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BIOCHEMISTRY STUDENTS' DIFFICULTIES WITH THE INTERPRETATION OF TEXTBOOK DIAGRAMS

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Abstract

Very little research has been reported on student difficulties with biochemistry, let alone with diagrams in biochemistry textbooks. Diagrams are external representations that help learners build mental models or internal representations of phenomena. Such models allow for comprehension, integration and connection of concepts and are, therefore, invaluable teaching and learning tools. Sometimes, however, students experience difficulties with the interpretation of diagrams and this may have a negative effect on their learning of science. This paper reports on three general categories of difficulties encountered by students with the interpretation of a diagrammatic representation of the structure of an immunoglobulin G (IgG) antibody molecule. The difficulties were identified and classified using the four-level framework of Grayson *et al*. (in press). Possible sources of the difficulties, their incidences, as well as implications of the results for teaching and learning are also discussed.

Introduction

Over the past few decades, a major focus of science education research has been the identification of students' reasoning and conceptual difficulties when learning science. This has especially been the case in physics (e.g. Harrison *et al*., 1999), chemistry (e.g. Garnett *et al*., 1995) and biology (e.g. Marek, 1986). In biochemistry, however, few such difficulties, which can hinder effective learning, have been identified. Limited results have been published by Fisher (1985) on student difficulties with protein synthesis, while Anderson and Grayson (1994) and Anderson *et al*. (1999) have identified a range of conceptual and reasoning difficulties in the area of metabolism. Recently, these workers also designed a methodological framework for the identification and classification of

such difficulties (Grayson *et al*., in press). In the present paper we apply this framework to the identification of various reasoning and conceptual difficulties that students show when using textbook diagrams to understand biochemistry.

The extensive use that science educators make of diagrams suggests that they are perceived to be invaluable tools for learning and teaching. This is because they are extremely useful for clarifying and connecting concepts, for the construction of useful mental models and for the mental representation of text (Winn, 1991; Schnotz, 1993). What has, however, not always been acknowledged is that the interpretation of diagrams is a highly cognitively demanding task (Lowe, 1996), which can lead to numerous alternative conceptions and incorrect ways of reasoning that tend to be resistant to change (Hill, 1988; Wheeler and Hill, 1990). Although a large body of literature exists on the general use of, and difficulties with, diagrams in other scientific fields (e.g. Johsua, 1984; Winn, 1988; Mayer, 1989; Lowe 1999), only a limited number of research-type reports have been published on the effectiveness of diagrams in the field of biochemistry. For instance, Nũnez de Castro and Alonso (1997) have shown that textbook diagrams of enzyme-catalysed reactions are often too simplified and exclude essential chemical steps. Further, Menger *et al*. (1998) have reported that the presentation of micelles in texts is not always accurate, especially when they are presented as 'spokes of a wheel'. Finally, our group has presented preliminary results that suggest that some students misinterpret diagrams of the electron transport chain occurring in the mitochondria (Crossley *et al*., 1996).

The aim of this study was to identify and classify students' difficulties with the interpretation of different types of diagrammatic representations of the structure of an immunoglobulin G (IgG) antibody molecule. In this paper we present evidence for three general categories of difficulties shown by students when interpreting one of the diagrams, and suggest possible sources and potential ways of remediating them.

Methodology

The study was undertaken in 2000 with 130 second-year and 21 third-year biochemistry students studying immunology. Written and oral questions were used in post-tests and interviews respectively, to probe for students' understanding. Ten one-hour long audio taped interviews were conducted and transcribed. The textbook diagram from Bohinski (1987), p.161, figure 4-46 (c)

used for the module had been supplied to students by means of printed lecture notes. Students were supplied with both the diagram and its caption (Fig. 1) when answering the post-tests and interview questions. The diagram represents the V (Variable) and C (Constant) regions of the tertiary structure of an immunoglobulin G (IgG) molecule by using different coloured shading (see Fig. 1). It shows the two heavy (γ) and two light (κ) polypeptide chains connected by interchain disulfide bonds. The characteristic 'Y' shape of the IgG molecule in its $\kappa_2\gamma_2$ structural designation is shown. The bivalent nature of the IgG molecule is represented by means of the inclusion of two antigen molecules indicating their possible and specific interaction with the variable regions at the antigen-binding domain.

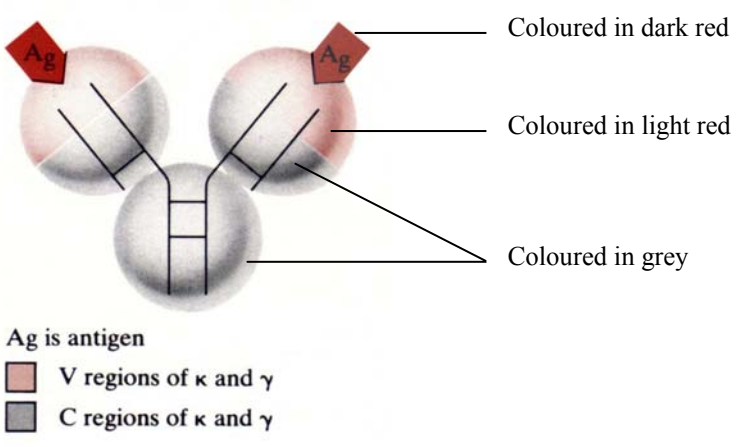

Figure 1: Schematic illustration of the three-dimensional structure of an IgG antibody molecule. Tertiary structure showing V and C regions. (From Bohinski (1987), Modern Concepts in Biochemistry 5th ed., p.161)

Data analysis

Student responses were analysed qualitatively by inductive analysis (McMillan and Schumacher, 1993), where interpretation patterns were sorted into categories according to the nature of the difficulty displayed (Lincoln and Guba, 1985, p.340-344). The four-level research framework of Grayson *et al*. (in press) was used for the classification of difficulties according to how much insight the researchers had into each difficulty. Thus difficulties that are well established across varying contexts 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 merely suspected on the basis of teaching or learning experience, while difficulties, which emerge unexpectedly from analysis of the data, are classified at Level 1. Since the researchers found no documented research on student difficulties with the interpretation of antibody diagrams, the written and interview questions were

designed to investigate various Level 2 suspected difficulties as well as any potential Level 1 difficulties that emerged from the student data. In each case the incidence of the difficulty was calculated and recorded.

Probe design

Initially, only free-response type questions were used to collect data. Examples of these, answered by both second and third-year students were:

a) Describe everything you think this diagram represents or shows.
b) Is there anything in the diagram that you don't understand or find confusing? If so specify.

As more insight was gained into each difficulty the questions became less free-response in nature and increasingly more focused on, and more specific for each difficulty. Only the second-year students responded to these and typical probes included the following:

c) With the aid of separate sketches, explain which part of the diagram represents:
   i) The antibody ii) The antigen
d) What do the various black lines on the diagram represent?
e) What do the coloured areas represent?
f) How do the coloured areas relate to the black lines on the diagram?
g) Use the diagram to explain what happens to the antigen (i.e. what does it do?) after it has bound to the antibody?

Even more specific information about each difficulty was obtained by means of clinical interviews conducted with ten second-year students. Interview strategies were similar to those outlined by White and Gunstone (1992), Posner and Gertzog (1982), Cohen *et al*. (2000) and Lincoln and Guba (1985). The interview data was used to elaborate several suspected difficulties, which had emerged from the written responses, as well as to expose unanticipated Level 1 difficulties.

Results and Discussion

Analysis of student response data revealed the existence of three general categories of difficulties as well as several sub-categories. This paper will focus only on the three general categories namely, *process-type* difficulties, *structural-type* difficulties, and *DNA-related* difficulties. These are discussed below.

Process-type difficulties

Students demonstrating the general process-type difficulty thought that the IgG diagram represented various complex processes, rather than a simple non-covalent binding interaction between antibody and antigen molecules. For instance, some students interpreted the diagram as showing an antigen either in the process of entering the antibody structure or attacking the antibody, similar to the way a virus would "attack" a host. This is illustrated by the following examples of student quotations:

> "Antigen entering the κ and γ. Shows the pathway on which the antigen goes through. The V region first, then the C region." [response to probe (a)]

> I: Now…the next step…then you told me a bit…now tell me related to this [points to antigen].
> S: Related to this…mmmm…I think it [antigen] goes straight and breaks those two strands [S-S bonds]… [interview extract: "I" denotes "Interviewer and "S" denotes "Student"]

> "The diagram is trying to represent regions…regions where an antigen may attack."
> [response to probe (a)]

Other students showing the process-type difficulty, when observing the diagram, thought that the antigen was actually destroyed by the antibody, rather than during subsequent steps of the immune response. This is shown by the following three student quotations:

> "After binding to the antibody, antigen will be destroyed due to a chemical rxn [reaction] that may take place between the binding sites" [response to probe (g)]

> I: Ok, once it is joined [Ag] what does it do?
> S: Umm…[pause]…then I would think that the antibody surrounds it [Ag] and kills it.
> [interview extract]

> "It forms a complex with it, and destroys the existing virus" [response to probe (g)]

Some students who thought that the diagram represented an antibody undergoing cell division showed another process-type difficulty. The following quote supports this interpretation:

> "Cell (C), cell division takes place, two cells (V) are formed. Cell C old mature structure attaches 2 cells with black lines or bonds. Young immature cells (V) are attacked by Ag." [response to probe (a)]

Finally, related to the above difficulty, some students in interviews interpreted the heavy and light chains as being able to grow from an origin within the structure of the antibody, as displayed by these two quotes:

> S: These strings [polypeptide chains]…they…I would say they originally came from this big black molecule [C-region]…
> I: Ja…
> S: …and it…they come apart [indicates], they bind into the antigens and they start…they know where to bind because they start at the C-region… [interview extract]

> I: All right, so, if the antibody was by itself here, if the antigens weren't here on this picture, how would it look?
> S: These black lines [heavy/light chains] wouldn't be out here [points], it will be compacted inside so there's just one sphere [lower]…

> I: …yes…
> S: …and then umm, it will come into contact with the antigens, and then sense the contact, and then these lines will protrude in and change the…[pause]. [interview extract]

The above difficulties suggest that, in many cases, the students were using "surface-level", rather than "deep-level" reasoning to interpret the features of the diagram (Ramsden, 1992, p. 46-49). This may originate from the way the features of the diagram are artistically presented. For example, students using surface-level reasoning could have simply interpreted the arrow-shape of the antigen (Fig. 1) as representing the antigen in the process of entering the antibody. This interpretation might have been further enhanced by the fact that, in the diagram, the antigen is both pointing at the space between the light and heavy chains and is of the same width as the space, suggesting a possible pathway of entry.

In the case of the cell division example, students may have also used surface-level reasoning, in this case to relate previously learnt biology concepts such as 'phagocytosis' and 'mitosis' to what was being graphically presented in the diagram. Also, in everyday language, we speak of the body being prone to an 'attack' by pathogens. Thus students may have been linking general, everyday language to their interpretation of only a single molecular event. It could have followed that their interpretation of the diagram encompassed the entire immune response reaction.

The process-type difficulty category initially emerged from student answers to free-response questions. Thereafter, more specific written probes and interview questions facilitated the development of greater insight into each difficulty, allowing them to be classified higher up the framework and at Level 3. At this level the incidence of the difficulty was found to range from 15% to 54% depending on whether second or third-year students responded to the probes and which probes they were given. Further studies are underway in order to try and further establish the nature of this difficulty and its related sub-categories.

Structural-type difficulties

Students who showed the structural-type difficulties incorrectly interpreted various conventions used in the diagram to represent structural features of IgG. These included conventions used to show disulfide bonds, variable and constant polypeptide regions, and light and heavy chains. The following student quotes illustrate these difficulties:

"Heavy and light chains and[with] H-bonds between them." [response to probe (d)]

"Show[s] how 3 atoms are bonded to form a molecule. The antigen binds to the V region of the molecule. It shows that all 3 atoms are bonded by the C region…" [response to probe (a)]

"Black lines [are] some form of bond or attachment holding the 3 cells together- blood cells, biconcave type shape." [response to probe (a)]

"The coloured areas represent different areas…of red blood cells" [response to probe (e)]

"The coloured (grey) region represents different amino acid residues attached to the backbone (black line) of the antibody." [response to probe (f)]

A possible source of these difficulties is the fact that biochemistry textbooks often use more than one convention to represent a single structural feature of a molecule. For instance, whereas the disulfide bond is represented as a short straight black line in the present diagram (Fig. 1), it is often also represented in other diagrams either as "-S-S-" or as a yellow coloured bar, presumably to denote the presence of sulphur (This in itself could cause a misconception since not all forms of sulphur are yellow in appearance). Student confusion might have been further compounded by the fact that, in the diagram (Fig. 1), the polypeptide chain is also represented as a straight black line. The way in which the variable and constant regions are depicted as large spheres, may also be a source of confusion. They look like separate, ball-like structural entities, possibly leading some students to believe that they are not part of the actual antibody structure.

Following the unexpected emergence of these difficulties in response to free-response type questions, more focused written and oral probes were designed to gain further insight into each difficulty. The results confirmed the existence of this category of structural-type difficulties with incidences ranging from 13% to 70% of students. The range of incidence displayed is attributed to the various sub-categories pertaining to this difficulty. The difficulties were classified on the framework as partially established at Level 3.

DNA-related difficulties

Students showing the DNA-related difficulty incorrectly interpreted the diagram as representing a form of DNA processing, such as replication or elongation. This difficulty is illustrated by the following quotes:

"Structure of DNA as it unfolds due to RNA interpretation of the DNA template. Ag is [a] protein molecule that is required according to the nitrogen base pairing of both the DNA and RNA. The whole process occurs in macrophages which are represented/shown by circles." [response to probe (a)]

"This is meant to represent a DNA molecule, leading strands and a lagging strand of DNA…" [response to probe (a)]

I: Ok…So, if I were to ask specifically about this line, the shorter one [light chain], what would you say?
S: It looks like a new replicating strand of DNA.
I: Yes…
S: [pause] ahhh…possibly…well ok, umm…possibly replicating the same information which is on this C region, and then building it onto the Ag molecules so you going to get identical molecules with the same DNA conformation.
I: …so there is a building process occurring here?
S: Ja…it is nucleotide synthesis, communication…
I: Alright…So, you called the shorter one a…
S: The new strand.
I: The new strand, ok, and the long one?
S: Like the parent strand, but…that is coding for the new strands. [interview extract]

Prior to this investigation, students had just completed a module on nucleic acids in which they had been exposed to diagrams of DNA replication and synthesis (e.g. as in Stryer, 1995, p. 804 and Hames *et al*., 1997, p. 137). Since the replication fork, with lagging and leading strands, in these diagrams looks similar in appearance to how the 'Y' shape of IgG's heavy and light chains are represented in Fig. 1, it is possible that some students were incorrectly transferring their knowledge from one context to the other. That is, as described by Salomon and Perkins (1989), students with this difficulty might have been inappropriately transferring constructed knowledge (e.g. of DNA) to novel learning domains (e.g. IgG). This difficulty initially emerged unexpectedly from second-year written responses showing an incidence of 11%. Following further investigations with the aid of interviews, this category of difficulty was reclassified from being suspected at Level 2 to Level 3 or partially established.

Conclusion

The results suggest that some students used a surface-level, instead of a deeper-level reasoning process to understand the diagram. This especially manifested itself when diagrammatic features were interpreted literally instead of students considering the abstract or stylised nature of the diagram. This was coupled to them struggling with an accurate interpretation of the conventions used to depict the structural features. Besides the former, it was shown that linking general, everyday language to a single molecular event might cause students to generalise incorrectly. Finally, some students inappropriately transferred graphical features from one learning domain to another.

Generally speaking, the origin of the difficulties presented could either be of diagram or of student origin. Those of a diagram origin could include factors such as: the artistic style used to represent

the elements of the diagram; lack of, or confusing diagram conventions; similarity of diagrams across contexts; portrayal of realism (in the sub-microscopic environment); and, the use of symbolic representation. A diagram that seems clear to an author may not be so for a learner, and, experts should not assume that novices will interpret diagrams and their conventions in the same way as they would. It follows that diagrams should contain sufficient 'visual support' to help students conceptualise relationships and build mental models (Lowe, 1996), and that students should be explicitly taught to 'read' diagrams (Wheeler and Hill, 1990; Gillespie, 1993). This should include the introduction and teaching of various conventions to make students aware of them (Wheeler and Hill, 1990) and, the use of conventions should be standardised or specially designed for this purpose.

Difficulties of student origin could include surface-level interpretations, for example, simple matching of graphical features to look-alike mental models (i.e. DNA-related difficulty), which may decrease the usefulness of the diagram. Also, it has been shown that the understanding of an abstract diagram is related to a learner's prior experience (Hill, 1988; Lowe, 1999), and, that interpreting diagrams is an acquired skill. Other aspects could consist of poor mental representation construction and weak mental models, where Lowe (1993) infers that superficial mental representations should be expected from novices, since they lack vast amounts of domain-specific knowledge.

This study confirms the results of other research reports (e.g. Johsua, 1984; Wheeler and Hill, 1990) in showing that incorrect interpretation of, and reasoning with diagrams can lead to misunderstandings and conceptual difficulties. In this regard, further research is in progress with more diagrams, to establish to what extent factors such as diagram quality, conceptual understanding, diagrammatic reasoning and interpretation contribute to each student difficulty. This will then enable the devising of interventions to supply learners with necessary skills to interpret diagrams effectively (Hill, 1988), one of the primary objectives of this project.

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