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NOTE

IRON-59 LABELLED SODIUM NITROPRUSSIDE

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SUMMARY

Sodium nitroprusside, a potent vasodilator in man, is synthesized with the gamma-emitting isotope ^{59}Fe in a single-step synthesis from potassium cyanide, sodium nitrite, and ferrous chloride. It is purified by anion exchange chromatography and assayed by ion-pair high pressure liquid chromatography. ^{14}C-nitroprusside is prepared by the same methods.

Key words: nitroprusside, sodium nitroprusside, ^{59}Fe-nitroprusside, ^{14}C-nitroprusside

INTRODUCTION

Sodium nitroprusside, (sodium pentacyanonitrosylferrate[II]), normally prepared as the crystalline hydrate, $Na_2Fe(CN)_5NO{\cdot}2H_2O$, is a potent vasodilator that is widely used clinically to rapidly lower blood pressure. When given intravenously it has a rapid onset of action and a very short duration, which necessitates administration by continuous intravenous infusion. It can cause a drop in blood pressure with a dosage less than 1 μg/kg body weight/minute, and can cause profound hypotension at doses between 1 and 10 μg/kg body weight/minute. The functional part of the molecule is the NO moiety which is believed to be cleaved off as free nitric oxide, although the chemistry involved is unknown[1]. Nitric oxide is known to cause vasodilation by a series of steps that are initiated by NO binding to a heme group in the enzyme guanylate cyclase in the cytoplasm of vascular smooth muscle[1]. The decomposition of

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nitroprusside also results in the release of free or simple cyanide and there have been deaths by frank cyanide toxicity when nitroprusside was given at doses in excess of 20 μg/kg/minute[2].

The chemistry of nitroprusside in biological tissue is difficult to study because the drug has a short half life in the body, is effective at low dosages, and apparently decomposes in multiple steps to nitric oxide, cyanide, and unknown iron salts which are all rapidly converted to other species. The only chemical assay for nitroprusside at pharmacological concentrations (1 μM and below) in biological samples uses high-performance differential pulse polarography[3]. The technique is technically demanding and is not applicable to following the metabolites or decomposition products of nitroprusside. The preparation of radioactive nitroprusside with a specific activity high enough to be detected at pharmacological concentrations has not been described. This paper describes the synthesis of sodium nitroprusside labelled with ^{59}Fe with a specific activity high enough to allow easy detection of the iron at concentrations well below those used clinically.

The usual synthesis of sodium nitroprusside uses the prolonged oxidation of potassium ferrocyanide by nitric acid, followed by neutralization with sodium carbonate and crystallization. That synthesis is difficult to perform with small starting quantites of iron. Instead, nitroprusside is obtained by the reaction of potassium cyanide, sodium nitrite, and ferrous chloride. The reaction is similar to the one below described by Hofmann in 1895, although the conditions are different[4,5].

$$5NaCN + 3FeCl_2 + 3NaNO_2 = Na_2Fe(CN)_5NO + 6NaCl + Fe_2O_3 + 2NO$$

In the synthesis described in this paper the ratio of cyanide to iron in the reaction is 6:1 with a large excess of nitrite, resulting in the rapid incorporation of about 50% of the iron and 40% of the cyanide into nitroprusside. This suggests a stoichiometry slightly different than the above reaction, but the only identified iron-containing products remain nitroprusside and precipitated ferric oxide or ferric hydroxide. This synthesis can be used to prepare nitroprusside containing ^{14}C or ^{59}Fe or both.

MATERIALS

^{59}Fe-ferric chloride and ^{14}C-KCN were purchased from Amersham. Ascorbic

acid, ferrous chloride, basic alumina, and Dowex 50 were from Sigma. Potassium cyanide, potassium ferrocyanide, potassium ferricyanide, sodium sulfide, sodium nitrite, HPLC-grade methanol, hydrochloric acid and potassium phosphate salts were from Fischer.

EXPERIMENTAL

^{59}Fe-ferric chloride (1 mCi, 78 micrograms Fe in 1 ml of 0.1 N HCl)was evaporated to dryness and reduced to ferrous chloride by redissolving it in 1 ml of 0.1 M ascorbic acid. The solution was applied to a 0.5 ml column of Dowex 50 equilibrated in 0.1 N HCl and the column rinsed with 1 ml of 0.1 N HCl. The iron was eluted with 3 ml of 2 M HCl. This solution was evaporated to dryness under vacuum and redissolved in water to a final concentration of 0.01 M $FeCl_2$. Parallel reactions using unlabelled ferric chloride demonstrated a complete conversion of ferric ion to ferrous ion as determined by reactions with ferricyanide and ferrocyanide.

300 µl of 0.01 M KCN was mixed with 25 µl of 2 M $NaNO_2$, and 50 µl of 0.01 M ^{59}Fe-ferrous chloride was added. The reaction was allowed to proceed at room temperature for 5 minutes, followed by the addition of 1 ml of methanol and immediate centrifugation at 10,000 x g for 2 minutes. Ferric oxide was precipitated and the supernate was applied to a column of alumina with a 0.5 ml bed volume equilibrated in 0.01 M sodium acetate buffer pH 4.7. The column was rinsed with 1.5 ml of the same buffer, which elutes unreacted cyanide and nitrite, and the nitroprusside was eluted with 3 ml of the same acetate buffer containing 0.2 M NaCl. The pooled fractions containing radioactive nitroprusside were evaporated to dryness under vacuum in the dark. The residue was redissolved in methanol, divided into aliquots and again evaporated to dryness under vacuum in the dark. Dried aliquots were stored in the dark and dissolved in methanol or aqueous buffer prior to use.

Reaction on a spot plate with alkaline sodium sulfide revealed the purple complex characteristic of nitroprusside. High pressure liquid chromatographic analysis was performed using a variation of the ion-pair method of Baaske et. al.[6] Chromatography on an octadecylsilica column equilibrated in a mobile phase of 30% methanol and 70 % (10 mM potassium phosphate, 1 mM tetrabutyl ammonium hydroxide, pH 6.0) revealed a single ^{59}Fe- containing peak which migrated with

authentic nitroprusside and could be clearly distinguished from ferricyanide or ferrocyanide (Fig. 1). Since the only source of iron in the synthesis was $^{59}FeCl_3$, the resulting nitroprusside had the same specific activity as the ferric chloride (750 mCi/mmole).

Synthesis of ^{14}C-nitroprusside started with 0.01 M $K^{14}CN$ (55 mCi/mmole), 2 M $NaNO_2$, and 0.01 M ferrous chloride and proceded as above. ^{14}C-nitroprusside with a specific activity of 275 mCi/mmole chromatographed as a single peak on HPLC.

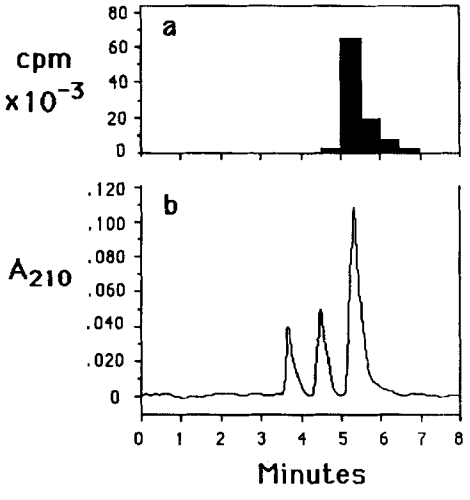

FIGURE 1. **ION -PAIR CHROMATOGRAPHY OF NITROPRUSSIDE.**
2 µl of a mixture of 0.5 mg/ml each of potassium ferrocyanide, potassium ferricyanide, and sodium nitroprusside, which also contained approximately 10^5 counts/minute of radiolabelled nitroprusside, were chromatographed as described above. Panel **a** shows radioactivity (counts/minute) collected in 30 second fractions. Panel **b** shows optical absorbance at 210 nm. The three cyanoferrates were well resolved with retention times of 3.6 min. (ferrocyanide), 4.5 min (ferricyanide), and 5.4 min (nitroprusside). All radioactivity migrated with nitroprusside.

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