STUDENTS' INTERPRETATION OF EXTERNAL REPRESENTATIONS IN BIOCHEMISTRY

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Abstract

The literature is very expansive when it comes to discussing the role of external representations (ERs), such as diagrams, in the learning and teaching of sciences such as physics, chemistry, geography and astronomy. The consensus is that ERs are both necessary and useful instruments for the conveying of scientific concepts and ideas. However. researchers also concur that the way students *interact* with and *use* ERs is of particular educational importance since reading diagrams can cause reasoning and conceptual difficulties (e.g. Cheng, Lowe & Scaife, 2001). In biochemistry, hardly any research has been done on student difficulties with ERs (e.g. Schönborn, Anderson & Grayson, 2003, 2002a). This study investigated student difficulties with five ERs of antibody-antigen binding using a four-level research framework (Grayson, Anderson & Crossley, 2001). Early identification and classification of the difficulties revealed four general categories of difficulties, namely the process-type, structural-type, DNA-type and binding-type difficulty. Deeper analysis of the data revealed at least one cognitive processing mechanism corresponding to each of the difficulties. Investigation of these processes allowed us to suggest possible sources for the Some of the cognitive processes that were explored included: classified difficulties. inappropriate transfer, surface-level reasoning, inadequate translation and inability to disentangle concepts. Using this information, future work will focus on using the source of each difficulty to inform the design of remediation techniques, guidelines for ER design, and decisions as to the effectiveness of each ER.

Introduction

Graphical external representations (ERs) refer to photographs, images, drawings, maps, diagrams, graphs and other graphics that are found in textbooks and educational resources such as software programs and the internet (e.g. Richardson & Richardson, 2002; Cox & Brna, 1995; Scaife & Rogers, 1996). The literature contains much information about the beneficial roles of ERs, such as diagrams and other pictorial images, in the learning and teaching of science (e.g. Peña & Quílez, 2002). However, many of these studies also suggest that ERs have the potential to cause reasoning and conceptual difficulties (e.g. Pintó & Ametller, 2002) that stem largely from the graphical language that is used within ERs to convey an idea or concept. To expand on this statement, unlike linguistic and verbal representations (e.g. spoken English or written Spanish), there is no self-standing or standardized *language* that one can apply exclusively to diagrams per se (e.g. Blackwell, 2001). For example, consider the science of biochemistry where there can be multiple ERs for communicating a single phenomenon such as antibody structure and its interaction with antigen. Such ERs can contain numerous graphical markings and signs that can be both abstract and idiosyncratic. For this reason, the viewer of the ER has to sometimes contend with markings that may be beyond their current or past experience. Thus it is not surprising that the background knowledge of the student will also play a role when reading an ER (e.g. Pintó & Ametller, 2002; Lowe, 1996). Authors such as Roth (2002) have referred to this issue as a type of 'chicken and egg' dilemma whereby one needs to possess the conceptual understanding in order to understand an ER while at the same time one needs to understand the signs used by the ER in order to acquire the conceptual understanding.

The literature also suggests that the mechanisms of visualization and reasoning that the viewer employs affect the way the ER is interpreted and can cause difficulties (Schönborn et al., 2003; van Dusen, Spach, Brown & Hansen, 1999). There appears to be only a few studies that have attempted to understand the cognitive processes that underpin interaction with ERs in science (e.g. Blackwell, Whitley, Good & Petre, 2001; Scaife & Rogers, 1996; Zhang & Norman, 1994). In Schönborn et al. (2003) we have suggested that students have to deal with at least three factors during interaction with an ER: the actual external graphical markings on the ERs, the conceptual understanding that the viewer brings to the ER, and the reasoning mechanisms that the viewer employs to process the ER.

The objectives of the current study were two-fold. One aim was concerned with filtering out and identifying difficulties that students have with the interpretation of selected ERs used in biochemistry and classifying them on an appropriate research framework. Following this, the second aim was to consider the cognitive processing associated with each of the identified student difficulties and to use this information to make suggestions as to the source of the difficulties.

Methods and Rationale

Student Samples and External Representations

The research reported in this paper was done from 2000 to 2002 and was concerned with investigating 166 undergraduate (2nd and 3rd year) biochemistry students' interaction with five ERs which all depicted the structure of immunoglobulin G (IgG) and its primary interaction with antigen (Fig. 1). The students who participated in the study consisted of the populations presented in Table 1. One hundred and thirty of the total participants were second-year biochemistry students who had completed a module on immunology and 21 were third-year students who had studied the same module the previous year. All of these students responded to written probes. In addition, 10 second-year students, 6 students with varying domain-specific knowledge in different years of study and a further 9 third-year biochemistry majors participated in interviews. The ERs used in the study are designated numbers 1-5 (Fig. 1 & Table 1).

 Table 1
 Student populations showing how data was collected from each group

Student	Year of	Responded to	Free-response	Focused	Participated in	ER under study
population	study	written probes	type probes	type probes	clinical interviews	(Fig. 1)
70	2^{nd}	Yes	Yes			1
21	3^{rd}	Yes	Yes			1
45	2^{nd}	Yes	Yes			2
69	2^{nd}	Yes	Yes			3
23	2^{nd}	Yes		Yes		2
13	2^{nd}	Yes		Yes		1
10	2^{nd}				Yes	1&2
6	Mix				Yes	4 & 5
9	3^{rd}				Yes	4 & 5



Fig 1 Five ERs (1-5) showing the structure of an IgG antibody molecule and/or its interaction with antigen. (1) Tertiary structure showing V and C regions, (2) Tertiary structure in chain form, (3) Three-dimensional structure; one of the H chains is shown in dark red, the other in dark blue. One of the L chains is shown in light red, the other in light blue. A carbohydrate unit attached to a C_H2 domain is shown in yellow, (4) Electron micrograph (x 1 000 000) of complexes formed on mixing divalent hapten with anti-hapten antibodies. The hapten links together the Y-shaped antibody molecules to form trimers (A), and pentamers (B), (5) Spacefilling model showing Fab antilysozyme and lysozyme molecules fitting snugly together. Antibody heavy chain, blue; light chain, yellow; lysozyme, green with its glutamine 121 in red. Fab and lysozyme models are also shown pulled apart in the second frame. ((1) From Bohinski, R.C., 1987, (2) from Silverton et al., 1977 and adapted in Bohinski, R.C., 1987: both reproduced by permission of Pearson Education, Inc., Upper Saddle River, NJ 07458; (3) From Silverton et al., 1977 and adapted in Stryer L., 1995, (4) From Valentine, R.C. and Green, N.M., 1967 and reproduced in Roitt, I.M., 1997: Reproduced by permission of Academic Press)

Investigation of Students' Interpretation of the ERs

Student understanding of the ERs was investigated by means of written tests and interview questions. In all cases students were supplied with both the ER and its caption when answering questions. Captions supplied to the participants were as per those provided for Fig. 1. For the written questions, interpretation of only a single diagram was required at a single test time. Written questions were given to all second-year students and one sample of third-year students (Table 1). The second-year sample of students answered both the free-response questions as well as the more focused questions, whereas the third year sample answered only free-response types. A total of three different sets of written questions were administered six times to the groups of students. All the written questions followed a similar format. The written questions were given to students either at the commencement of lectures, laboratory sessions or tutorials and were designed to take approximately 5-10 minutes to answer.

More detailed information was obtained by means of clinical interviews (e.g. Posner & Gertzog, 1982). Participants were asked about their understanding and interpretation of the ERs. The general interview methods for gathering information about student understanding proposed by Cohen, Manion and Morrison (2000), Rubin and Rubin (1995), White and Gunstone (1992), Lincoln and Guba (1985) and Posner and Gertzog (1982) were used. The interviews followed a natural, neutral, semi-structured and flexible approach (e.g. Simonneaux, 2000; Sumfleth & Telgenbüscher, 2001). If no significant patterns of reasoning or conceptual understanding emerged at the time, the interviewer asked specific questions that were similar to those used in the more focused written questions. The interviews lasted about one hour each and were audio taped and transcribed. Transcripts were qualitatively analysed in order to identify conceptual and reasoning difficulties (e.g. Kindfield, 1993/1994). In particular, the interview data was used to elaborate several difficulties which had emerged from the written data, as well as exposing some unexpected difficulties.

Initially, only free-response type probes were used to collect data during the written tests and interviews. This ensured that students were free to respond with what came to mind and reveal their understanding of the ER, without being led into giving a particular answer. As more insight was gained into the nature of each difficulty, the probes became increasingly more focused, and more specific for each difficulty.

Analysis of the Data

The data analysed in the study consisted of written responses, videotapes, audio-transcripts and student-generated diagrams, all of which provided information on the nature of student difficulties. In addition, important information about the nature of the cognitive processes (Glynn, 1997) associated with each student difficulty were obtained from viewing videotapes of students' drawing behaviour, such as beginning, annotating or modifying a diagram (e.g. Kindfield, 1993/1994; Lowe, 1993), and gesturing such as "pointing" or "indicating" (e.g. Sumfleth & Telgenbüscher, 2001) when interacting with the diagram. In total, 134 student-generated diagrams were analysed.

To delve deeper into the nature of each difficulty, student responses to written questions and interviews were subjected to an iterative and inductive analysis process (McMillan & Schumacher, 1993) in which the categories of student difficulties emerge from the data themselves, rather than being pre-determined (e.g. Anderson & McKenzie, 2002). A method of triangulation was also employed via a multi-method approach for collecting data. In light of this, written probes, interview probes and other continuous observation methods were utilised. Furthermore data was not only collected from single, but multiple samples of participants, as well as from at least two different time frames (e.g. Verma & Mallick, 1999; Anderson & Arsenault, 1998; see Table 1). Moreover, new methods were sometimes especially developed to carry out evolving objectives of the study. Some of these methods are dealt with in other papers (e.g. Schönborn et al., 2002b).

During the sorting of students' responses, the nature of the categories, and hence the underlying difficulties, became clearer and sub-categories emerged (Lincoln & Guba, 1985). The four-level methodological framework of Grayson et al. (2001) was used for the classification of difficulties according to how much information and understanding the researchers had about the nature of each difficulty. According to this framework, difficulties that are well established across varying contexts (e.g. different courses, student groups and

institutions) and for which there is a stable description are classified at Level 4 or established, while those that are known to researchers but have not been extensively explored are classified at Level 3 or partially established. Level 2 difficulties are those that are suspected on the basis of teaching or learning experience. Difficulties that emerge unexpectedly from analysis of the data are classified at Level 1. In each case the incidence of the difficulty was calculated and recorded.

Results and Discussion

The results of the study revealed four main categories of student difficulties, which we termed the *process-type*, *structural-type*, *DNA-type* and *binding-type*. For the purposes of this paper, only the four general categories will be discussed and the sub-categories that emerged from the data will be ignored. Also, only the highest incidences recorded for each difficulty as well as their highest level of classification on the Grayson et al. (2001) framework are presented. Possible sources of the difficulties and the nature of the cognitive processing associated with each difficulty are also discussed. For the purposes of clarity, each student quotation, or student-generated diagram, is labelled with the corresponding ER number (See Fig. 1; ER 1-5.) used by the student when generating that particular response.

Process-Type Difficulty

The *process-type* difficulty was characterised by students viewing the ERs (Fig. 1) as representing various complex processes (e.g. secondary responses of the immune system), rather than a simple primary and non-covalent binding interaction between antibody and antigen molecules. Consider the following two student quotations and an example of a student-generated diagram (Fig. 2) that showed this difficulty:

"...It [1] shows where the antigens attack / go through the antibody. Shows us that antigens enter into the V regions first and then move into the C regions." [1]

S: There was probably interaction between the antibody and the lysozyme...yeah [points to 5] and that interaction caused the glutamine to break down and join with the antibody [points on 5]. The antibody is actually working on the glutamine [circular pointing on 5]... [5]



Fig 2 Student generated diagram showing a fusion of antibody and antigen resulting in digestion of antigen rather than a non-covalent interaction

In consideration of the first quote above, due to the portrayal of antigen as an arrow-like entity in ER 1 (Fig. 1), students may have thought that antigen actually entered the antibody structure itself. The second student above thought that ER 5 (Fig. 1) was showing the glutamine 121 amino acid residue as being actively "broken down" by the antibody, probably due to the use of the same red colour used to represent both the contact regions between amino acids as well as the glutamine 121 molecule itself. A similar interpretation of ER 5 (Fig. 1) is shown in Figure 2 in which a student sketched an antibody-antigen interaction as a type of fusion resulting in antigen degradation. This biochemical process would be characteristic of a secondary immune response and not a primary response. As discussed by

Cheng et al. (2001) and Henderson (1999), overemphasis of the graphical markings on a display during processing may lead to the construction of superficial and unwarranted mental models. It follows in the present study that students' formulation and construction of ideas such as the antibody itself being responsible for elimination or digestion of the antigen may be a consequence of this. The above results, and other data not shown in this paper, suggest that students often relied heavily on perceptual organisation during processing (e.g. Olivier, 2001), and were employing surface-level reasoning rather than deep-level reasoning (Chi, Feltovich & Glaser, 1981). In other words, students made extensive use of perceptual cognitive operators to make sense of an ER, and did not link such information to their conceptual understanding when interpreting the ER. The result of this was an over reliance on the visual signs, which resulted in the construction of scientifically flawed concepts and mental models. Furthermore, this reasoning was worsened when perceptual groupings were filtered by poor or flawed conceptual knowledge (e.g. Olivier, 2001; Henderson, 1999). The results also showed that the manner in which an ER was processed depended largely on the knowledge that the user brought to the ER. As shown by Cheng et al. (2001) and Roth (2002), we too found that reasoning with an ER was indeed modulated by this knowledge.

The process-type difficulty initially emerged unexpectedly from the free-response data. Its reemergence during focused written and oral probes allowed it to be classified at Level 3 as partially established (Grayson et al., 2001). The highest recorded incidence of this difficulty was 70%.

Structural-Type Difficulty

Students who showed the *structural-type* difficulty erroneously interpreted the way in which various structural features of IgG were externally represented on the ERs. These included features representing disulfide bonds, variable and constant amino-acid regions, light and heavy chains, α -carbons, antigen molecules, antigen binding sites and level of protein structure. Evidence of this difficulty was provided by the following data:





Fig 3 Student generated diagrams showing the 'black lines' divorced from the black 'spheres' (left), and interpretation of antibody as 'spheres' only (right)

"The coloured areas represent different areas... of red blood cells." [1]

"...The yellow parts might be a metal and the other elements bound to it to form a ligand to give some oxidation state." [3]

The first quote above shows an erroneous student interpretation of the α -carbon backbone represented by ER 2 in Fig. 1. The "small circles" were thought of as oxygen atoms rather than amino acid centres. Fig. 3 shows two students' diagrammatic depictions of antibody structure after interpretation of ER 1 in Fig. 1. Instead of incorporating both the "spherical

shapes" and "black lines" into one diagram, meaning that as in ER 1 they visualized them as together representing traits of antibody structure, these students divorced the visual marks from one another and either used lines or spheres in their drawing. A similar observation was made with another student (second quote) who suggested that the "spherical shapes" in ER 1 were red blood cells. During interpretation of ER 3, a student (see third quote) thought the yellow "spheres" representing carbohydrate units were representative of a metallic oxidation state. In view of this visual processing, the results suggested that some students were unable to translate between different representations of an identical construct. In relation to this, they found it difficult to map between one representation system and another, probably because students treated each as a unique situation (e.g. Ainsworth, Bibby & Wood, 1998). This suggests that some students did not seem to possess a single and integrated mental model of the phenomenon of antibody structure. Instead, there appeared to be many alternative mental models available to a student depending on the function of the cognitive task (Gobert & Clement, 1999).

The highest recorded incidence for the structural-type difficulty was 70%. It was classified as partially established (Level 3) after it had originally emerged from our analysis at Level 1, and then at Level 2 (Grayson et al., 2001).

DNA-Type Difficulty

Interpretation of the ERs as a form of DNA structure or processing constituted the *DNA-type* difficulty. The following two student quotations are examples of this type of difficulty:

"This is meant to represent a DNA molecule, leading strands and a lagging strand of DNA..." [1]

"This diagram is meant to show how DNA molecule IgG fights the antigen. It has an antigen binding site where antigen binds and will be killed after it is locked by this molecule" [3]

The quotes above suggest that some students performed inappropriate transfer (Salomon & Perkins, 1989) when interpreting the ERs. Since a DNA replication fork, with lagging and leading strands, looks similar in appearance to the typical 'Y' shape of antibody heavy and light chains (see Fig. 4 below) and the helical nature of DNA (see ER 3 in Fig. 1), it is possible that some students were inappropriately transferring their knowledge of DNA elongation or processing to the context of IgG structure.



Fig 4

A schematic diagram showing antibody structure (left) and a typical diagram showing DNA replication (right)

The above inappropriate transfer may have been a consequence of surface-level reasoning in which only the surface features or graphical markings of the diagram are considered (e.g. Chi et al., 1981). Furthermore, the results showed an inappropriate "superimposing" of one concept upon another when students interpreted the ERs. It is clear from the above two quotations that these students inappropriately fused immunology knowledge with DNA-related knowledge. Like Grayson (submitted) has shown in the context of students' understanding of electric circuits in physics, it is possible that the above students were unable

to disentangle distinctively different concepts from one-another when interpreting certain ERs.

The DNA-related difficulty was suspected after its emergence during written free-response probes. Its recurrence in interviews allowed it to be classified at Level 3 as partially established. It was found that the highest recorded incidence of its presence was 40%.

Binding-Type Difficulty

Students who manifested what we termed the *binding-type* difficulty suggested that the IgG antibody had only *one* possible binding site for the antigen and that an antigen was accommodated within the entire V-shaped 'cleft' of the Y-shaped antibody, instead of sharing an interaction with the antibody's *two* variable binding domains. Pictorially, this difficulty can be represented as follows (Fig. 5):



Fig 5 Diagram demonstrating the binding-type difficulty. Antigen is erroneously interpreted as being accommodated within the entire 'V-cleft' (thick arrow) instead of at one of the two variable sites (thin arrow)

What was revealed *prior* to students' interaction with the ERs, was the concrete and ingrained nature of a particular conceptual schema. It was found that *all* the students in this part of the study, just like some textbooks and research manuscripts (e.g. Roitt, 1997; Amit, Mariuzza, Phillips & Poljak, 1986), readily used the "lock-and-key" analogy, normally used to explain enzyme-substrate interactions, to explain antibody-antigen interaction. The use of this analogy was demonstrated by the following student responses given prior to student interaction with any of the ERs:

S: ...the binding of the antibody to the antigen and how there was a lock-and-key method. And, yeah... it is almost like specific... the antibody binding to the antigen. They have to have similar shapes in order for the binding to take place.

S: It [antigen] is kind of like an upside down pyramid that tries to fit into that Y-shape."

Our view of the existence of an ingrained schema causing the binding-type difficulty among some students, was well supported by various student generated diagrams like that given in Figure 6 below for ER 4 in which the student uses a "lock and key" model to fit the antigen into the cleft between the 3 antibodies, rather than at the variable regions of each antibody.



Fig 6 A student-generated diagram showing interpretation of a trimer (ER 4) as incorporating a single antigen fitting into the 'V-clefts' of three antibody structures.

A possible source of the binding-type difficulty is as follows. Since most textbooks (e.g. Ritter, 1996) only give *single*-enzyme-*single*-substrate lock-and-key analogies, the possibility arises that students may think that a *single* immunoglobulin G molecule can only bind to a *single* antigen molecule. The student-generated diagram (Fig. 6) showing interaction between IgG antibody and antigen, illustrates that this analogy was indeed erroneously understood and depicted. Clearly, the above student-generated diagram serves as an example of how some students thought that the entire 'V-cleft' accommodated the antigen, instead of each binding site being viewed as its own "lock-and-key" system. With regard to the binding-type difficulty, the study illustrated that some students' employed inappropriate analogical reasoning (Sumfleth & Telgenbüscher, 2001) when reading the ERs.

During interviews, the binding-type difficulty emerged unexpectedly at an incidence of 67%, and was therefore classified at Level 1, or as unanticipated. In future, the difficulty shall be suspected at Level 2.

Conclusions and Implications

This study has identified and classified four general categories of difficulties with students' interaction with ERs used in biochemistry. We have also shown how deeper analysis of the data on student difficulties has allowed us to identify some of the cognitive processes associated with students' interaction with the ERs. This in turn, has yielded an analysis of the possible sources for the respective difficulties. We suggest that understanding the source of a particular difficulty is pivotal if we are to make any beneficial inroads into: ER design, learning and teaching with diagrams, suggesting which ERs are of optimum use, and remediation of difficulties with ERs (e.g. Peña & Quílez, 2002; Brna, Cox & Good, 2001). Such issues will be the focus of future research in which modelling the cognitive processes associated with students' interaction with ERs will be of central importance. Once this has been accomplished, researchers would be in a position to suggest how skills for interpreting ERs could be learnt by students and taught by instructors.

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