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The in vitro stability in human plasma of two different technetium-99m-fibrinogen compounds

E. Spicher, B. Gieux and R. Fridrich

Department of Nuclear Medicine, University Hospital Basel, CH-4031 Basel (Switzerland), 6 July 1983

Summary. Two ^{99m}Tc-labeling methods of human fibrinogen resulted in different complexes. This was concluded from different dissociation rates in human plasma. The dissociation could be described by a simple exponential function.

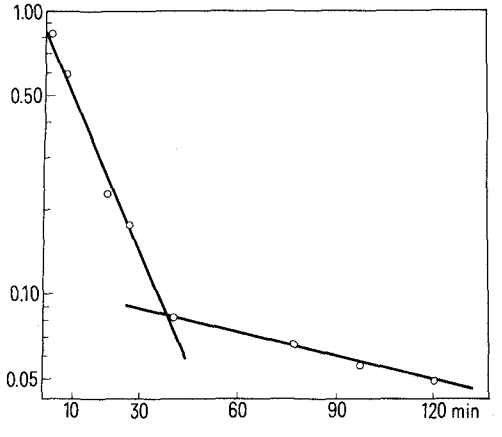
Radiolabeled human fibrinogen (HF) is used in the diagnosis of intra- and extravascular coagulation. While ^{125}I and ^{131}I are covalently bound to the aromatic ring of tyrosine[1], ^{99m}Tc is incorporated into a complex the structure of which has not yet been determined. As the complex may vary according to the labeling conditions, we compared the stability of 2 samples of ^{99m}Tc-HF labeled under different conditions. One method was published recently[2]. The reduction of $^{99m}TcO_4^-$ is obtained by Sn^{2+}. During the whole labeling procedure a physiologic pH (7.4) can be maintained, due to the use of a citrate/carbonate buffer. Column chromatography with Sephadex G 25 is reported to yield 81.1 ± 1.5% radioactivity attributable to HF. The other method replaces citrate by glycine buffer[3]. After adding $^{99m}TcO_4^-$ which is reduced by Sn^{2+} as well, the pH of the mixture is about 10. According to the authors, column chromatography with Biogel P6 yielded 90–95% radioactivity attributable to HF, while the isotopic clottability reached only 65–70%. We investigated the stability in human plasma at 37 °C to obtain comparable results with a view to eventual in vivo investigations.

Methods. One preparation of ^{99m}Tc-HF (complex A) was labeled according to the published method[2]. The other preparation (complex B) was obtained by adding $^{99m}TcO_4^-$ to a commercially available vial containing lyophilized HF, tin chloride and glycine (Sorin SA., Italy). Details of the labeling solutions are listed in table 1. After adding 1 drop of the labeling solution to fresh human plasma the ^{99m}Tc-HF was incubated at 37 °C. The labeled and unlabeled HF were separated from other plasma components by clotting with thrombin. 200 µl plasma samples were brought to 1 ml with Michaelis buffer. After addition of 100 µl thrombin (120 U/ml) the mixture was incubated 2 min at 37 °C. The clot was sedimented at about 2000 × g for 15 min, decanted and washed with 2 ml isotonic NaCl solution. The clot radioactivity was measured by a gamma counter and related to the radioactivity of a plasma sample. Solutions of $^{99m}TcO_4^-$, ^{99m}Tc-human serum albumin (HSA) and ^{125}I-HF (2 mCi ^{99m}Tc in 5 ml respectively 110 µCi ^{125}I in 1.1 ml) were prepared. 1 drop of each solution was added to human plasma and treated as described above.

Results and discussion. The efficiency of our separation method was checked on the one hand by investigating the coprecipitation of $^{99m}TcO_4^-$ and ^{99m}Tc-HSA. These compounds, we thought, have to be considered first after ^{99m}Tc-HF dissociation. $^{99m}TcO_4^-$ might be coprecipitated because about 80% is bound to proteins even if weakly[4]. The table 2 shows neither $^{99m}TcO_4^-$ nor ^{99m}Tc-HSA coprecipitating to an extent worth mentioning with HF. On the other hand, we checked the clot yield with ^{125}I-HF. About 90% radioactivity could be precipi-

Table 1. Details of the labeling solutions

	Complex A	Complex B
Human fibrinogen	3 mg	5 mg
Sodium citrate	3.6 mg	–
Sodium bicarbonate	0.17 µg	–
Glycine	–	q.s.
Tin chloride dihydrate	11 µg	0.2 mg
^{99m}Technetium in isotonic saline	2 mCi (74 MBq)	2 mCi
Total volume	1.6 ml	2 ml
pH	ca. 7.4	ca. 10
Incubation	1 h, 28 °C	20 min



The dissociation of complex A in human plasma at 37 °C, the clot/plasma-radioactivity-ratio incubation time.

Table 2. The plasma radioactivity in the fibrin clot (N = 3). Incubation at 37 °C

	$^{99m}TcO_4^-$	^{99m}Tc-HSA	^{125}J-HF	^{99m}Tc-HF Complex A	^{99m}Tc-HF Complex B
3 min	0.1 ± 0.1%	0.9 ± 0.5%	89.1 ± 3.0%	74.3 ± 4.6%	66.0 ± 8.9%
10 min	0.6 ± 0.9%	1.8 ± 0.9%	88.9 ± 3.0%	39.3 ± 2.6%	65.6 ± 8.8%
3 h	0.4 ± 0.5%	1.7 ± 0.6%	86.4 ± 2.5%	2.7 ± 1.2%	55.3 ± 8.0%

Table 3. The dissociation of ^{99m}Tc-HF in human plasma at 37 °C. K (dissociation constant in sec^{-1}), A (amplitude), V (variation coefficient of the regression line), N (number of curves).

	K	A	V	N
Complex A	$1.13 \pm 0.11\ 10^{-3}$	0.855 ± 0.073	3.4 ± 0.4%	3
	$1.18 \pm 0.24\ 10^{-4}$	0.092 ± 0.009	7.2 ± 4.9%	3
Complex B	$1.07 \pm 0.23\ 10^{-5}$	0.654 ± 0.107	2.3 ± 1.3%	7

tated (table 2). The difference 3 h after incubation at 37 °C was small. Thus we could neglect fibrinolytic or other decomposing effects. A second wash of the clot with 2 ml saline solution removed only about 1.5% radioactivity, compared with the plasma sample, and could therefore be omitted.

The behavior of the two ^{99m}Tc-HF preparations in human plasma differed considerably (table 2). Complex A lost its Tc much more rapidly than complex B. It was possible to express the dissociation rate by a simple exponential function. While the complex A showed 2 phases in the first 3 h with half-lives of 10.2 min and 1.6 h (fig.), the complex B showed 1 phase with a half-life of 17.9 h. The dissociation constants and amplitudes are listed in table 3. The amplitudes mean that 85.5 ± 7.3% resp. 65.4 ± 10.7% radioactivity was bound to HF when the complex A or B had been added to the plasma (t = 0). While the amplitude for complex A was in good agreement with the results of column chromatography mentioned above, the amplitude for complex B was substantially lower, but confirming the reported isotopic clottability. Either the chromatographic separation was not effective enough in the latter case or a considerable part of the activity was bound to the fibrinopeptides which are split off at coagulation.

It was suspected that citrate could compete with proteins for Tc, which would explain the low labeling-yields found in the presence of citrate[5]. Our results with the complex A, however, show that there is no doubt about the labeling of HF with Tc even in acceptable yields (> 80%), but rapid dissociation takes place in plasma. The instability of complex A and the low labeling yield of complex B do not recommend these products for in vivo use.

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Formation of 2-methoxy-1,4-benzoquinone from vanillic acid by fungal laccases at various pH values[1]

A. Leonowicz and J.-M. Bollag

Laboratory of Soil Microbiology, Department of Agronomy, The Pennsylvania State University, University Park (Pennsylvania 16802, USA), 3 August 1983

Summary. Laccases of various fungi metabolize vanillic acid, but contradictory reports were found concerning the formation of 2-methoxy-1,4-benzoquinone. We could establish that the pH of the medium was the major factor affecting the formation of this product.

Several methoxyphenolic acids have been reported as degradation products of lignin by various wood-rotting fungi and other microorganisms[2]. In particular, vanillic acid and its derivatives were found to result from softwood lignin biotransformation[3]. These compounds, however, are not the end products; they can be further transformed by various enzymes such as fungal phenol oxidases[4], and peroxidases[5], which may cause the formation of humic acid-like polymers[6].

Among phenol oxidases a fungal laccase (benzenediol:oxygen oxidoreductase, E.C. 1.10.3.2) can be involved in the transformation of vanillic acid. The formation of different products was established, but there exists a controversy concerning the generation of methoxyhydroquinone or methoxybenzoquinone. Several researchers determined methoxyhydroquinone as an intermediate of vanillic acid metabolism in media of laccase-producing fungi[7,8] or as a product of a partially purified enzyme[9]. During incubation of vanillic acid with a laccase of *Trametes sanguineus* at pH 4.0, 2-methoxy-1,4-benzoquinone was produced[10], whereas the same substrate incubated with a laccase of *Rhizoctonia praticola* at pH 6.9 did not form this metabolite[4]. This report attempts to clarify whether the pH or the specificity of a particular laccase is the actual factor causing the formation of methoxybenzoquinone as a product.

Laccases were isolated from the culture media of the following fungi: *Botrytis cinerea, Fomes annosus, Pholiota mutabilis, Pleurotus ostreatus, Podospora anserina, Rhizoctonia praticola* and *Trametes versicolor*. We received the culture of *B. cinerea* from Dr B. Donèche, *F. annosus* from Dr A. Hüttermann, and *P. anserina* from Dr K. Esser. The other fungi were from our own laboratories[4,11]. All cultures were grown on the same basal medium which was developed from Czapek-Dox and Lindeberg media[12], and the laccases were isolated from the culture filtrate as described earlier[13]. Laccase activity was determined on a Bausch and Lomb Spectronic 2000 spectrophoto-