Micronized Gel Electrophoresis: Construction and Its Versatile Applications

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We have constructed a one-inch-sized apparatus for gel electrophoresis (μGE). This apparatus was shown to be of high performance and to have potentials to apply to versatile purposes mainly due to its speed and economy. The temperature gradient gel electrophoresis as well as the conventional gel electrophoresis of multi-wells was successfully performed with both DNAs and proteins. Secondary gel electrophoreses in the two alternative modes, band-cut and zone-cut, were shown to be operable.

Gel electrophoresis has been made a great contribution to the advance of biochemistry and the other life sciences in the 20th century. It is still advancing and widening its applications because of low initial-threshold and high utility. Currently, science is progressing to analyzing a large number but a small amount of samples at a time in many fields.[1] This is also the case with the disciplines that deal with DNAs and proteins by way of gel electrophoresis. Accordingly, such an electrophoretic system that enables us to perform parallel gel electrophoresis in a large scale has been waited, especially in the fields of microbe-related science.[2] For example, recently, collaborators of Nishigaki have developed a genotype-based species identification system which also depends on a parallel operation of TGGE (Temperature Gradient Gel Electrophoresis).[3,4] On the other hand, trials of micronization in gel electrophoresis have been successfully done; e.g., slide glass sequencing of a 6 cm run way using RI-labeled DNA,[5] micronized 2D gel for separation of protein,[6] and discontinuous ultrathin slabgel electrophoresis of DNAs.[7]

In this context, we have constructed a thermo-controllable micronized gel electrophoresis apparatus (μGE) as shown in Figure 1(a, a'), which has a dimension of 5.5 cm × 8.5 cm (2.5 cm × 2.5 cm for the gel) and is made of acrylic resin. To operate this tiny cassette electrophoretic apparatus under the temperature-controlled conditions, we had to newly build a temperature-control unit, of which a part is appearing in Figure 1(a, a') (dark plates named as flat heat sources attached to a Peltier effect actuator). This apparatus can accommodate samples in the following two fashions: loaded on a comb-like strip that is made of nitrocellulose (multi-well mode) or loaded directly pouring into the wide well (single well mode or TGGE mode). The result is shown in Figure 1a (center) for the multiple well mode of μGE applied to separation of DNAs. Evidently, clearly discernable band separation was attained. Proteins also were separated by this system with 8% SDS polyacrylamide gel (data not shown). The result is shown in Figure 1b for the TGGE mode applied to analysis of protein structural stability. In this case, red fluorescent protein (RFP) was observed to lose its original fluorescence at more than 70 °C, reflecting the destruction of its native structure. The processes of thermal melting of DNAs were also observed by the similar experiments.[8] The third application of the band-cut mode was also effective (Figure 1c). This method is especially useful when the further analysis about a particular band is required: whether it is single or degenerated, or what kind of DNA/protein is it viewed from the thermal stability. As such problems need a different type of separation principle, they have not been easy to solve conventionally. Figure 1c shows clear separation of two stacked bands as well as featuring those bands from the viewpoint of thermal stability. The fourth application is far more convenient and economical than any other methods since it requires no additional materials in order to obtain the further information. As shown in Figure 1d, the monotonous parallel bands were specifically featured by the secondary electrophoresis of TGGE performed on the same gel having been used for the first separation. The method introduced here, which we call μGE, has great advantages in its operation: i) It takes only several minutes to carry out (~10 times rapider). ii) The amounts of samples and reagents are much reduced in proportion to the gel size (by 1/10~1/100). iii) It does not need special, sophisticated instruments except for the μGE apparatus. Thus, such experiments as had been tedious or too costly to perform have altered to be easily accessible. Such handiness removes psychological barriers from the scientists and seems to have an effect to let them devise novel applications.

References and Notes

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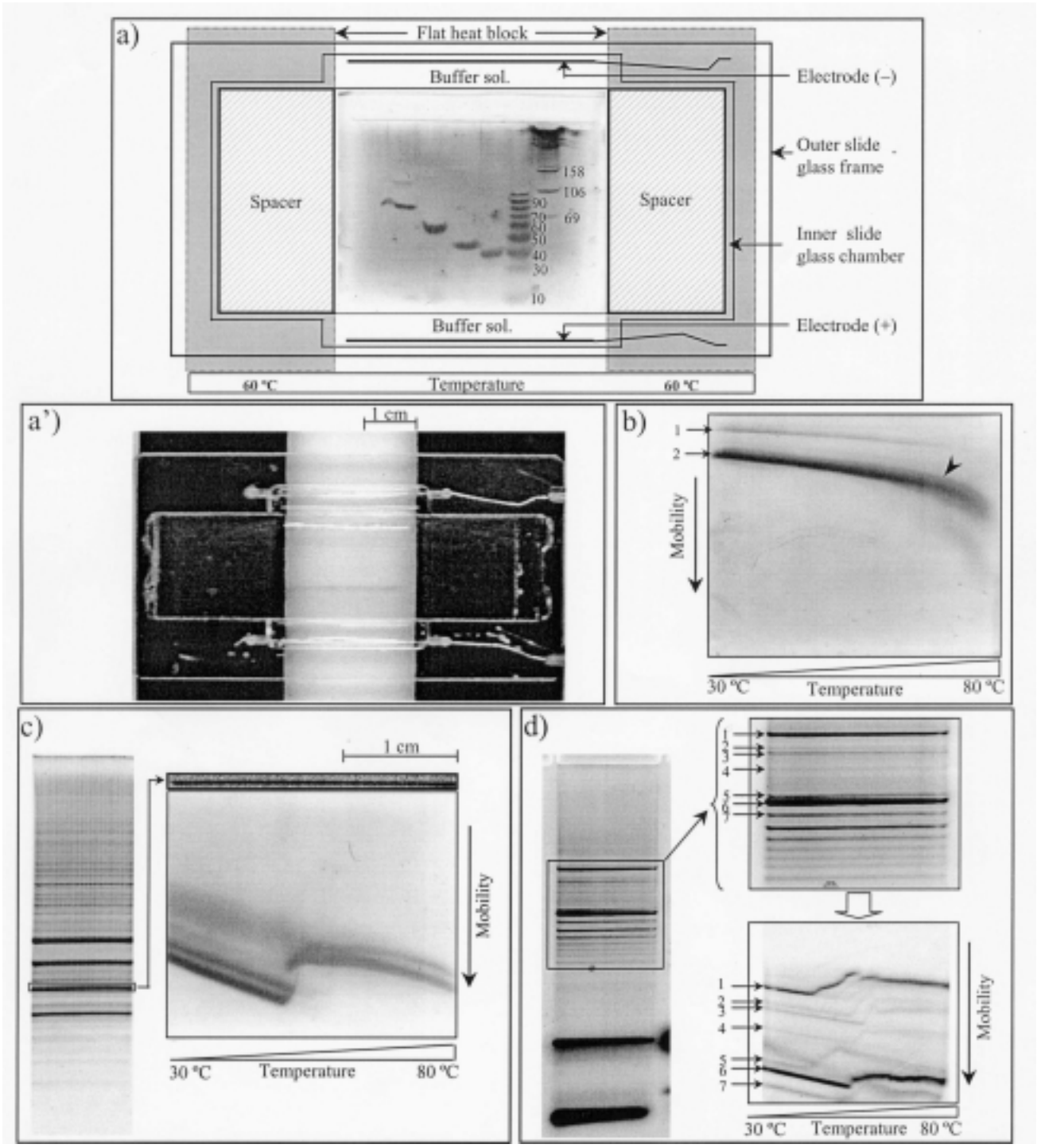

Figure 1. Overview of μGE and its typical applications.
a. An experimental result of the multi-well mode framed in the drawing of μGE apparatus. a'. Photograph of μGE. A slide glass, which contains a gel portion, is embedded in the outer frame that is made of acrylic resin, equipped with electrodes, and made reusable. Plate a is equivalent to this photograph. b. TGGE mode. Red fluorescent protein (RFP) was charged in a line on the top of the gel and then subjected to electrophoresis under the temperature gradient (30 °→80 °C), which we call μTGGE. The mark shows the position, from where the fluorescence of RFP fades away. The bands assigned, 1 and 2, are supposed to correspond to the higher aggregates of RFP and the dimer, respectively. c. Band-cut mode. A band generated in a conventional gel electrophoresis was cut out and loaded on the top of μGE. The single band was shown to separate into two bands by μTGGE, which also reveals the transition temperature of DNAs. d. Zone-cut mode. A piece of gel containing a zone of DNA bands was excised from the conventional gel, then mounted on the slide glass for μGE, and then subjected to μTGGE. The same number indicates the same band throughout. The gels used were 4% acrylamide and contained 8 M urea except in b, where 8M urea &20% formamide (10% acrylamide gel) was used in 0.27 M Tris-borate (pH 8.7). All migration times were less than 10 min at 100 V except for b (34 min).