Monoclonal Antibody Register

Monoclonal Antibodies Specific to Polynuclear Aromatic Hydrocarbons

(PAH / polycyclic aromatic hydrocarbon / determination / ELISA / monoclonal antibody)

M. SUCHÁNEK[1], T. SCHARNWEBER[2], M. FISHER[3], D. KNOPP[2], R. NIESSNER[2]

[1]EXBIO Praha a.s., Prague, Czech Republic
[2]Institute of Hydrochemistry, Technical University Munich, Germany
[3]Technion-Israel Institution of Technology, Haifa, Israel

Background

The interest in polycyclic aromatic hydrocarbons (PAHs), especially benzo[a]pyrene, in environmental samples (e.g. city aerosols) is derived from their known mutagenic potential (Topinka et al., 1998) and their adjuvant effect for the causation of allergies (Diaz-Sanchez, 1997; Bömmel et al., 2000). Analysis of PAHs is routinely performed by gas chromatography/mass spectrometry (GC/MS) or high performance liquid chromatography/fluorescence detection (HPLC/FD). However, these methods are time consuming, mainly due to complex procedures involved in the preparation of samples, which usually includes removal of disturbing matrix compounds and concentration of the target analytes. The objective of our project is to develop a rapid immunoassay which can be applied to screening of a large number of samples without the need for demanding sample preparation. In this communication we describe monoclonal antibodies BAP-13 and BAP-14 specific for a range of PAHs.

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Corresponding author: Miloslav Suchánek, EXBIO Praha a.s., Vídeňská 1083, 14220 Praha 4, Fax: +420 (2) 475 2151; e-mail: suchanek@exbio.cz.

Abbreviations: BSA – bovine serum albumin, ELISA – enzyme-linked immunosorbent assay, EPA – Environmental Protection Agency, GC/MS – gas chromatography/mass spectrometry, HPLC/FD – high performance liquid chromatography/fluorescence detection, PAHs - polycyclic aromatic hydrocarbons.

Description of the antibodies BAP-13 and BAP-14

Preparation of the antigen

Because benzo[a]pyrene (BaP) does not contain any functional groups, its derivative, benzo[a]pyrenyl-1-butyric acid (BBA) was used for the synthesis of conjugates with carrier proteins thyroglobulin (TG) and bovine serum albumin (BSA), respectively. The BaP-BSA conjugate was used for immunization and the BaP-TG conjugate for screening of the hybridoma supernatants. The coupling was carried out following the mixed anhydride procedure. Fifty μmol of BBA were dissolved in 2 ml of dry 1,4-dioxane and 50 μmol of tri-n-butylamine were added. The solution was cooled to about 12°C and 50 μmol of isobutyl-chloroformate were added, and the reaction mixture was stirred for 20 min at room temperature. Subsequently, this mixture was added dropwise to the solution of 50 mg of a carrier protein in 3 ml of distilled water adjusted to pH 8.5 with 40 μl of 0.1 M NaOH. The reaction solution was incubated 4 h with gentle stirring. The pH value was monitored during incubation and adjusted to 8.5 with 0.1 M NaOH, when necessary. The final solution was dialyzed for 2 days against 0.01 M glycine, pH 9, with several buffer changes, and lyophilized afterwards.

Production

Hybridoma cell lines producing antibody to PAHs were obtained after immunization of BALB/c mice with the BaP-BSA conjugate and fusion of spleen cells with myeloma cells Sp2/0. The methodology of fusion, screening by ELISA and cloning have been described previously (Viklický et al., 1982).

Table 1. The cross-reactivity (CR) of BAP-13 and BAP-14 antibodies with selected PAHs

Tested compound	(CR[a] ± SD) [%]	
	BAP-13	BAP-14
Benzo[a]pyrene	100	100
Naphthalene	< 1	0
Acenaphthene	1 ± 0.2	2 ± 0.2
Acenaphthylene	< 1	2 ± 0.2
Fluorene	5 ± 1	5 ± 0.5
Phenanthrene	23 ± 4	25 ± 3
Anthracene	25 ± 4	74 ± 7
Fluoranthene	60 ± 9	81 ± 8
Pyrene	47 ± 7	25 ± 3
Benzo[a]anthracene	44 ± 7	58 ± 6
Chrysene	42 ± 6	83 ± 8
Benzo[b]fluoranthene	26 ± 4	63 ± 6
Benzo[k]fluoranthene	15 ± 2	12 ± 1
Dibenzo[a,h]anthracene	9 ± 1	0
Benzo[g,h,i]perylene	15 ± 2	0
Indeno[1,2,3-c,d]pyrene	63 ± 10	18 ± 2
Benzo[e]pyrene	55 ± 9	36 ± 4
Perylene	8 ± 1	24 ± 3

[a] Cross-reactivity [%] was expressed as × 100.

IC_{50} (concentration of an analyte required to reduce the maximum assay signal by 50%) of each tested PAH was determined simultaneously with IC_{50} of BaP, which served as the reference analyte.

Characteristics

Out of 15 different monoclonal antibodies that were selected during ELISA-screening assays, the antibodies BAP-13 (IgG_1) and BAP-14 (IgG_1) exhibited properties suitable for their intended use. The ELISA developed with the BAP-13 antibody had an IC_{50} of 11 $\mu g \cdot l^{-1}$ of benzo[a]pyrene. The ELISA developed with the BAP-14 antibody had an IC_{50} of 30 $\mu g \cdot l^{-1}$ of benzo[a]pyrene. The limit of detection (LOD) for the ELISA was determined at 2 $\mu g \cdot l^{-1}$ of benzo[a]pyrene. Cross-reactivity of BAP-13 and BAP-14 antibodies with the 16 EPA PAHs and 2 other selected PAHs was determined according to the method of Abraham (1969). The results summarized in Table 1 showed that both BAP-13 and BAP-14 antibodies are specific not only for a target analyte (BaP), but for the whole group of tested compounds. Thus, results obtained by ELISA at practical applications (e.g. monitoring of the pollution by PAHs) would reflect the sum of detected PAHs. However, there are also perspectives allowing the estimation of the content of an individual compound. Long-term monitoring of PAHs at the same site revealed "patterns" in the ratio of individual PAHs to each other, which depend on the sources emitting the PAHs (Hall, 1999). The knowledge of such "patterns" and the availability of immunoassays based on different antibodies with defined cross-reactivity would lead to a meaningful interpretation of collective results.

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