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Assay of Cysteine in Human Serum with Quinine–Ce^{4+} Chemiluminescence System

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Abstract: A sensitive and selective chemiluminescence (CL) method was developed for the determination of cysteine. This method is based on that the weak CL of cysteine oxidized with cerium (IV) can be greatly enhanced by quinine, and the total cysteine in human serum can be detected through simply diluting with water, showing a simpler analytical characteristic.

Key words: Chemiluminescence, cysteine, quinine-Ce^{4+} system, human serum.

Cysteine is one of amino acids containing thiol groups. It not only constitutes protein but also plays an important role in several biological processes, such as redox, methyl transfer and carbon fixation reactions in which CoA participates. Therefore, the detection of cysteine and its derivatives has attracted much attention[1-3].

In this work, a CL method was developed for the determination of cysteine, which was based on oxidation of the thiol group of cysteine by cerium sulfate. This reaction produced a weak CL but the CL intensity could be enhanced significantly in the presence of quinine. The conditions for assay of cysteine were optimized and the proposed method was used to detect cysteine in human serum. Compared to other methods for the detection of cysteine, the present one does not require any prior derivatization and separation, and has the merits of simplicity, high sensitivity and selectivity.

Experimental

Lumat LB 9507 (EG & G BERTHOLD, Bad Wildbad, Germany) was used for CL measurements. This apparatus is equipped with a variable automatic volume injector and has a function of monitoring kinetic behavior of light emission; the emitted light is measured by a selected high-sensitivity, low noise photomultiplier. Its spectral sensitivity covers a range between 390-620 nm.

All solutions were prepared with deionized-distilled water. Cerium sulfate was purchased from Beijing Chemical Reagent Co. L-Cysteine and quinine were purchased from Sigma (St. Louis, MO, USA) and Shanghai Chemical Co., respectively.

A 100 μL sample solution containing no more than 2×10^{-5} mol/L of L-cysteine was mixed with 50 μL of 6.2 mmol/L quinine solution in a 5 mL tube. Then appropriate volume of Ce^{4+} solution was automatically injected into this mixture solution. The peak

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height signal was recorded as the CL intensity, and each data in this work was the mean of 5 determinations.

Results and Discussion

It is well established that the weak CL from a redox system can be greatly amplified by introducing a strong fluorescent species through a mechanism of matchable intermolecular energy transfer. In this system, Ce^{4+} and quinine were used as an oxidant and a sensitizer, respectively, since the former can oxidize thiol groups[4] and the latter is a good fluorescent substance (Φ =0.57). The conditions for detecting cysteine were studied in details. 1 mmol/L Ce^{4+}, 0.18 mol/L H_2SO_4 and 0.45 mmol/L quinine were found to be optimal. Under this condition the peak height of the CL intensity is directly proportional to the concentration of cysteine in the range of 3.5×10^{-9}-3.5×10^{-6} mol/L. The regression equation was determined to be $I = 3 \times 10^9 C$ (cysteine/ mol/L) – 11, r = 0.9992, n = 12. The relative standard deviation was 8.4% by 10 replicate determinations of 2.89×10^{-8} mol/L cysteine, and the detection limit was 2.5×10^{-9} mol/L (*S/N* = 3). In order to assess the applicability of the CL method, the effects of various metal ions (Fe^{3+}, Zn^{2+}, Pb^{2+}, Mg^{2+}, Cu^{2+}, Ca^{2+}, Na^+, K^+, Cl^-, SO_4^{2-}, NO_3^-) and some other amino acids present commonly in human serum were examined by analyzing synthetic sample solutions containing 3×10^{-8} mol/L cysteine. The results showed that the selectivity of the method was very high, though larger amounts of methionine and especially tryptophan (>5-fold of cysteine) yielded interferences. Yet, their concentrations in healthy human serum are usually not more than 5 times of that of cysteine, suggesting that the present method may be directly applied to the determination of cysteine in human serum.

Table 1 shows such an attempt on determining directly total cysteine (including free and protein-bound cysteine[3]) in serum mixtures from 30 normal persons, and the results obtained are consistent with the reported values[2,3]. Also, recoveries of the method ranged in 92.0-104.2%, which are good enough for practical use. Further studies are being continued to understand the mechanism of this CL enhancement.

Table 1 Concentration and recoveries of cysteine in human serum

Cysteine added/μmol/L	Cysteine found/μmol/L Mean ± SD (n = 5)	Recovery (%)
0	265.2 ± 16.9	-
93	362.1 ± 22.1	104
135	389.4 ± 7.2	92.0
173	424.5 ± 17.6	92.1
306	564.9 ± 19.5	97.9

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