대한의생명과학회지: 제4권 제1호 1998
Korean J. Biomed. Lab. Sci.
Vol. 4, No. 1, p.73~76, June, 1998

Brief Communication

SDS-PAGE and Immunoblot Patterns of *Echinostoma hortense* in Experimentally Infected Rats

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Abstract: The authors characterized the antigen proteins and some specific antibodies from *Echinostoma hortense*. Crude antigen extracted from *E. hortense* worm was analyzed by SDS-PAGE of the crude antigen showed 46 profiles betweeen 200.2 - 8.2kDa, among which 200.2, 107.9, 86.8, 75, 69.8, 46.8, 43.5, 34.5, 20.9, 13.6, 12.6, 11.7, and 8.2kDa, protein profiles were strong. EITB resolved the specific IgG antibody into 17 profiles between 193 - 13.7kDa, among which 198, 123.4, 100.8, 91.1, 88.1, 62.8, 34.2, 32, 29.9, 18, 15.7, 13.7kDa profiles showed strong immunostain.

Key Words: *Echinostoma hortense*, Crude antigen, SDS-PADE, Immunoblot, Profile

The authors analyzed the protein profiles of the crude antigen extracted from *E. hortense* worm by SDS-PAGE. And the authors; also characterized some specific antibodies by EITB of the antibody serum from rats collectively infected with 150 *E. hortense* metacercariae, and serum from rats injected with *E. hortense* crude antigen and adjuvant[1].

E. hortense adult worms[11] were collected from rats 4 weeks after the infection with the metacercariae. The worms were washed by PBS several times and homogenized by ultrasonicating 5 times, at 100W and for 30 seconds at each time. The homogenized specimen was let to stand for 24 hours at 4℃, and centrifuged at 20,000g for 1 hour[5,10].

The supernatant was used as crude antigen. Protein of the crude antigen was quantified by the method described by Lowry et al. (1951) The antibody for *E. hortense* was acquired as follows. 200μl of the crude antigen and 200μl of Freund's complete adjuvant were mixed to emulsion, and the emulsion was injected intraperitoneally to rats. Two weeks later, the emulsion of the crude antigen 200μl and Freund's incomplete adjuvant 200μl was injected intraperitioneally for the second time. Another 2 weeks later, only the crude antigen was injected as booster injection[6]. After three or four days, the rat sera were separated and used as antibodies. SDS-PAGE was done by the method described by Laemmli (1970), using X Cell II Mini-Cell (10cm x 10cm, NOVEX, USA) and 4.0% stacking gel and 4~20% linear gradient separating gel (8cm x 8cm x 1mm, NOVEX, USA)[2]. The crude antigen wasdiluted 1:4 in sample buffer solution, heated at 100℃ for 3 miniutes, cooled and then injected in volume of 15μl (protein content 17μg/15μl) to each well. Maker protein for SDS-PAGE had molecular weight range of 200,000 - 6,000 Dalton (NOVEX, USA). After electrophoresis, the gel was stained in Coomassie blue R-250 by the method described by Morrissey (1981).

*Received May 15 1998,
Accepted after revision July 1, 1998.
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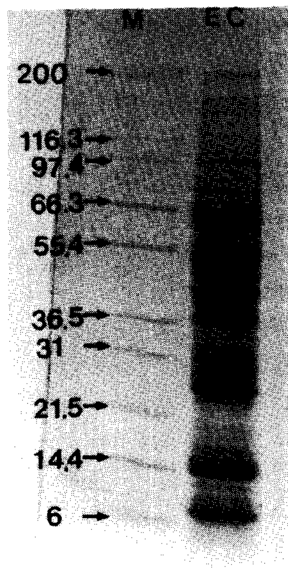

Fig. 1. SDS-PAGE of the crude antigen from *E. hortense*. 4~20% gradient separating gel was used. Molecular masses in kDa were estimated with standard markers of NOVEX (U.S.A).

EITB (enzyme-linked immunoelectrophoretic transfer blot) was done through electrophoretically tranferring the proteins resolved on the gel to nitrocellulose (NC) membrane by the method supplied by NOVEX, referring to the methods described by Towbin et al., 1979; Tsang et al., & Grogl et al., 1985. The proteins were blotted from SDS-PAGE gel to NC membrane (Bio-Rad, USA) by electrophoresis in Tris-glycine transfer buffer (20mM Tris, 144mM glycine, pH 8.3 / 20% methanol) at 4℃ and 100V, for 2 hours[1]. The NC membrane was washed in TBST solution (10mM Tris, 150mM NaCl, 0.05% Tween 20) 3 times, for 10minutes at each time, and was blocked by 1% BSA/TBST (bovine serum albumin/ Tris buffered saline Tweeen 20) at 37℃ for 1 hour. The membrane was washed in TBST solution 3 times, at each time for 10 minutes, and reacted at 37℃ for 1 hour with each antibody serum diluted 1,000 in TBST. The membrane was washed in TBST solution 3 times, for 10 minutes at each time, and reacted

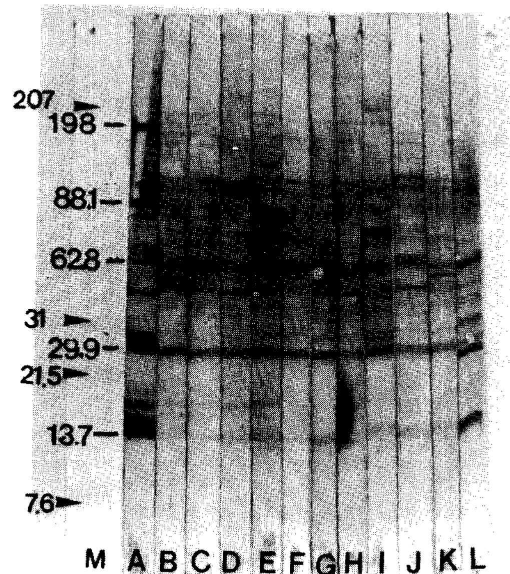

Fig. 2. Immunoblot analyses of *E. hortense*. Immunization of *E. hortense* crude antigen and adjuvant (A), immunodifficiency after oral infection from 1 week to 4 weeks (B, C, D, E), and oral infection from 1 week to 8 weeks (F, G, H, I, J, K, L). Molecular massed in kDa were estimated with Prestained SDS-PAGE Standard markers of Bio-Rad. Closed liner bar mean 207 and 7.6 kDa antigen bands that were observed remarkably.

at 37℃ for 1 hour with alkaline phosphatase-conjugated anti-rat IgG (blotting grade, Sigma, USA) which was diluted, 1:3000. Staining reaction was done for 10 miniutes at room temperature in the dark, using BCIP (5-bromo-4-chloro-3-indoyl phosphate p-toluidine salt, Bio-Rad, USA) and NBT (p-nitro blue tetrazolium chloride, Bio-Rad, USA) as substrates, after washing in TBST solution 3 times, for 10 minutes at each time. staining extent was confrimed after stopping the reaction by blocking vuffer slotion (20mM Tris, 50M EDTA, pH 80). Molecular weight of the polypeptide bands transferred to NC membrane were calculated on the calibration curve prepared from Prestained SDS-PAGE Standards Broad Range (Bio-Rad, USA)[12].

SDS-PAGE of the crude antigen from *E. hortense* showed 46 polypeptide profiles (Fig. 1). Molecular Weight for each band was 200.2,

166.9, 149.7, 144.3, 129.4, 116.1, 107.9, 100.3, 96.8, 93.3, 86.8, 83.7, 80.7, 75, 69.8, 62.5, 56.1, 54.1, 52.2, 50.3, 48.5, 46.8, 45.1, 43.5, 41.9, 40.4, 39.8, 35, 34.5, 28.1, 26.1, 24.3, 23.4, 22.6, 21.8, 20.9, 19.4, 16.9, 15.7, 13.6, 12.6, 11.7, 10.2, 9.4, and 8.2kDa, Among these, protein profiles of 200.2, 107.9, 86.8, 75, 69.8, 46.8, 43.5, 34.5, 20.9, 13.6, 12.6, 11.7, and 8.2kDa were particularly strongly visualized. Specific IgG antibodies analyzed by immunoreaction of the crude antigen and the antibody sera were mainly 198, 123.4, 100.8, 91.1, 88.1, 67.2, 62.8, 58.7, 47.9, 37.8, 35.4, 34.2, 32, 29.9, 18, 15.7 and 13.7kDa proteins. Among these, 198, 123.4, 100.8, 91.1, 88.1, 62.8, 34.2, 32, 29.9, 18, 15.7, 13.7kDa proteins showed strong immunostain (Fig. 2)

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=국문초록=

SDS-PAGE 및 면역이적법에 의한 호르텐스극구흡충 항원분획과 항체반응 양상

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저자들은 호르텐스극구흡충의 항원 단백질과 일부 특이 항원을 알아보기 위해 다음과 같이 실험을 실시하였다. 호르텐스극구흡충 충체를 추출하여 제조한 조항원을 재료로 SDS-PAGE를 실시하여 항원 단백질을 분석하였다. 그리고 흰쥐 (rat)에게 호르텐스극구흡충 피낭유충을 감염시켜 얻은 혈청 항체와, 호르텐스극구흡충 조항원과 면역증강보조제를 함께 투여하여 얻은 혈청 항체를 재료로 하여 EITB를 실시하여, 일부 특이 항원을 규명하였으며 성적은 다음과 같다. 즉, 조항원으로 SDS-PAGE를 실시한 결과는 200.2-8.2kDa까지 46개의 분획이 관찰되었고, 이중에서 특히 강하게 관찰된 단백분획은 200.2, 107.9, 86.8, 75, 69.8, 46.8, 43.5, 34.5, 20.9, 13.6, 12.6, 11.7, 8.2 kDa이었다. 그리고 EITB를 실시하여 분석한 특이 IgG 항체는 198-13.7 kDa까지 17개의 분획을 보였고, 특히 면역염색이 강하게 나타난 분획은 198, 123.4, 100.8, 91.1, 88.1, 62.8, 34.2, 32, 29.9, 18, 15.7, 13.7 kDa이었다.

[대한의생명과학회지 4(1): 73-76, 1998년 6월]

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