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Acridinium ester conjugated to lectin as chemiluminescent histochemistry marker

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Abstract

Cell differentiation/dedifferentiation includes changes in oligosaccharide composition and distribution in the cell surface glycoconjugates. Lectins have been used as auxiliary tools in histopathological diagnosis of mammary, uterus and brain pathologies. Acridinium ester (AE) conjugated to biomolecules has been employed in chemiluminescent analytical applications. This work aimed to use a lectin, concanavalin A (Con A), conjugated to AE as a chemiluminescent histochemistry tool. Biopsies of normal and infiltrating duct carcinoma (IDC) of mammary tissues were treated by a Con A–AE derivative. Photon emission, observed during the breakage of the chemical bound between Con A and AE, was quantified, expressed in relative light units (RLU) and correlated to the labelling of the normal and transformed tissues. The results demonstrated that RLU presented a linear relationship with the labelled tissue area in the range 0.125–1.0 cm^2 ($r = 0.98$). Furthermore, RLU was much higher for the IDC ($1283.920 \times 10^3 \pm 220.621 \times 10^3$) than the normal tissue ($2.565 \times 10^3 \pm 0.247 \times 10^3$), namely, about 500 times higher. The Con A–AE conjugation efficiency, differential staining of normal and IDC tissues, and quantification of results contribute to a decrease in the subjectivity in routine histopathological diagnoses and indicate that acrydinum ester can join other lectin marker to be used in histochemistry.

Keywords: *Chemiluminescence, concanavalin A (Con A), human mammary tissues, histochemistry*

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Introduction

Chemiluminescence (CL), which is defined as a process in which excited molecules or atoms generated from chemical reactions release the excess of energy in light form, has been known to be a powerful analytical technique that exhibits high sensitivity and selectivity. The benefits of chemiluminescent methods include ultrasensitive detection limits (attomole-zeptomole), rapid assays and a broad range of analytical applications (Kricka 2003).

Luminol and isoluminol were the first chemiluminescence compounds to be used as labels (Campbell et al. 1985), but the more sensitive acridinium ester (AE) labels

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quickly superseded them. As effective labels for biomacromolecules, AEs have received great interest as a basis for the development of high-performance chemiluminometric assays using labelled molecules. For instance, an electrochemiluminescent method for the rapid and sensitive detection of AE in neutral solution was described based on the emission from the *N*-methylacridone, which is the oxidization product by the nascent oxygen generated on the surface of working electrode in the course of oxidization of water (Yang et al. 2002).

Lectins, (glyco)proteins that reversibly bind specifically free or conjugated carbohydrates, have found an increasing number of applications in medicine and biological processes (Kennedy et al. 1995). They are traditionally conjugated to peroxidase, fluorescein isothiocyanate (FITC) or biotinylated to be used in histochemistry and flow cytometry analysis (Brooks & Leathen 1995, Nishimura et al. 2000).

As histochemical marker, lectins have been applied to characterize mammary (Beltrão et al. 1998), uterine (Remani et al. 1994) and cerebral neoplasic tumours (Nishimura et al. 2000, Beltrão et al. 2003), and many others. Lectin histochemistry has been used as an extra marker besides the traditional immunohistochemistry to characterize normal and transformed tissues.

In this work, AE is proposed as the labelling molecule for concanavalin A (Con A) to be used as an auxiliary histochemistry tool to help the clinical–pathological evaluation of infiltrating duct carcinoma, a human mammary tumour of high incidence in the State of Pernambuco, Northeast Brazil.

Materials and methods

Human mammary specimens

Twelve formalin-fixed and paraffin-embedded malign mammary tissues, diagnosed as infiltrating duct carcinoma, and four normal mammary tissues were obtained from the archives of the Hospital do Câncer of the State of Pernambuco, Brazil.

Con A conjugation with AE

AE was conjugated to Con A according to Weeks et al. (1986). Briefly, Con A (500 µl containing 2 mg of protein) was incubated with 15 µl of acridinium ester solution (0.2 mg diluted in 400 µl of N,N-dimethylformamide) for 1 h at 25°C. The conjugate (Con A–AE) was applied to a column of Sephadex G-25 (10 × 1 cm), previously equilibrated with 10 mM phosphate buffer, pH 7.2 (100 ml) and eluted with this buffer containing 300 mM glucose (50 ml). Aliquots (200 µl) were collected and their protein content was spectrophotometrically determined at 280 nm. The aliquots corresponding to the protein peak had their chemiluminescence assayed, pooled, dialysed overnight against 10 mM phosphate buffer, pH 7.2, and kept at 4°C until use. After conjugation, Con A–AE was evaluated regarding the maintenance of its carbohydrate recognition property (haemagglutinating activity) using glutaraldehyde treated rabbit erythrocytes according to Beltrão et al. (1998).

Lectin histochemistry

Paraffin sections (4 µm) were cut and transferred to Petri dishes containing xylene (total of four) for deparaffinization followed by four dishes containing graded alcohols

(3 × 100% and 1 × 70%) for hydration. Tissue slices were transferred to test tubes and then incubated with Con A–AE (100 μg ml^{-1}) for 2 h at 4°C. After lectin incubation tissues were washed twice with 2 ml of 10 mM phosphate buffer, containing 0.15 M NaCl (PBS), pH 7.2, and transferred to polypropylene test tubs with a volume of 5 μl of PBS. Lectin binding inhibition assays were accomplished by incubating each lectin solution with D-glucose (0.3 M) for 15 min at 25°C prior to its incubation with the tissue. Following steps were as described previously for the binding protocol.

Chemiluminescence measurement

Luminometry was performed using a Magic Lite Analyzer (Ciba Corning Diagnostics Corp., East Walpole, MA, USA). The emission intensity was determined as relative light units (RLU) using 5 μl of sample. A counting time was of 5 s per sample. Duplicate measurements routinely exhibit precision rate lower than 5%.

Results and discussion

Conjugation of AE to antibodies and enzymes has been widely used in analytical and clinical research (Goto et al. 2002, Kricka 2003). Figure 1 depicts its molecular structure and the protein labelling reaction (García-Campaña et al. 2003). Here, lectin labelling with AE was carried out using Con A and the conjugate, Con A–AE, was employed as a chemiluminescent histochemistry tool. Typical profile of the Con A–AE obtention by Sephadex G-25 filtration is demonstrated in Figure 2. In our laboratory, Con A conjugated to peroxidase used in the histochemistry analysis of mammary tissues already showed that neoplastic tissues present a higher intensity of staining than normal ones (Beltrão et al. 1998).

First, luminescence was evaluated as a function of tissue area. It was observed that there was a linear relationship between photon emission (RLU) and the tissue area (IDC sample) incubated with Con A–AE in the range 0.125–1.0 cm^2 (Figure 3). Furthermore, RLU was much higher for the IDC ($1283.920 \times 10^3 \pm 220.621 \times 10^3$) than the normal tissue ($2.565 \times 10^3 \pm 0.247 \times 10^3$) as can be seen in Table I. These data also showed that lectin inhibition binding by D-glucose (0.3 M) resulted in a dramatic reduction in RLU. They also assured that resulting counting of RLU was

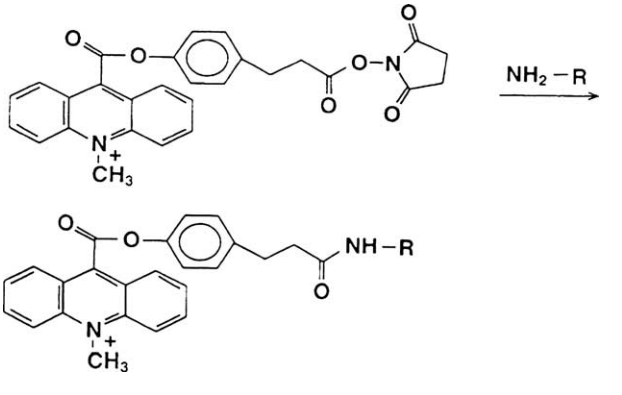

Figure 1. Protein (NH_2-R) labelling reaction employing acridinium N-hydroxysuccinimide ester (García-Campaña et al. 2003).

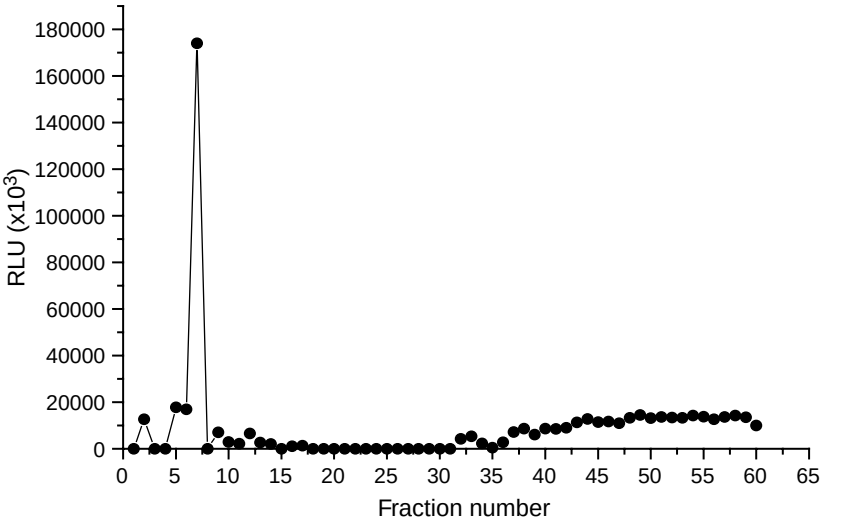

Figure 2. Concanavalin A–acridinum ester conjugate (Con A–AE) elution profile from Sephadex G-25 filtration. The conjugate was applied to a column of Sephadex G-25 (10 × 1 cm) and eluted with buffer containing 300 mM glucose. Fractions (aliquots of 200 µl) were collected and chemiluminescence was assayed.

obtained via lectin carbohydrate binding site that recognized glucose/mannose residues in cell membrane and not by unspecific binding between acridinium ester and such residues of glycoconjugates also present in cell membrane. Therefore, these results are according to Beltrão et al. (1998) who previously demonstrated that Con A specifically labelled IDC. Change in the glycosilation pattern of glycoconjugates in the cell membrane, mainly as over expression, it is a feature observed in many tumours.

These results is of great importance since the use of others labels such as peroxidase and FITC showed to be effective to indicate difference in staining intensity in transformed and normal tissues but not to quantify how much is correspondent to

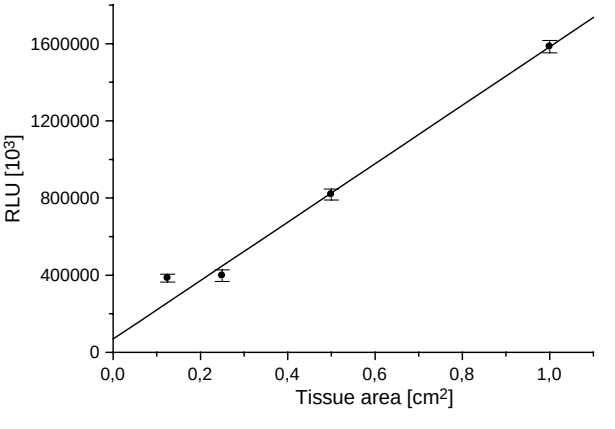

Figure 3. Relationship between chemiluminescence and the area of mammary tissue diagnosed as an infiltrating duct carcinoma. The photon emission, expressed in relative light units (RLU), was detected from different tissue samples treated with concanavalin A–acridinum ester conjugate (Con A–AE) (quadruplicates ± standard deviation).

Table I. Chemiluminescence of normal and infiltrating duct carcinoma of mammary tissues treated with concanavalin A–acridinum ester conjugate (Con A–AE) and previously inhibited with glucose.

Tissue	Labelled (RLU)*	Lectin inhibited (RLU)*
IDC	$1283.920 \times 10^3 \pm 220.621 \times 10^3$	$0.647 \times 10^3 \pm 0.046 \times 10^3$
Normal	$2.565 \times 10^3 \pm 0.247 \times 10^3$	$0.192 \times 10^3 \pm 0.032 \times 10^3$

*Mean $\pm$ SD.
IDC, infiltration duct carcinoma tissue ($n = 12$); normal tissue ($n = 4$); Con A–AE, concanavalin A–acridinum ester conjugate; RLU, relative light unit.

such transformations per area. The sensitivity, dynamic range and diversity of chemiluminescent assays, notably in immunoassays, protein and nucleic acid blotting, have found another application in lectin histochemistry as showed here in this work.

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References

Beltrão EIC, Correia MTS, Figueredo-Silva J, Coelho LCBB. 1998. Binding evaluation de isoform Cratylia mollis lectin to human mammary tissues. Applied Biochemistry and Biotechnology 74:125–134.

Beltrão EIC, Medeiros PL, Rodrigues OG, Figueredo-Silva J, Valença MM, Coelho LCBB, Carvalho Jr LB. 2003. Parkia pendula lectin as histochemistry marker for meninge tumor. European Journal of Histochemistry 27(2):139–142.

Brooks SA, Leathen AJC. 1995. Expression of alpha-GalNac glycoproteins by breast cancers. British Journal of Cancer 71:1033–1038.

Campbell AK, Hallet MB, Weeks I. 1985. Chemiluminescence as an analytical tool in cell biology and medicine. Methods in Biochemistry Analysis 31:317–416.

García-Campaña AM, Gámiz-Gracia L, Baeyens WRG, Barrero FA. 2003. Derivatization of biomolecules for chemiluminescent detection in capillary electrophoresis. Journal of Chromatography B 793:49–74.

Kennedy JF, Paiva PMG, Correia MTS, Cavalcanti MSM, Coelho LCBB. 1995. Lectins, versatile proteins of recognition: a review. Carbohydrates and Polymers 24:219–230.

Kricka LJ. 2003. Clinical applications of chemiluminescence. Analitica et Chimica Acta 500:279–386.

Nishimura A, Sawada S, Ushiyama I, Yamamoto Y, Nahagawa T, Tanegashima A, Nishi K. 2000. Lectin-histochemical detection of degenerative glycoconjugate deposits in human brain. Forensic Science International 113(1–3):265–269.

Remani P, Pillai KR, Haseenabeevi VM, Ankathil R, Bhattathiri M, Nair MK, Vijayakumar T. 1994. Lectin cytochemistry in the exfoliative cytology of uterine cervix. Neoplasma 41:39–42.

Weeks J, Sturgess ML, Brown RC, Woodhead JS. 1986. Immunoassay using acridinium esters. Methods in Enzymology 133:366–387.

Yang M, Liu C, Hu X, He P, Fang Y. 2002. Electrochemiluminescence assay for the detection of acridinium esters. Analitica et Chimica Acta 461:141–146.