The Consecutive Determination of Serum Ammonia and Leucine Amino Peptidase with an Ammonia Gas-sensing Electrode

Masakatsu HATO,* Yukio SHIMURA, Tomiko AKIYAMA, and Hiroko NAKAYAMA
Research Institute for Polymers and Textiles, Yatabehigashi 1-1-4, Tsukuba-gun, Ibaraki-305
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Synopsis. A simple method for the consecutive determination of serum ammonia and the activity of leucine amino peptidase (LAP) with an ammonia gas-sensing electrode is described. The values determined by the present method agree with those determined by conventional methods within 6%.

The serum ammonia and LAP level is of clinical significance; *i.e.*, elevated levels have been found in a number of conditions of the liver.[1] Many procedures have been proposed for the determination of serum ammonia.[2-5] However, these methods have various shortcomings;[6] *e.g.*, the ammonia concentration is considerably affected by the diffusion conditions, such as the temperature and the diffusion time. Nitrogenous compounds other than ammonia are destroyed by alkali during the diffusion period, liberating ammonia and giving erroneously high ammonia values. The method proposed by Dienst,[7] which was later improved by Fenton,[8] seems to be complicating.[1] More recently, Okuda *et al.*[9] have developed a usable method. However, their method needs a fairly large amount of serum (0.5—1.0 ml)[10] and requires the deproteinization of the serum and a long incubation time.

In the previous paper, we have reported on the use of an ammonia gas-sensing electrode (abbreviated to an NH_3-electrode) in a kinetic assay of serum LAP,[11,12] where the time course of ammonia production in the reaction, L-leucine amide $\xrightarrow{\text{LAP}}$ L-leucine$+NH_3$, is directly monitored in buffered serum by means of an NH_3-electrode. The LAP activity thus determined agreed well with that determined by the conventional methods.[12,16] This suggests that, with a minor modification of the procedure, the method can be extended to the determination of serum ammonia as well. In the present communication, the consecutive determination of serum ammonia and LAP with an NH_3-electrode is described.

Experimental

Apparatus and Procedures. The experimental apparatus is illustrated in Fig. 1a. An Orion model 95-10 NH_3-electrode (a) was dipped in a buffer (1.5 ml; 0.2 M TrisHCl pH=8.5, 10 μM NH_4Cl) stirred by a magnetic stirrer (c). The whole apparatus was kept at 25±0.1 °C by circulating thermostated water through a glass jacket (d). The electrode was pre-equilibrated with the buffer. At time $t=0$, male rabbit serum was added through an inlet (e) and the electromotive force (E) was recorded as a function of the time by means of an Orion model 801 mV meter and a Yokogawa 3047 strip-chart recorder. After the equilibrium E value was reached, 0.1 ml of a 110 mM L-leucine amide buffer (a substrate for LAP, 0.2 M TrisHCl pH=8.5) was added to the buffered serum in order to initiate the LAP reaction; the E was recorded as a function of time. The pH was constant throughout the experiment. To examie the accuracy of the present method, the serum ammonia was also determined by means of an Ammonia Test Wako® (Wako Chem. Co.). The LAP activity was also determined by the method of Hjemdahl-Monsen *et al.*[12,16]

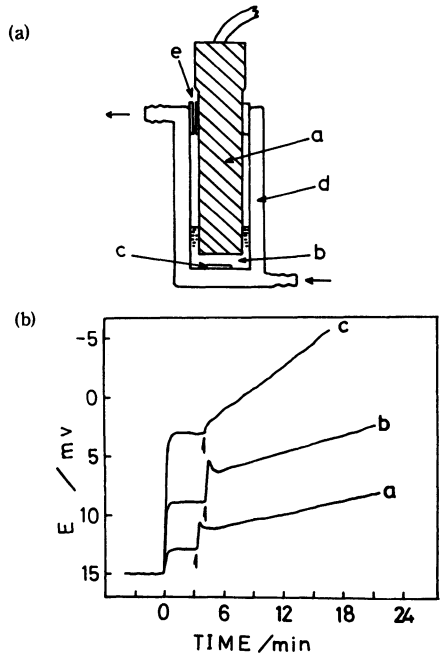

Fig. 1.(a) Schematic illustration of experimental apparatus.
(b) Typical electrode response to serum ammonia and LAP as a function of volume of male rabbit serum (25 °C).

Results and Discussion

Electrode Response to Serum Ammonia and LAP Reaction. Figure 1b shows a typical electrode response to serum ammonia and LAP. With the addition of 10 μl (Curve a), 20 μl (Curve b), and 50 μl (Curve c) of serum at time $t=0$, the E decreased steeply and attained equilibrium values within 1—2 min. The substrate buffer was then added at the times indicated by arrows (+). The initial irregular changes in the E were attributable to the electrode response to the ammonia present in L-leucine amide. The E decreased continuously with time. This reflects the continuous increase in the ammonia concentration due to the LAP reaction in the reaction mixture. The E *vs.* time curves were converted into concentration *vs.* time curves *via* an E *vs.* log (concentration) curve (Fig. 2). The activity of LAP was calculated from the slope of the concentration *vs.* time curve.[12] Measurable activity range is estimated to be from 0.1 $\mu M \cdot min^{-1}$ to 20 $\mu M \cdot min^{-1}$.[11,12] The addition of NH_4Cl in advance to the buffer is important not only to eliminate errors caused by the contamination of ammonia (from the atmosphere or from the

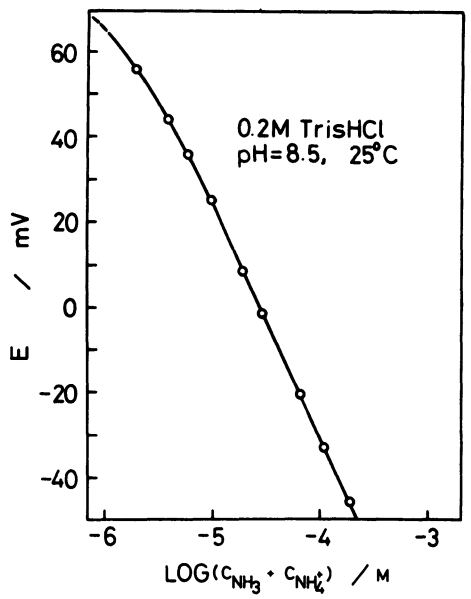

Fig. 2. Electromotive force (E) of the electrode as a function of total ammoniacal concentration (calibration curve).
1 M=1 mol·dm^{-3}. 1 μM=1.4 μg·NH_3-nistrogen/dl.

human body), but also to increase the electrode-response rate.[11] When there is no need to determine the LAP activity, several serum samples can be analyzed consecutively. Five different samples could be analyzed within 7—8 min. The serum ammonia concentration C was calculated from a calibration curve (Fig. 2) and from the equation;

$$C = [(V_i+v)C_f - C_iV_i]/v, \quad (1)$$

where V_i, v, C_i, and C_f are the initial volume of the buffer (or buffered serum), the volume of the added serum, and the initial and final ammonia concentration of the reaction mixture respectively. The measurable ammonia concentration range is estimated to be from 70 to at least 10^4 μgNH_3-nitrogen/dl at $v/V_i=0.01$ and from 20 to at least 10^4 μgNH_3-nitrogen/dl at $v/V_i=0.1$.

Accuracy and Stability of the Electrode Response.

Table 1. Comparison of the serum ammonia and LAP determined by the present method with those determined by conventional methods

Sample	Serum ammonia (μg·NH_3-nitrogen/dl)		LAP activity (μM/min[d])	
	This method[a]	O-F method[b]	This method[a]	H-P-R method[c]
1	134	128	—	—
2	97	92	—	—
3	168	171	0.21	0.20
4	170	175	0.62	0.65

a) Average of 2 measurements. The relative standard deviation for three identical measurements was 6%. b) Average of three measurements[10] c) Average of two measurements[12,16] d) 1 M=1 mol·dm^{-3}.

The serum ammonia and LAP activity determined by the present method are compared with those determined by conventional methods in Table 1. The analytical values agreed with each other within 6%. This indicates that the present method is effective in the determination of serum ammonia and LAP.

Practical difficulties accompanying an NH_3-electrode, *e.g.*, the clogging of the gas-permeable membrane in biological fluid, have often been pointed out.[13,14] However, provided a volume fraction of serum ranging from 0.005—0.25 was used, the electrode response in the buffered serum was stable for at least 3 weeks (10 assays/day). When the fraction exceeded 0.3, errors tended to increase because of the adsorption of serum components on the membrane. Similar effects have also been observed for the case of urea determination in whole blood with an NH_3-electrode based urea electrode.[15] When the fraction was less than 0.005, the electrode response became too small to get accurate results. The NH_3-electrode seems to have no serious practical limitations when employed in moderately diluted serum.

The advantages of the present method are: (1) The consecutive determination of serum ammonia and LAP is possible. (2) Only a small amount of serum is needed; *e.g.*, 10—50 μl is sufficient. (3) A long color-development time for the conventional method is unnecessary. (4) Strong acidic and alkaline reagents are unnecessary, and therefore the procedures for waste disposal are simple.

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