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Anomalous behavior of human leukocyte interferon subtypes on polyacrylamide gel electrophoresis in the presence of dodecyl sulfate

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^{35}S-labeled human leukocyte interferon (IFN) subtypes produced in a cell-free system derived from *Escherichia coli* were analyzed by polyacrylamide gel electrophoresis in the presence of SDS (SDS-PAGE). Some IFN subtypes anomalously showed lower electrophoretic mobilities than those expected from their formula molecular masses. The results with hybrid IFNs and esterification suggest that this anomaly of IFN subtypes on SDS-PAGE is due to the introduction of one or two negative charges in the middle of the molecule.

SDS; Polyacrylamide gel electrophoresis; Interferon; cDNA; Interferon production; (Human leukocyte)

1. INTRODUCTION

Rubinstein et al. characterized several molecular forms of human leukocyte interferon (IFN) by polyacrylamide gel electrophoresis in the presence of SDS, amino acid composition, and antiviral activity [1], and reported that their apparent molecular masses obtained by SDS-PAGE ranged from 16 to 21 kDa, although IFN genes identified thus far encode the polypeptides with nearly the same formula molecular masses of about 19 kDa [2,3]. Since unusual behavior on SDS-PAGE has been known with various proteins [4], the differences in apparent molecular masses of IFN subtypes have been accounted for as abnormal behavior on SDS-PAGE [5]. Since IFN subtypes have very similar amino acid sequences, the differences in electrophoretic behavior of IFN subtypes might help identify the amino acid sequence affecting the interaction of protein with SDS. Accumulation of sequence data responsible for the abnormal interaction of protein with SDS should help us to understand the protein-SDS interaction on a molecular basis, which would be valuable to biochemists since SDS is now routinely used in the estimation of protein molecular masses. For this purpose, we examined the electrophoretic behavior of IFN subtypes produced in a cell-free system derived from *Escherichia coli* by using cloned IFN subtypes cDNAs.

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Abbreviations: IFN, human leukocyte interferon; PAGE, polyacrylamide gel electrophoresis

2. MATERIALS AND METHODS

cDNAs for IFN subtypes were isolated from a cDNA library of human lymphoblastoid cells stimulated for interferon production. cDNAs for IFN-A, IFN-B′, and IFN-G were completely sequenced by the chain termination method [6] with

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a sequencing kit from Takara Shuzo Co. (Kyoto, Japan), and other cDNAs were assigned by comparing the restriction cleavage maps with the published ones [2,3]. The expression plasmids for a mature form of IFN subtypes were constructed essentially as described elsewhere [7]. The expression plasmids for hybrid IFNs were constructed from those for IFN-B and IFN-G; the nucleotide sequences encoding amino terminal parts of mature IFN-B and IFN-G were exchanged through the restriction enzyme cleavage site for *Xba*I, which is located at the same position (between amino acid residue 82 and 83) in DNA sequences for IFN-B and IFN-G. ^{35}S-labeled IFN subtypes were synthesized in a cell-free coupled transcription-translation system derived from *E. coli* (Amersham International) with [^{35}S]methionine according to the supplier's instructions.

SDS-PAGE was carried out with a miniaturized slab gel apparatus (1 × 50 × 80 mm, Marysol Industry Co., Tokyo, Japan) using the discontinuous buffer system of Laemmli [8]. For a Ferguson plot analysis, a continuous buffer system (40 mM Tris-acetate, pH 7.4) was employed. The gel porosity was expressed as acrylamide concentration (w/v), and the weight ratio of acrylamide to methylenebisacrylamide was constant (30:0.8). Proteins used as size markers were purchased from Sigma (Dalton Marker VII-L), and labeled with 5-dimethylamino-1-naphthalenesulfonyl chloride for easy identification in a gel [9].

Esterification of IFN subtypes was carried out by the methanol-HCl method, and the ester groups were removed by treatment with NaOH [10]. Immunoprecipitation was performed using sheep anti-IFN antiserum (G-026-502-568), a gift from NIH (USA).

3. RESULTS AND DISCUSSION

The alphabetical nomenclature of IFN subtypes described by Pestka [2] was used, except for IFN-B′ and hybrid IFNs; IFN-B′ carried a deduced amino acid sequence identical to that of IFN-alpha8 [11] except for deletion of five residues and amino acid substitution from Arg to Asp at the carboxyl-terminal. The amino acid sequence of IFN-alpha8 is very similar to that of IFN-B; hybrid IFNs were designated as IFN-BG and IFN-GB, where the penultimate and final letters indicate the sources of the amino- and carboxyl-terminal portions, respectively.

To obtain mature IFN subtypes in the cell-free system derived from *E. coli*, their cDNAs were tailored and the nucleotide sequences encoding mature IFN subtypes were placed under the control of a *trp* promoter with a translation initiation site as described elsewhere [12]. In this system, post-translational modification such as glycosylation or phosphorylation would not occur. Fig.1 shows SDS-PAGE patterns of immunoprecipitated mature IFN subtypes produced in the cell-free system. IFN-A, IFN-BG, IFN-F, and IFN-J showed apparent molecular masses ranging from 18 to 20 kDa, according to our expectation. However, the electrophoretic patterns for IFN-B, IFN-B′, IFN-G, and IFN-GB were strikingly different from those for other IFN subtypes; the apparent molecular masses of IFN-B and IFN-B′ estimated from molecular mass markers were 27 and 25 kDa, respectively, although the formula molecular masses for all of these species should be about 19 kDa, and the product for IFN-G gave two bands with apparent molecular masses of 17 and 19 kDa. Both striking features were preserved

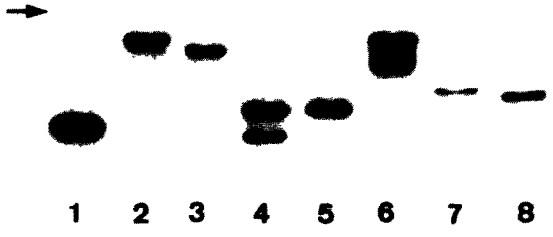

Fig.1. SDS-PAGE (15%) of IFN subtypes produced in the cell-free system after immunoprecipitation. Since IFN subtypes were labeled with [^{35}S]methionine, the bands were detected by fluorography. The arrow indicates the position of the band containing pre-β-lactamase, the product of the ampicillin-resistance gene. The formula molecular mass of pre-β-lactamase is about 31500 Da. Lanes: 1, IFN-A; 2, IFN-B; 3, IFN-B′; 4, IFN-G; 5, IFN-BG; 6, IFN-GB; 7, IFN-F; 8, IFN-J.

for hybrid IFN-GB, but not for IFN-BG. IFN-GB gave two bands with mobilities that decreased to the level of that of IFN-B, but IFN-BG moved normally as a single band with an apparent molecular mass of 19 kDa. Therefore, the nucleotide sequences encoding the amino-terminal half of mature IFN-G and the carboxyl-terminal half of IFN-B were responsible for the production of two polypeptides corresponding to the electrophoretic bands and for the anomalously low electrophoretic mobility on SDS-PAGE, respectively.

The production of two polypeptides observed for IFN-G was ascribed to unexpected translation initiation at an internal ATG codon, as described elsewhere [13]. The polypeptide in the upper band was a complete IFN-G molecule, whereas the polypeptide in the lower band was an incomplete IFN-G which was derived from unexpected translation initiation at the ATG codon for Met-18 in mature IFN-G (Edman degradation, not shown). The ATG codon for Met-18 was fortuitously accompanied by a purine-rich sequence (GGAGG), i.e., a putative Shine-Dalgarno sequence [14] with appropriate spacing to the ATG codon to provide a translation initiation site in *E. coli*. This easily explains the production of two polypeptides observed for IFN-GB but not for IFN-BG.

The deviations of apparent molecular masses of IFN-B and IFN-B′ from their expected masses were beyond the general range of deviation in molecular mass estimation by SDS-PAGE [4]. Since the electrophoretic mobilities of IFN-B and IFN-B′ did not change significantly with or without boiling in the presence of 2-mercaptoethanol, the intra- and/or intermolecular disulfide bond was not responsible for the anomalously low mobilities. From Ferguson plots of IFN-A, IFN-B, IFN-B′, and marker proteins, the values of the logarithm of relative mobilities extrapolated to zero gel concentration were 0.21 for IFN-A, 0.16 for IFN-B and IFN-B′, and 0.17–0.19 for marker proteins. Furthermore, the retardation coefficients for IFN-B and IFN-B′ were nearly the same as that of a marker protein with a molecular mass of 24 kDa. These results demonstrated the anomaly of IFN-B and IFN-B′ on SDS-PAGE, but their interpretation on the molecular basis would be difficult.

The behavior of hybrid IFNs indicated that the carboxyl-terminal half of IFN-B (from residue 83 to the carboxyl-terminal) was responsible for its anomalous behavior on SDS-PAGE. As shown in fig.2A, esterification of IFN-B and IFN-B′ increased their mobilities to levels identical to those of other IFN subtypes with mobilities not affected

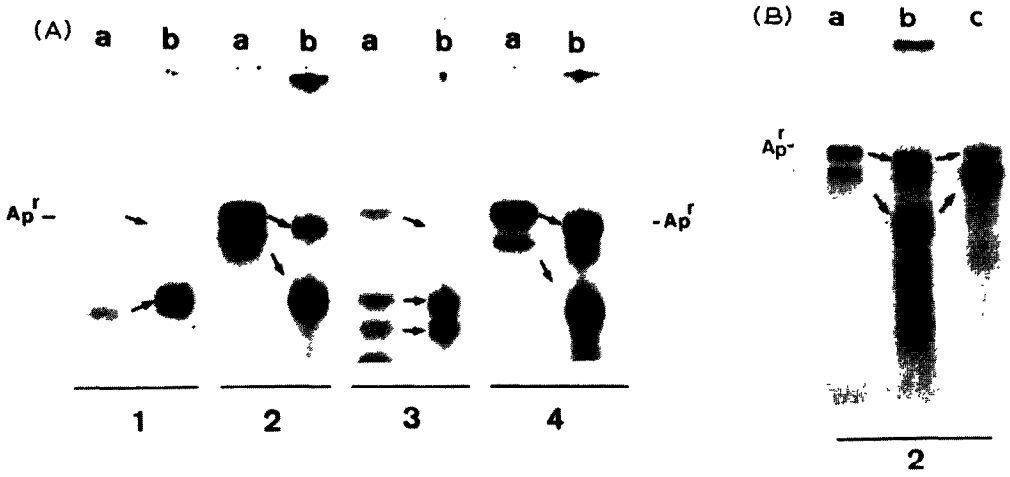

Fig.2. Effect of esterification on electrophoretic mobilities of IFN subtypes. SDS-PAGE was carried out on a 15% gel. (A) SDS-PAGE pattern of IFN subtypes before (a) and after (b) esterification. The products in the cell-free system were esterified without removal of the product of the ampicillin-resistance gene by immunoprecipitation. Ap^r indicates the band of the product of the ampicillin-resistance gene. Lanes: 1, IFN-A; 2, IFN-B; 3, IFN-G; 4, IFN-B′. (B) The product of IFN-B expression plasmid was esterified, and then treated with NaOH. (a) Before esterification, (b) after esterification, (c) after removal of ester groups.

as much by esterification. Removal of ester groups on IFN-B decreased its mobility to the original level (fig.2B). These results showed that negative charges on IFN-B and IFN-B′ play a crucial role in determining their electrophoretic mobilities on SDS-PAGE.

Consideration of this behavior of hybrid IFNs suggests that Asp and/or Glu located in the carboxyl-terminal half of IFN-B and IFN-B′ in common (from residue 83 to the carboxyl-terminal) must be responsible for the anomaly. Since IFN-GB and IFN-BG contained the same number of acidic residues in a molecule but behaved differently on SDS-PAGE, the total number of acidic residues does not seem to be related to the anomaly of IFN subtypes on SDS-PAGE. Comparison of the amino acid sequences of IFN subtypes reveals that IFN-B and IFN-B′ contain acidic residues in common at positions 84 and 90 while other IFN subtypes do not (fig.3). Therefore, the anomalous behavior of IFN-B and IFN-B′ was concluded to result from only one or two amino acid substitution(s) to an acidic residue located just in the middle of the IFN molecule. Some amino acid substitutions to an acidic residue have been reported to decrease the electrophoretic mobility of protein on SDS-PAGE in some cases [15,16], but the change in apparent molecular mass is generally within 10% of the expected mass. Therefore, it is remarkable that the apparent molecular masses of IFN-B and IFN-B′ obtained by SDS-PAGE deviated by more than 30% of their formular masses although they carried only a subtle change in the distribution of their negative charges in comparison with other typically behaving IFN subtypes.

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 1        10        20        30        40        50        60
MCDLPQTHSLGSRRTLMLLAQMRKISLFSCLKDRHDFGFPQEEF GNQFQKAETIPVLHEM      (A)
----------N--A-I------R--P----------E------DDK-----QA-S-----      (B)
----------N--A-I------R--P----------E------DDK-----QA-S-----      (B')
----------SN-----IM---GR--P----------------D-------QA-S-----      (G)
----------N--A-I-----GR--P----------------D-------QA-S-----      (F)
----------RN--A-I-----GR--P--------E-R--E---D-H----TQA-S-----      (J)

        70        80        90        100       110       120
IQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDLEACVIQGVGVTETPLMKEDSILAV      (A)
---T------------L------E--I--D-------VLCD-E---I-S---Y-------      (B)
---T------------L------E--I--D-------SCVM-E---I-S---Y-------      (B')
---T----------T----------------------MM-E---ED----NV----T-      (G)
---T----------T-EQS--E--S---N-----M------E---E-----NV------      (F)
---T------E------EQS--E--S----------------E---E-----N--F----      (J)

       130       140       150       160
RKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLRSKE 165   (A)
----------T-----S----------------I---KR-K---  166   (B)
----------T-----S----------------I---KD      161   (B')
----------T----------------------A----R--R--  166   (G)
K---------T----------------------KIF--R--R--  166   (F)
----------M--------------------F----KKG--R-D 166   (J)
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Fig.3. Amino acid sequences of IFN subtypes deduced from the nucleotide sequences of their cDNAs. The nucleotide sequences of IFN-A, IFN-B′ and IFN-G were confirmed by DNA sequencing. The residues are numbered according to the structure of mature IFN subtypes, although IFN subtypes produced in the cell-free system derived from *E. coli* carried formyl-Met (or Met) at their amino terminals. (−) A residue identical to that of IFN-A. Amino acid residues are expressed with one-letter abbreviations.

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