequency in the Figs. 6(a), 6(b), and 6(c) correspond to the masses of thiol, streptavidin, and biotin, respectively. These results show quantitatively good agreement with those of a PMOSFET-type biosensor. Therefore, it is confirmed that the variation of the drain current in a PMOSFET-type biosensor was caused by biomolecules.

7. Conclusions

A PMOSFET-type biosensor was fabricated in order to detect streptavidin-biotin protein complexes. SAM was used in order to bind streptavidin. After the reaction of SAM, streptavidin, and biotin, the variation in the drain current was investigated. After the immobilization of thiols which were composed of the head group of SAM, the drain current of the PMOSFET-type biosensor increased due to the negative charges of thiols. The hydroxyl group of SAM was bound with the amine group of streptavidin. As a result, the drain current decreased due to the positive charges of the amine group. Then, biotin was injected to identify streptavidin-biotin complexes. The drain current decreased due to the binding of biotin. Since the length of the streptavidin-biotin complexes was as long as 10 nm, the drain current decreased due to the capacitance effect caused by the characteristic length of biomolecules. In the same measurement conditions, a QCM measurement was performed to verify interactions among SAM, streptaividn, and biotin. The shift in the resonant frequency verified that SAM, streptavidin, and biotin were attached to the Au surface.

Therefore, it is concluded that the proposed PMOSFET-type biosensor can be used to detect streptavidin-biotin protein complexes. In addition, it is expected that such a PMOSFET-type biosensor can detect various protein molecules.

(a)

(b)

(c)

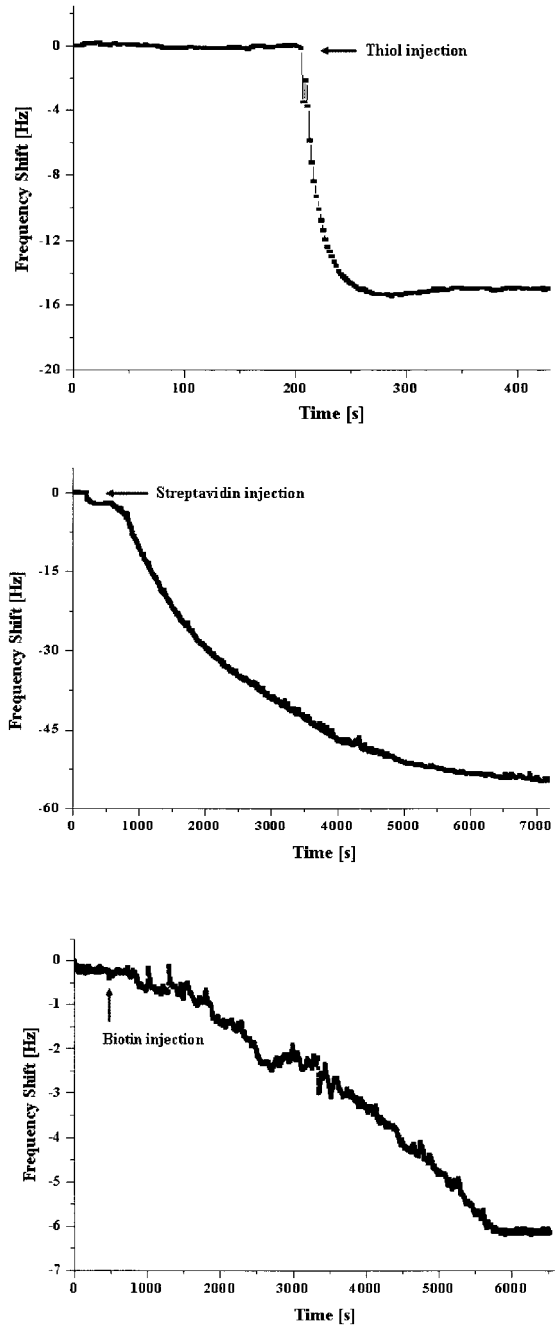


Fig.6. Real–time detection of SAM, streptavidin, and biotin according to variation in resonance frequency using QCM. (a) Resonance frequency versus time for thiol. (b) Resonance frequency versus time for streptavidin. (c) Resonance frequency versus time for biotin.

Acknowledgement

This work was supported in part by the BK21 program in Korea.

References

1 E. Souteyrand, J. P. Cloarec, J. R. Martin, C. Wilson, I. Lawrence, S. Mikkelsen and M. F. Lawrence: J. Phys. Chem. **101** (1997) 2980.
2 H. Zhu, M. Bilgin, R. Bangham, D. Hall and A. Casamayor: Science **293** (2001) 2101.
3 C. Yuan, A. Chen, P. Kolb and V. T. Moy: Biochemistry **39** (2000) 10219.
4 D. Piscevic, W. Knoll and M. J. Tarlov: Supramolecular Science **99** (1995) 2.
5 H. Zhu, M. Bilgin, R. Bangham, D. Hall, A. Casamayor and P. Bertone: Science **293** (2001) 2101.
6 F. K. Perkins, L. M. Tender and S. J. Fertig: NRL REV. **65** (2002).
7 A. Star, J. P. Gabriel, K. Bradley and G. Gruner: American Chem. Soc. **3** (2003) 459.
8 S. Nakata, N. Kido, M. Hayashi, M. Hara, H. Sasabe, T. Sugawara and T. Matsuda: Biophys. Chem. **62** (1996) 63.
9 J. P. Cloarec, N. Deligianis, J. R. Martin, I. Lawrence, E. Souteyrand, C. Polychronakos and M. F. Lawrence: Biosens. & Bioelectron. **17** (2002) 405.
10 L. Kircka: J. of IFCC **6** (1994) 59.
11 Y. J. Kim, Y. C. Lee, B. K. Sohn, J. H. Lee and C. S. Kim: J. Korean Phys. Soc. **43** (2003) 769.
12 H. K. Lyu, D. S. Kim, H. J. Park, J. K. Shin, P. Choi, J. H. Lee, C. S. Park and G. Lim: J. Korean Phys. Soc. **44** (2004) 162.
13 J. H. Park, S. H. Seo, I. S. Wang, J. K. Shin, P. Choi, Y. C. Jo and H. Kim: J. Korean Phys. Soc. **44** (2004) 157 .
14 S. Schouten, P. Stroeve and M. L. Longo: Langmuir **15** (1999) 8133.
15 K. E. Nelson, L. Gamble, L. S. Jung and M. S. Boeckl: Langmuir **17** (2001) 2807.
16 L. Haussling and H. Ringsdorf: Laxngmuir **7** (1991) 1837.
17 L. A. Klumb, V Chu and P. S. Stayton: Biochemistry **37** (1998) 7657.
18 R. Schumacher , Angew Chem. Int. Edit. Engl. **29** (1996) 329 .
19 I. Rickert, T. Weiss, W. Kraas, G. Jung, W. Gopel: Biosens. & Bioelectron. **11** (1996) 591.
20 M. J. Schoning and A. Poghossian: Analyst **127** (2002) 1137.