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On the autofluorescence of aged fingermarks

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ABSTRACT

Fingermark autofluorescence changes with time, both spectrally and in total intensity. In this study we investigate which components in the aged fingermarks cause this change in autofluorescent signal. Thin layer chromatography combined with fluorescence spectroscopy was used to identify fluorescent aging products. Based on our results, tryptophan derivatives, including indoleacetic acid, (nor)harman and xanthurenic acid are indicated as important contributors to the autofluorescence of aged fingermarks. Knowledge about which fluorescent aging products are present in fingermarks might be useful in the development of fingermark age estimation methods. This work is part of a larger project of which the major goal is to develop a method to estimate the time of deposition of fingermarks. Additionally, by selective targeting of aging products the development of aged fingermarks might be improved.

1. Introduction

Fingermarks have the ability to emit fluorescence upon illumination with ultraviolet and visible light [1,2]. In 1992 Roland Menzel wrote a letter in which he described unpublished work on the age estimation of fingermarks based on their fluorescence emission color [3]. He and his co-workers had embarked on a fairly extensive project trying to link the fluorescence emission color to the age of the fingermark. They did find a correlation between age and fluorescence color, fresh fingermarks showed a more yellow while older fingermarks showed a more red fluorescence emission. The inter- and intra-person variability however was too large for this observation to be used for age estimation purposes. We have taken this work as the basis of our research on the age estimation of fingermarks using fluorescence emission and the shift towards a longer wavelength upon aging. We partially solved the problem of inter- and intrapersonal variation by taking into account the aging rate of the fingermarks and were able to estimate the age of fingermarks aged under controlled conditions up to three weeks old with an accuracy of 1.9 days [4]. In order to improve the age estimation more knowledge on the compounds responsible for fingermark autofluorescence and the change upon aging is needed.

We have proposed protein bound tryptophan to be the main source of autofluorescence in fresh fingermarks and have demonstrated that protein fluorescence decreases upon fingermark aging [2,4]. We thus hypothesize that tryptophan and its derivatives play a major role in the fluorescent properties of aged fingermarks. The aim of this study is to identify which components in fingermarks cause the change in autofluorescence signal. We will use thin layer chromatography (TLC) to separate the different fluorophores in fingermarks at different time points after deposition, and subsequently use fluorescence spectroscopy to identify the fluorophores in the fingermark, by comparison with tryptophan and its derivatives. Fingermarks were stored and aged for 0, 1, 2 and 3 weeks under two different conditions, storage in a dark chamber and storage under office conditions.

2. Materials and methods

Materials and instruments used in our experiments are listed in Table 1.

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Table 1
Overview of type of surfaces used including the supplier's name.

Material	Supplier	Address information
Chloroform (288306), L-tryptophan (A6376), L-kynurenine (K8625), xanthurenic acid (D120804), 4-aminoacetophenone (A9653), kynurenic acid (K3375), indole acetic acid (I2886), norharman (N6252), harman (103276), tryptophan (T0254), anthranilic acid (10680), hydroxyl quinaldic acid (55088), hydroxyl anthranillic acid (148776), hydroxykunurenine (H1771), Ehrlich reagent (03891) and bovine serum albumin (BSA) (A7906)	Sigma–Aldrich	Zwijndrecht, the Netherlands
Methanol (1.06009.1000) and TLC Silicagel 60 aluminium sheets (1.05553.0001)	Merck KGaA	Darmstadt, Germany
N-Formylkynurenine (purified using RP-HPLC)	Synthesized in the laboratory of Prof Dr. Hoffmann [5]	Institute of Bioanalytical Chemistry, Leipzig University, Germany
Sterile Medical Wire MWSCS Taper-Tip swabs (B22715-100)	WA products	Essex, UK
Crime-lite®2 torches: UV (365 nm, 10% band width 350–380 nm), Blue (445 nm, 10% band width, 420–470 nm) Plastic goggle: Clear (D-21000, optical density 190–400 nm 5+), and orange (I-505600, optical density: 190–534 nm 3+) Camera filters: Yellow (GG495, 1% nom 476 nm, long pass filter) and a polarizer filter	Foster and Freeman	Worcestershire, UK
Nikon D40× digital camera	Nikon	Tokyo, Japan
LS 55 Luminescence spectrometer Fiber optic accessory	PerkinElmer	Groningen, the Netherlands

Fingermark preparation: Volunteers were asked to wash their hands with water and soap, rinse well, and dry them with a paper towel according to the protocol described by Lambrechts et al. [2]. After drying, fingertips were rinsed with 70% ethanol and air-dried by waving the hands through the air. Directly, after air-drying the hands, volunteers were asked to wipe their fingertips across the forehead and from the bridge of the nose to the cheeks. Volunteers wearing facial cream were asked to wipe behind the ears instead of wiping the fingertips on facial regions. After that volunteers were asked to deposit their fingermarks on the requested surface. Donor specific information can be found in Tables S-1–S-4 of the supporting information. Fingermarks were deposited directly upon an activated thin layer chromatography (TLC) plate, for activation of the TLC plate, see method section 'Thin layer chromatography'. To obtain a stronger fluorescent signal five (from all five fingers of one hand) or ten (from all ten fingers of both hands) fingermarks were deposited on top of each other. Fingermarks aged at different time periods, 1 week, 2 weeks and 3 weeks, were developed and compared with the results obtained from fresh fingermarks. The study described in this article was performed in parallel with the work described in "On the Autofluorescence of Fingermarks" [2], in which we elaborately describe the fluorescent behaviour of 19 fingermarks at $t = 0$. To these results we have added three more $t = 0$ fingermarks that were not previously reported, for reason that this data was included in a later stage of the research project (Table S-3). *Storage conditions*: Volunteers deposited 5 or 10 fingermarks on top of each other on the TLC plates. Two different approaches were utilized, which causes the difference in number of stacked fingermarks. In the first approach the effect of light and dark storage conditions on the aging of fingermarks and the formation of aging products was studied. Therefore two sets of 5× stacked fingermarks were deposited per donor, one set was aged under office conditions (office room with TL-lightning during working hours) and one set was aged under dark conditions (closed closet) up to three weeks prior to development. Spots with corresponding fluorescent color, Rf-values, fluorescence spectra and color reaction were observed in fingermarks obtained from different donors. To increase the fluorescent signal to obtain fluorescence spectra of the different spots using a luminescence spectrometer, a second approach was included. Different donors were asked to deposit 10 fingermarks on top of each other on the TLC plate. At least six different donors were used per experiment, for donor information see Tables S-1–S-3 (supporting information). Upon aging, TLC plates were developed, as described in the method section 'Thin layer chromatography'.

Reference compounds: Stock solutions of reference compounds were freshly prepared in 1% methanol in milliQ. Concentrations of solutions are described in the supporting information. The following reference compounds were tested: tryptophan, aminoacetophenone [6], anthranilic acid [6,7], N-formylkynurenine [8], 3-hydroxyanthranilic acid [6,7], 3-hydroxykynurenine [6,7], 8-hydroxy quinaldic acid [9], indoleacetic acid [10,11], kynurenic acid [7,12], kynurenine [2,6], norharman [13], harman [12,13] and xanthurenic acid [2,9]. Samples of 2 μl were applied to the TLC plate. 20 μl aliquots of 50 mg ml^{-1} BSA were pipetted onto a TLC plate to serve as fluorescence reference for fingermark residues. *Aged reference compounds:* Stock solutions of fresh tryptophan and 3-indoleacetic acid were aged in the same manner as the stacked fingermarks. Tryptophan and indoleacetic acid were aged on TLC-plates for one, two and three weeks under office conditions or in a dark room. Also, fresh tryptophan and indoleacetic acid were developed on a TLC-plate.

Thin layer chromatography: The TLC method was used as introduced by Bramble et al. and modified by Jones et al. [6,7]. Silica coated TLC plates were pre-washed in methanol and activated for 30 min at 120 °C. Chloroform/methanol (1:4) was used as mobile phase. Dried plates were studied with UV and blue Crime-lite®2 torches in combination with the clear and yellow goggles. UV (365 nm, 10% band width 350–380 nm) and blue (445 nm, 10% band width 420–470 nm). Crime-lite®2 torches were used to visualize the spots on the developed TLC plates. Digital images were taken using the polarizer filter (to reduce direct reflection) and the yellow (GG495) filter (to block any residual excitation light and pass longer wavelengths above 495 nm) supplied with the blue Crime-lite®2 torches. To achieve equal distribution of light on the TLC plate and to visualize the whole TLC plate in one image, the light sources and the digital camera were placed 40 cm above the TLC plate. The camera was placed directly above the TLC plate, the lamps were placed at an angle in such a way that the light incident angle on the TLC plate was as close as possible to 90°. The following settings were employed for developed fingermarks directly left on TLC plate: aperture f/5 and iso-800. The amount of fluorescence originating from the background and fingermarks depended on the Crime-lite®2 torches used, resulting in a different exposure time used for different light sources: UV:25 s; blue: 8 s. Next, excitation and emission spectra of the observed spots were obtained using the fiber optic accessory of the luminescence spectrometer. Excitation and emission wavelengths to obtain fluorescence spectra from the eluted spots were based on previous studies, but also based on the excitation/emission wavelength of the