

Possibility of real-time measurement of an airborne *Cryptomeria japonica* pollen allergen based on the principle of surface plasmon resonance

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Received 23 November 2000; accepted in final form 18 June 2001

Key words: airborne pollen, *Cryptomeria*, pollen, pollen allergen, real-time measurement, SPR

Abstract

In order to establish a real-time measurement system for the concentration of airborne pollen allergens, we examined Cry j 1, one of the major allergens of *Cryptomeria japonica* pollen, as an example to establish the system. The feasible system consisted of: collection of airborne pollen allergens using the Virtual Impactor or Cyclone sampler, extraction of Cry j 1 using 10 mM HEPES buffer containing 0.125 M NH_4HCO_3 and finally, real-time measurement of Cry j 1 using the BIACORE 3000 system. The sensitivity of the system was 5 ng/ml, and 0.1 ml sample volume and at least 500 pg of Cry j 1 were required for each measurement. Quantification of Cry j 1 in the air can be determined 30 min after collection, i.e. 15 min for extraction, 10 min for separation from particulate matters and 5 min for the measurement.

1. Introduction

Information on the amounts of pollen allergens and pollen in the air was first reported in Finland in 1998 (Rantio-Lehtimäki A., 1998). It took about one day to obtain the information because the ELISA technique was time-consuming. Several antibody-based methods have been developed that enabled measurement of the amount of airborne pollen allergens (Razmovski V., 1999; Acevedo F., 1998; Holmquist L., 1999; Yli-Panula E., 1997; Rantio-Lehtimäki A., 1994; Takahashi Y., 1993). However, none of these methods allowed immediate quantification of pollen allergens because these were based on an end-point assay and an indirect method using primary and secondary antibodies.

The BIACORE system is based on the principle of surface plasmon resonance (SPR) which detects even minimum shifts of the wavelength of the reflected

light intensity caused by association or dissociation events (Markey F., 1999). The distinctive feature of the system is measurement of the target substances without labeling procedure. Therefore, application of the system for rapid measurement of airborne pollen allergens is possible. The present paper focuses on the possibility of real-time measurement of pollen allergens using the system based on the principle of surface plasmon resonance.

2. Materials and methods

2.1 Collecting airborne pollen and extraction of allergens

Two sampling devices (Cyclone sampler, Burkard C90M, Burkard Manufacturing Co. Ltd. England and Virtual Impactor, our own device) were used for

collecting airborne pollen allergens (Mullin J. and Emberlin J., 1997). Airborne samples were collected in 1.5 ml-Eppendorf tube (dry condition), in the case of the Cyclone Sampler. Virtual Impactor consists of a slit ($0.5 \text{ cm} \times 7 \text{ cm}$) and a sampling vessel with a trough ($0.5 \text{ cm} \times 7 \text{ cm} \times 1.2 \text{ cm}$) located right under the slit. Samples were collected in the trough containing 3 ml distilled water. For the extraction (15 min), the water was lyophilized then 10 mM HEPES buffer (pH 7.4) containing 150 mM NaCl, 0.125 M NH_4HCO_3 , 3 mM EDTA and 0.005% Tween 20 (0.125 M NH_4HCO_3 in HEPES buffer), was added to make a total volume of 200 μl . Suction volume of the Cyclone sampler and Virtual Impactor were 1 and 2 m^3/hr , respectively. In principle, a Cyclone sampler collected airborne particles, namely allergens, on a daily basis. These particles were measured in 24 m^3 volume air and Virtual Impactor collected different volumes (from 4 to 48 m^3) of air at various time-length, depending on the number of pollen in the air. The Cyclone sampler and Virtual Impactor have high efficiency over a wide range of captured particles, i.e. 90% for particles in the 1 and 7 micron range, respectively. The collected samples were extracted with 0.125 M NH_4HCO_3 in HEPES buffer for 15 min then separated from particulate matters by centrifugation at 4,000 rpm for 5 min.

2.2 Preparation of sensor surface

Anti-Cry j 1 monoclonal antibody (anti-Cry j 1 Mab, KOWA; KW-S131 or KW-S10) was immobilized on a sensor chip CM5 using BIACORE 3000 (Biacore AB, Uppsala, Sweden). About 14,000RU of anti-Cry j 1 Mab was immobilized on the chip. Resonance units (RU) were used to express the SPR signal, and 1000RU is equivalent to a change of about 1 ng/mm^2 in surface protein concentration. The sensor chip consists of a thin layer of gold deposited on a glass support. The gold layer has been covered with a non-crosslinked carboxylated dextran hydrogel (Löfås Sweden, 1990). All procedures were run at 25 °C and the flow rate was 10 $\mu\text{l}/\text{min}$ using HEPES buffer. Initially, the carboxylated dextran layer was activated with 50 mM N-hydroxysuccinimide (NHS) and 200 mM N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) for 15 min. Anti-Cry j 1 monoclonal antibody (anti-Cry j 1 Mab) was then coupled for 15 min (50 $\mu\text{g}/\text{ml}$ in sodium acetate pH 4.7). Finally, the remaining active group was blocked with 1 M ethanolamine hydrochloride-NaOH

(pH 8.5) for 15 min. After blocking, the surface was washed by injection of 50 mM NaOH for 1 min.

2.3 Concentration determination of Cry j 1

The concentration of the standard Cry j 1 and extracted airborne samples were measured using the BIACORE system. The standard Cry j 1 was provided by the Japanese Society of Allergology. The standard Cry j 1 was diluted in HEPES buffer to concentrations from 3.9–500 ng/ml. In order to confirm the reproducibility of the analysis, each injection was replicated two times. Bound Cry j 1 was completely removed from the sensor chip by injection 25 μl of 10 mM Glycine-HCl (pH 2.0) at a high flow rate of 50 $\mu\text{l}/\text{min}$. The unmodified dextran surface in flow cell was used as a reference surface.

2.4 Extraction medium and extraction time

0.125 M NH_4HCO_3 in HEPES buffer is used as the extraction medium. The extraction time is 15 min because extraction efficiency of Cry j 1 showed no significant difference between 15 min and 2 hrs (Figure 1).

3. Results

3.1 Measurement of Cry j 1 with BIACORE system

Figure 2a shows raw data of standard Cry j 1 (3.9–500 ng/ml) obtained the BIACORE system, which is a plot of the SPR signal against time. Figure 2b was subtracted the reference data, and data sets were zeroed on Y-axis just prior to the start of the injection. In the calibration curves (data not shown), vertical axis is the slope values (RU/s) calculated from binding rate of SPR signal and collected over a total period of 30 s after injection start. The curves showed a linear relationship between Cry j 1 concentration and slope. The sensitivity of this system is ca. 5 ng/ml (h of Figure 2b) and the sample volume required is 0.1 ml. Therefore detection limit of Cry j 1 is 500 pg. The procedure was reproducible without decrease in capacity.

In order to confirm the specificity of the assay system, mouse IgG was immobilized on the surface of a sensor chip and analyzed at the same time as a reference cell. SPR signals were not found when standard Cry j 1 was applied to the system. The specificity of the assay system was also examined using immobilized Cry j 1 (antigen) sensor chip and anti-Cry j

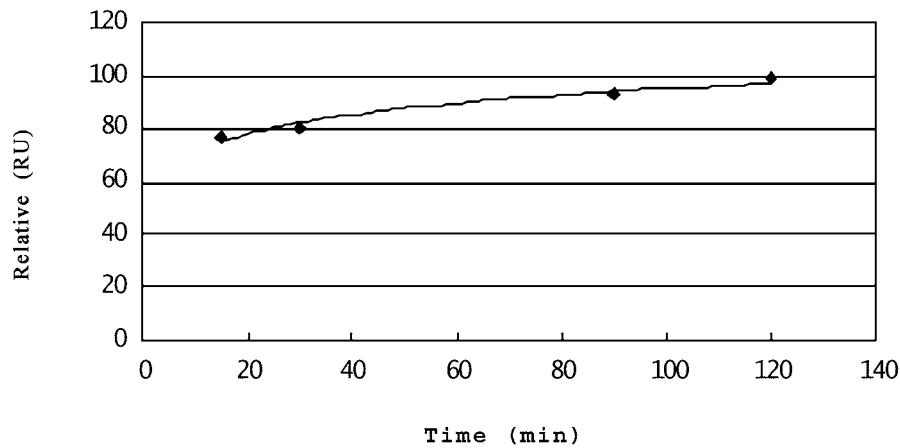


Figure 1. Time course of the extraction efficiency of Cry j 1 from pollen using NH_4HCO_3 in HEPES buffer.

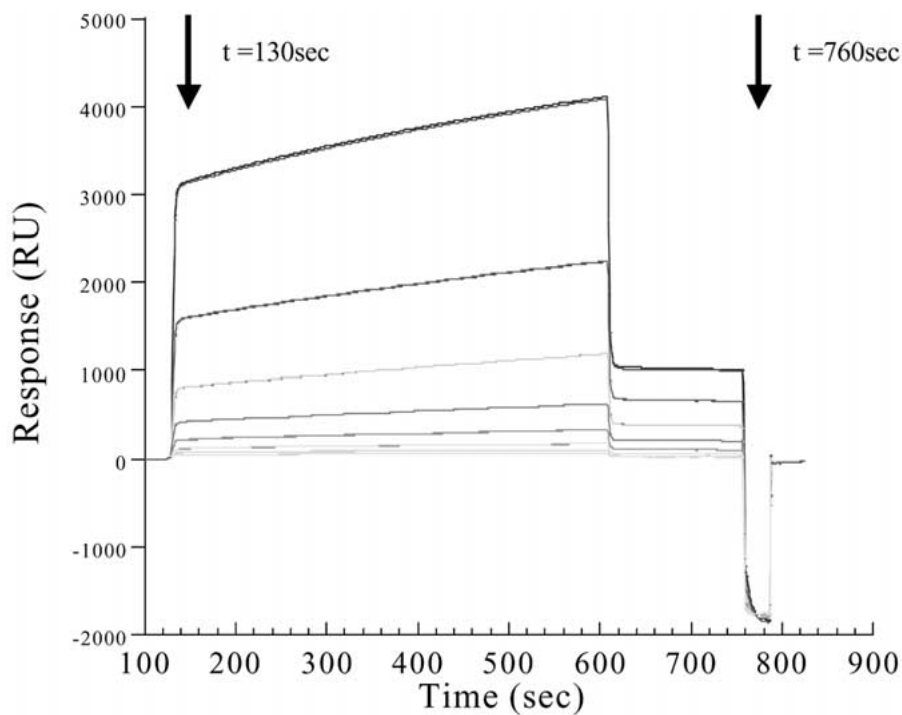


Figure 2a. Typical overlay plot of raw data from BIACORE for Cry j 1 injected over Mab surface. Increasing standard concentrations of Cry j 1 were injected at $t = 130$ sec followed by injection of regeneration solution at $t = 760$ sec.

1 Mab. Anti-Cry j 1 Mab bound specifically on the immobilized Cry j 1 chip. These results indicate that our assay system is specific to detection of Cry j 1.

Figure 3 describes the procedure for measurement of airborne Cry j 1 after sample collection. Briefly, sampled airborne allergens were extracted with 0.125 M NH_4HCO_3 in HEPES buffer for 15 min, and separated from pollen grains for 5 min by centrifugation,

then finally applied to BIACORE 3000 system. Each step required minimal time, with the whole procedure being complete in about 30 min.

3.2 Field examination

Figure 4 indicates the daily fluctuation of the amount of airborne Cry j 1 and the number of *C. japonica*

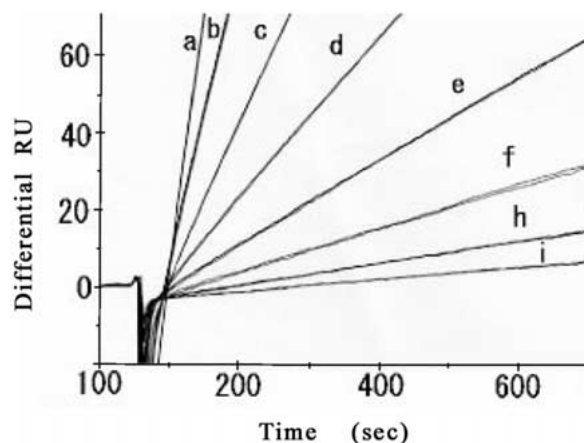


Figure 2b. Time course of response of the standard at various concentrations. Values from the reference surface was subtracted from the values from the Mab surface. The values were calculated from the slope of response values of SPR. Letters are as follows: a) 500 ng/ml, b) 250 ng/ml, c) 125 ng/ml, d) 62.5 ng/ml, e) 31.25 ng/ml, f) 15.625 ng/ml, g) 7.81 ng/ml, h) 3.9 ng/ml, i) 0 ng/ml.

pollen from the start to the end of the *C. japonica* pollen season in 2000. The amount of Cry j 1 from the supernatant and the number of pollen was measured from the pellets of the same samples. The concentrations of Cry j 1 by SPR do not always parallel to the number of pollen and the date of maximum value of Cry j 1 and pollen did not agree. Cry j 1 existed several days before *C. japonica* pollen appeared in the air. This fact coincides with the finding that symptoms appear among sensitive pollinosis patients before the pollen can be detected in the air (Baba K., 1998).

Table 1 shows the result of the samples collected with Virtual Impactor and various time-length. Because of the detection limit of the BIACORE 3000 system is 5 ng/ml, it is sufficient for every one-hour measurement at the time of the pollen peak season (Table 1 sample Nos. 7 and 8).

4. Discussion

The specificity of the assay depends on the specificity of the used antibody. The specificity of the used anti-Cry j 1 Mab is strictly restricted against *C. japonica* and stained only with *C. japonica* pollen grains (Takahashi Y., 1993). The poor correlation between the amount of Cry j 1 and the number of pollen in the Cyclone sampler may be caused by the more efficient collection of amorphous material from *C. japonica* pollen. Another possible cause may be due to a wide

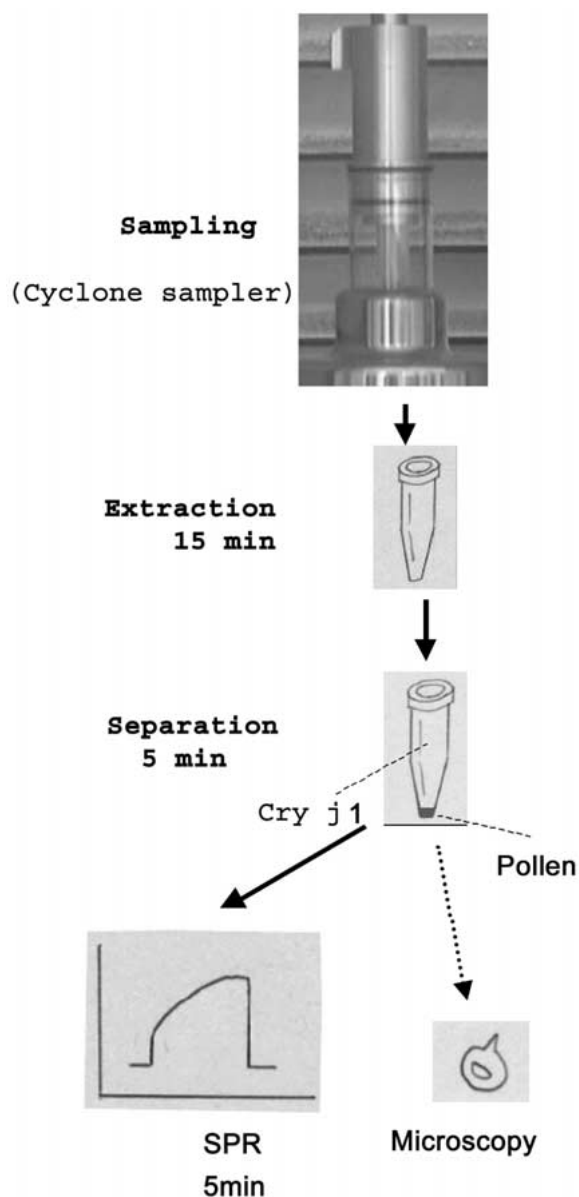


Figure 3. Procedure for the measurement of airborne antigen(Cry j 1) by SPR.

variation of the amount of Cry j 1 in pollen collected at each tree (Seido et al., 1999).

It is conceivable from this study that real-time measurement of pollinosis causing-allergens in the air might become realistic in the future. However, a problem is to determine how to combine the sampling and measuring parts of the system. The BIACORE system cannot be used outside because surface plasmon resonance is quite sensitive to temperature

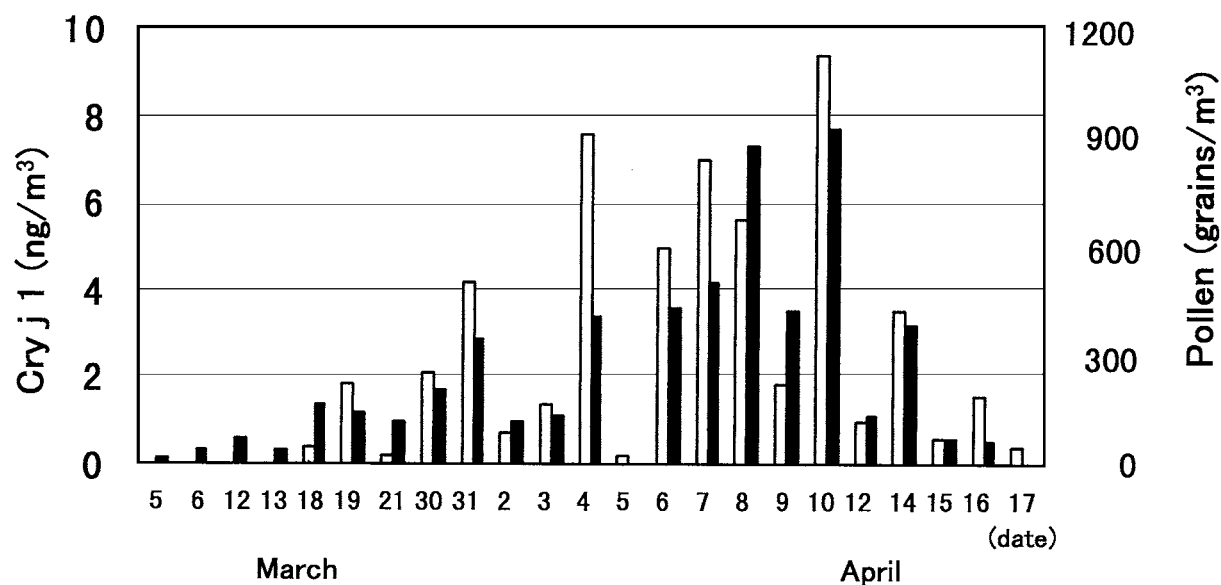


Figure 4. Comparison of the amount of Cry j 1 (black bar) and the number of *Cryptomeria japonica* pollen (white bar) in samples collected with Cyclone Sampler.

Table 1. The amount of Cry j 1 and the number of *Cryptomeria japonica* pollen in samples collected with Virtual Impactor

Sample No.	sampling periods	duration	Cry j 1 pollen			
			hrs	ng/ml	ng/m ³	grains/ml grains/m ³
1	6 March 13:00–16:00	3	0	0	0	0
2	6 March 16:00–8:00 (7 March)	16	6.8	0.2	10400	325
3	23 March 16:00–8:00 (24 March)	16	47.9	1.5	86600	2706.3
4	30 March 13:30–16:00	2.5	10.8	2.2	3900	780
5	30 March 16:00–8:00 (31 March)	16	15.5	0.5	10000	312.5
6	2 April 16:00–8:00 (3 April)	16	9.2	0.3	4000	125
7	4 April 12:00–15:00	3	73.9	12.3	17700	2950
8	4 April 15:00–17:00	2	84.2	21.1	23200	5800
9	4 April 17:00–8:00 (5 April)	15	356.9	11.9	110900	3696.7
10	6 April 8:30–16:00	7.5	140.2	9.3	52600	3506.7
11	6 April 16:00–8:00 (7 April)	16	46.6	1.5	22600	706.3
12	7 April 16:00–16:00 (8 April)	24	440.3	9.2	296000	6166.7
13	10 April 8:00–16:00	8	26.2	1.6	10800	675
14	13 April 8:00–13:00	5	106.2	10.6	44200	4420
15	13 April 16:00–8:00 (14 April)	16	81.5	2.5	58400	1825

and must be set in an air-conditioned room. On the other hand, air sampler must be set outside for collecting suitable pollen allergens. Bringing each sample to the BIACORE system one by one would be time-consuming and troublesome. Therefore, the development of handy-type SPR equipment for outdoor use is needed.

It is clear that rapid quantification of airborne pollen allergens, i.e. Cry j 1, was possible using our system. The technique will be applied not only to pollen allergens but airborne indoor/outdoor allergens existing at more than nano-gram level, such as mites (Sakaguchi M., 1995), mold, insects, animal allergens, etc. The BIACORE 3000 system can measure four

different kinds of allergens at the same time and in addition, enable our system to quantify the important allergens simultaneously existing in the air.

5. Conclusions

The current study indicates the possibility of real-time measurement of airborne pollen allergens using the BIACORE 3000 system based on the principle of surface plasmon resonance. The sensitivity of the system was enough to measure the amounts of allergens every hour at the time of pollen peak season and every 24 hrs at the start time of the pollen season. The whole procedure can be completed in about 30 min.

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