Determination of the Affinity and Kinetic Constants for the Interaction between the Human Virus Echovirus 11 and Its Cellular Receptor, CD55*

Susan M. Lea§§, Robert M. Powell¶¶, Tom McKee*¶¶, David J. Evans¶¶, David Brown*¶¶, David I. Stuart*¶¶, and P. Anton van der Merwe§§

From the §Laboratory of Molecular Biophysics, Department of Biochemistry, University of Oxford, Rex Richards Building, South Parks Road, Oxford OX1 3QU, United Kingdom, the ¶¶Virology Research Group, School of Animal and Microbial Sciences, University of Reading, Whiteknights, Reading RG6 5AF, United Kingdom, the *Department of Pathology, Cambridge University, Tennis Court Road, Cambridge CB2 2QP, United Kingdom, the §§Oxford Centre for Molecular Sciences, New Chemistry Laboratory, South Parks Road, Oxford OX1 3QT, United Kingdom, and the §§§MRC Cellular Immunology Unit, Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE, United Kingdom

The biochemical properties of the molecular interactions mediating viral-cell recognition are poorly characterized. In this study, we use surface plasmon resonance to study the affinity and kinetics of the interaction of echovirus 11 with its cellular receptor decay-accelerating factor (CD55). As reported for interactions between cell-cell recognition molecules, the interaction has a low affinity (KD ~3.0 nM) as a result of a very fast dissociation rate constant (koff ~100 M⁻¹s⁻¹, koff ~0.3 s⁻¹). This contrasts with the interaction of soluble ICAM-1 (sICAM-1, CD54) with human rhinovirus 3 which has been reported to have a similar affinity but 10²-10⁶-fold slower kinetics (Casasnovas, J. M., and Springer, T. A. (1995) J. Biol. Chem. 270, 13216–13224). The extracellular portion of decay-accelerating factor comprises four short consensus repeat domains (domains 1–4) and a mucin-like stalk. By comparison of the binding affinity for echovirus 11 of various fragments of decay-accelerating factor, we are able to conclude that short consensus repeat domain 3 contributes ~80% of the binding energy.

Echovirus 11 (EV11) is a member of the genus Enterovirus, family Picornaviridae. While most enterovirus infections either are asymptomatic or result in mild symptoms, EV11 infection has been associated with disorders of the central nervous, cardiac, respiratory, and gastrointestinal systems (2). The interaction of the virus with a specific cell surface receptor is a prerequisite for cell infection, and individual members of the Picornaviridae have evolved to use many different cell surface molecules for attachment and entry (3–6). EV11 strain 207 (hereafter referred to as EV11) is one of several enteroviruses that use decay-accelerating factor (DAF, CD55) as a cell surface receptor; anti-DAF antibodies block infection of cells by these viruses in vitro (7, 8). DAF is a widely expressed glycosylphosphatidylinositol-anchored cell surface glycoprotein that protects cells from damage by autologous complement-mediated lysis (reviewed in Ref. 9) by accelerating the decay of the C3/C5 convertases (hence DAF). DAF is also commonly used as a cell surface receptor by various pathogenic viruses and bacteria, thereby providing an initial site of host-pathogen interaction. Both the complement-regulatory and pathogen recognition functions are located within the membrane distal portion of the molecule which contains four contiguous, 60-amino acid-long short consensus repeats (SCR) (10–12). The SCR domains are connected to the glycosylphosphatidylinositol anchor by a Ser/Thr-rich stalk containing many O-linked oligosaccharides (13, 14). The position of the virus-binding site on DAF appears to vary, even between closely related viruses. For example, SCR domains 2 to 4 (DAF234) are required for binding and infection by most DAF-utilizing picornaviruses (e.g. Coxsackie B3 (15) and echovirus 7 (7, 11, 16)) whereas domains 1 and 2 are sufficient for Coxsackie A21 binding (17).

In the present study, we have used surface plasmon resonance to measure the affinity and kinetics of the binding of soluble DAF fragments, expressed in the yeast Pichia pastoris (16), to EV11. Our results indicate that DAF binds to EV11 with a low affinity and very fast kinetics and that most of the binding energy is contributed by the DAF SCR domain 3.

EXPERIMENTAL PROCEDURES

The expression of soluble DAF in the yeast P. pastoris has been described in detail elsewhere (16). In brief, soluble recombinant DAF (hereafter termed DAF) and DAF domain deletion mutants were expressed with a carboxyl-terminal oligohistidine tag and purified to >95% purity using nickel-nitrilotriacetic acid columns (Qiagen, Dorking, United Kingdom). The proteins migrated at the expected size on reducing and nonreducing SDS-PAGE analysis, indicating that they did not form disulfide-linked multimers (data not shown). The concentrations of the DAF constructs were calculated from the absorption at 280 nm using extinction coefficients (DAF1234 = 36,840 M⁻¹cm⁻¹; DAF234 = 29,870 M⁻¹cm⁻¹; DAF12 = 17,780 M⁻¹cm⁻¹; DAF23 = 17,780 M⁻¹cm⁻¹; DAF34 = 19,060 M⁻¹cm⁻¹) computed from the amino acid composition (Trp, Tyr, and Cys) on the Expasy server (17). The recombinant DAF fragments were judged to be correctly folded by two criteria: they had NMR spectra typical of proteins with SCR domains; they were able to inhibit the binding of a range (~30 in total) of different CD55 monomeric antibodies to erythrocytes (19).

EV11 was grown in confluent monolayers of HT29 human intestinal epithelial cells. Cells were infected at a multiplicity of infection of 5–10, and infection was allowed to proceed for ~18 h. Cells remaining attached

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Royal Society Dorothy Hodgkin Research Fellow. To whom correspondence and requests should be addressed. Tel.: 44 1865 275181; Fax: 44 1865 275182; E-mail: susan@biog.ox.ac.uk.

‡ Supported by the Medical Research Council.

§§ Support for the work in Cambridge was provided by The Wellcome Trust (Grant 043512).

The abbreviations used are: EV11, echovirus 11; DAF, decay-accelerating factor; SCR, short consensus repeats; HRV3, human rhinovirus 3; ICAM-1, intercellular adhesion molecule-1; sICAM, soluble intercellular adhesion molecule-1.

1 T. McKee et al., manuscript in preparation.

2 The DAF constructs are named so that the subscript denotes which SCR domains are present; e.g. DAF234 consists of domains 2, 3, and 4.
to the flasks were removed by scraping, and the medium was frozen and thawed three times. Debris was removed by low speed centrifugation, and the virus was pelleted through a 30% sucrose layer. The pelleted material was then subjected to two rounds of rate zonal centrifugation on 15–45% sucrose gradients. All sucrose solutions contained 10 mM Tris-Cl, pH 7.4. Virus was pelleted from gradient fractions following dilution in 10 mM Tris-Cl, pH 7.4, and finally resuspended in the same buffer. Purified virus was concentrated to 8 mg/ml using Centricon 10-kDa cutoff microcentrators (Millipore, Watford, UK).

Surface plasmon resonance experiments were performed on a BIAcore2000 (BIAcore AB, Stevenage, UK). EV11 was covalently immobilized to the carboxylated dextran matrix on the surface of CMS sensor chips via primary amino groups using the amine-coupling kit (BIAcore AB) as directed (20) with the following modifications. After the activation step, purified virus was at 80 μg/ml in 10 mM sodium formate (pH 3.0) for 5 min. In separate experiments, we showed that incubation at low pH does not affect virus infectivity (data not shown), indicating that such treatment does not disrupt virion structure. This is not unexpected since the virus replicates in the intestine, having passed through the very low pH environment of the stomach. Different levels (1,300–16,200 response units or RU) of virus were immobilized by varying the length of the activation step from 30 s to 5 min. Unless otherwise indicated, all experiments were performed at a flow rate of 40 μl/min at 25 °C using as running buffer commercially obtained (BIAcore AB) HEPES-buffered saline (10 mM HEPES, pH 7.4, 150 mM NaCl, 3.4 mM EDTA, and 0.005% surfactant P20).

## RESULTS

Binding of recombinant DAF to EV11 was measured by injecting the proteins, such as DAF234, through a BIAcore flow cell with EV11 covalently coupled to the sensor surface as well as through a mock-coupled control flow cell in which the sensor surface had been subjected to the coupling reaction in the absence of EV11 (see, e.g. Fig. 1A). The traces, or sensograms, reveal that a background response is observed when DAF234 is injected (Fig. 1A, bar) over the control (mock-coupled) surface. This background response is seen because the BIAcore measures the refractive index near the sensor surface and therefore detects any changes in bulk refractive index of the injected sample. It does not represent nonspecific binding. A much larger response is observed when DAF234 is injected over the EV11-coupled flow cell. Subtraction of the sensogram obtained in the control flow cell from the latter sensogram gives the actual binding response (Fig. 1A, inset). Inspection of these corrected sensograms reveals that the kinetics of DAF234 binding is rapid; binding reached the theoretical maximum if it is assumed that there are 60 binding masses of 8

### Table I

| Construct | \( K_D \) | ΔG° |
|-----------|----------|-----|
| DAF1234   | 3.4 ± 0.4* | −7.5 |
| DAF234    | 3.0 ± 0.04 | −7.6 |
| DAF23     | 9.3 ± 0.8  | −6.9 |
| DAF34     | 19.2 ± 0.4 | −6.5 |

* Mean ± SD of 5 to 10 independent experiments.

Because the full binding activity resided in DAF234 (Refs. 11 and 16 and this study), this construct was used for a more detailed analysis. Measurement of the affinity at different temperatures revealed that there was a decrease in the affinity as the temperature increased from 15 to 40 °C (Table II). This indicates that the interaction is exothermic at physiological temperatures. A (van’t Hoff) plot of In(1/KD) versus 1/T was not linear (data not shown), indicating that the enthalpy varies with temperature.
sociation ($k_{\text{on}}$) rate constants were obtained by simultaneously fitting numerically integrated rate equations derived from the simple Langmuir binding model ($A + B \rightarrow AB$) to all the sensorgrams (global analysis). Excellent fits were obtained ($\chi^2$ values were $0.87 \pm 0.41$; see, for example Fig. 1B), providing the $k_{\text{on}}$ and $k_{\text{off}}$ values shown in Table III (once again, these values are the average after five repeated experiments with the errors reflecting the variation between individual experiments). The same $k_{\text{on}}$ and $k_{\text{off}}$ values were obtained at different flow rates (data not shown) and with two different levels of virus immobilized to the sensor surface, demonstrating that binding under these condition is not limited by mass transport. Furthermore, the calculated $K_D$ values agree well with the $K_D$ determined by equilibrium binding (Table I).

**DISCUSSION**

The affinity constants reported here for DAF binding to EV11 are much lower than affinities measured for biological interactions involving soluble macromolecules such as hormones, cytokines, and antibodies but are typical of interactions between cell-cell recognition molecules (22). This is appropriate since the interaction between echovirus virions and cell surface DAF is likely to be highly multivalent; the virus surface presents 60 identical sites due to the symmetry of the capsid.

There is one other reported study of the affinity and kinetics of an intact virus with its receptor (1). Although Casasnovas and Springer reported a similar affinity for sICAM-1 binding to HRV3, a virus belonging to the same family as EV11, the kinetics were much slower (see below). A higher affinity ($K_D$ 40–400 nM) has been reported for the binding of the human immunodeficiency virus envelope glycoprotein gp120 to its cell surface receptor CD4 (23). The affinity of DAF$_{234}$ to EV11 are essentially the same, whereas DAF$_{23}$ and DAF$_{34}$ bind with affinities between 3- and 6-fold lower (Table I). Comparison of the calculated binding energies of all these constructs indicates that SCR domain 3 contributes ~80% of the binding energy (Table I). In this context, it is noteworthy that two of the antibodies that most effectively inhibit in vitro infection by the closely related echovirus 7 (1H4 and 3D11) have been shown to bind domain 3 (7, 11). Furthermore, *Escherichia coli* Dr and related adhesins have also been shown to bind DAF SCR domain 3 (12).

The $k_{\text{on}}$ for the interaction between EV11 and DAF$_{234}$ falls within the usual range for macromolecular interactions (22), while the $k_{\text{off}}$ is unusually fast, a feature characteristic of interactions between cell-cell recognition molecules (22). These results differ from the findings of Casasnovas and Springer (1), who reported kinetic constants for the sICAM-1/HRV3 interaction that were two to three orders of magnitude slower ($k_{\text{on}}$ 130–2450 M$^{-1}$s$^{-1}$ and $k_{\text{off}}$ 0.0013 s$^{-1}$). Casasnovas and Springer (1) proposed that the unusually slow $k_{\text{off}}$ rate was either due to the relatively inaccessible nature of the sICAM-1 binding site on HRV3 (the tip of sICAM-1 binding in a depression on the surface of the virus capsid (24)) or because of a requirement for conformational change in the HRV3 binding site before sICAM-1 binding can occur. In contrast, we show that a domain in the “middle” of DAF is crucial for binding to EV11, making it unlikely that DAF binds in a similar way, i.e. to a deep depression on the surface of EV11. The binding of EV11 to DAF can also be fully described using a simple 1:1 binding model, consistent with the “docking” of two preformed sites. In contrast, the sICAM-1/HRV3 interaction exhibited biphasic binding kinetics, suggesting a more complex binding scheme. Finally, analysis of the temperature dependence of the sICAM-1/HRV3 interaction indicated it was endothermic, which is somewhat unusual for a protein-protein interaction, whereas our analysis of the EV11/DAF interaction indicates that it is exothermic.

In conclusion, we have shown that DAF binds to EV11 with a low affinity and very fast kinetics. These binding characteristics are typical of the molecular interactions mediating cell-cell recognition but differ substantially from the sICAM-1/HRV3 interaction, the only other whole virus cellular-receptor interaction studied to date (1). We also show that the bulk of the binding energy is contributed by the third SCR domain.

**Acknowledgment**—We thank for Christine Harley for assistance in virus preparation.

**REFERENCES**

1. Casasnovas, J. M., and Springer, T. A. (1995) *J. Biol. Chem.* 270, 13216–13224
2. Melnick, J. L. (1996) in *Fields’ Virology* (Fields, B. N., Kuhn, D. M., Hewley, P. M., Chanock, R. M., and Melnick, J. L., eds) Lipincott-Raven Publishers, Philadelphia, PA
3. Bernhardt, G., Harber, J., Zibert, A., Decrombrugghe, M., and Wimmer, E. (1994) *Virology* 203, 344–356
4. Greve, J. M., Davis, G., Meyer, A. M., Forte, C. P., Connolly-Yost, A., Marler, C. W., Kamarck, M. E., and McClelland, A. (1989) *Cell* 56, 839–847
5. Staunton, D. E., Merluzzi, V. J., Rothlein, R., Barton, R., Martin, S. D., and Springer, T. A. (1989) *Cell* 56, 849–853
6. Bergelson, J. M., Cunningham, J. A., Draguet, G., Kurt Jones, E. A., Krithivas, A., Hung, J. S., Horwitz, M. S., Crowell, R. L., and Finberg, R. W. (1997) *Science* 275, 1320–1323
7. Bergelson, J. M., Chan, M., Solomon, K. R., St John, N. F., Lin, H., and Finberg, R. W. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 6245–6248
8. Ward, T., Pipkin, P. A., Clarkson, N. A., Stone, D. M., Minor, P. D., and Almond, J. W. (1994) *EMBO J.* 13, 5070–5074
9. Nicholson Weller, A., and Wang, C. E. (1994) *J. Lab. Clin. Med.* 123, 485–491
10. Coyne, R. E., Hall, S. E., Thompson, E. S., Arce, M. A., Kinoshiita, T., Fujita, T., Anstee, D. J., Rosse, W., and Lublin, D. M. (1992) *J. Immunol.* 149, 2906–2913
11. Clarkson, N. A., Kaufman, R., Lublin, D. M., Ward, T., Pipkin, P. A., Minor, P. D., Evans, D. J., and Almond, J. W. (1995) *J. Virol.* 69, 5497–5501
12. Nowicki, B., Hart, A., Coyne, K. E., Lublin, D. M., and Nowicki, S. (1995) *J. Exp. Med.* 176, 2115–2121
13. Medof, M. E., Lublin, D. M., Holers, V. M., Ayers, D. J., Getty, R. R., Leykam, J. F., Atkinson, J. P., and Tykocinski, M. L. (1987) *Proc. Natl. Acad. Sci. U. S. A.* 84, 2007–2011
14. Caras, I. W., Weddell, G. N., Davitz, M. A., Nussenzweig, V., and Martin,
Echovirus-CD55 Binding Analysis

D. W. J. (1987) Science 238, 1280–1283
15. Bergelson, J. M., Mohanty, J. G., Crowell, R. L., St. John, N. F., Lublin, D. M., and Finberg, R. W. (1995) J. Virol. 69, 1903–1906
16. Powell, R. M., Ward, T., Evans, D. J., and Almond, J. W. (1997) J. Virol. 71, 9306–9312
17. Shafren, D. R., Dorahy, D. J., Ingham, R. A., Burns, G. F., and Barry, R. D. (1997) J. Virol. 71, 4736–4743
18. Appel, R. D., Bairoch, A., Hochstrasser, D. F. (1994) Trends Biochem. Sci. 19, 258–260
19. Daniels, G. L., Green, C. A., Powell, R. M., and Ward, T. (1997) Transfusion 38, 332–336
20. Karlsson, R., Michaelsson, A., and Mattsson, L. (1991) J. Immunol. Methods 145, 229–240
21. Schuck, P. (1996) Biophys. J. 70, 1230–1249
22. Van Der Merwe, P. A., and Barclay, A. N. (1994) Trends Biochem. Sci. 19, 354–358
23. Fischer, P. B., Collin, M., Karlsson, G. B., James, W., Butters, T. D., Davis, S. J., Gordon, S., Dwek, R. A., and Platt, F. M. (1995) J. Virol. 69, 5791–5797
24. Olson, N. H., Kolatkar, P. R., Oliveira, M. A., Cheng, R. H., Greve, J. M., McClelland, A., Baker, T. S., and Rossmann, M. G. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 507–511